CELLULAR RESPONSES TO DNA DAMAGE

E. C. Friedberg and B. A. Bridges, Organizers
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Plenary Sessions

Poster Sessions

Alternations in DNA Chemistry

0914 DNA DAMAGE AND MUTATION: AN OVERVIEW, William A. Haseltine, Department of Biochemical Pharmacology, Sidney Farber Cancer Institute, Boston, MA 02115

Carcinogens and mutagens are for the most part agents which damage DNA. Much to the distress of workers in this field, most of these agents modify DNA in a number of ways. In the past it has been very difficult to determine which, if any, of the DNA modifications are precursors to the biological effects. Recently advances in cell biology and molecular biology have opened the possibility that the precise nature of the pre-mutagenic and carcinogenic events can be studied. These advances come principally from genetic definition of precise sites of mutation, DNA sequencing methods, and the ability to introduce DNA into prokaryotic and eukaryotic cells.

A number of workers have begun to avail themselves of the power of these methodologies. This work integrates studies of the chemistry of DNA modification, the mutagenic specificity of a variety of carcinogens and mutagens, and DNA sequencing methodologies. All of these methods are focused on single genes. As a result of these efforts by a number of groups, a picture of some of the key events in mutagenesis is slowly emerging.

This talk will summerize some of the methods and approaches that are in current use in this field. Specific examples from work in my laboratory and work in other laboratories will illustrate these approaches. In many cases this work has confirmed some of our earlier notions regarding mutagenesis. In other cases we have been surprised by the results of such analysis.

0915 Z-DNA--IMPLICATIONS FOR DNA DAMAGE AND REPAIR, Marc Leng, Centre de Biophysique Moléculaire, C.N.R.S., 45045 Orléans, France

Left-handed double stranded DNA, named Z-DNA,was first discovered by an X-ray analysis of the alternating hexanucleotide d(CpGpCpGpCpG) crystals (1) and then of several oligo $d(C\text{-}G)_n$ crystals (2,3). Studies of poly(dG-dC).poly(dG-dC) in fibers, films and in solution have lead to the conclusion that this polymer can adopt the Z-form (general review 4).

Several factors can stabilize poly(dG-dC).poly(dG-dC) in the Z-conformation as the nature and the concentration of salts, the presence of organic solvents, the chemical modification of the base residues... (general review 4). In physiological conditions, $(dC\text{-}dG)_n$ blocks in plasmids can be in left-handed conformation if the DNA is sufficiently negatively supercoiled (5,6). The role of the topological constraints can be shown by the study of form V DNA (7).

The dynamic structure of the B and Z forms are strikingly different. This difference might be important in biological processes that require molecular recognition. The effects of some drugs which bind to DNA in B or Z form by covalent bond or by intercalation will be discussed.

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The Repair of DNA I

0916 PROPERTIES AND REGULATION OF THE UVRABC NUCLEASE, W. Dean Rupp, Aziz Sancar, Gwen Sancar and Barry Kacinski, Departments of Therapeutic Radiology and Molecular Biophysics & Biochemistry, Yale University School of Medicine, New Haven, CT 06510 USA

Because the classical biochemical approach had provided only minimal information concerning the initial steps of nucleotide excision repair in E. coli, we have used DNA recombinant methodology for the cloning and characterization of the uvr genes. The uvrA, uvrB, and uvrC genes were cloned on plasmids. The maxicell technique was developed in order to specifically label the proteins encoded by cloned genes, and when the cloned genes were inactivated by the insertion of transposons, the gene products of the 3 uvr genes were identified. In order to obtain larger quantities of these gene products for biochemical studies, the genes were subcloned onto expression vectors in which the yields of the gene products were greatly increased. When the 3 purified proteins, UVRA, UVRB and UVRC were mixed together, active enzyme was reconstituted that specifically cuts damaged DNA but not undamaged DNA. By using restriction fragments, the location of the cuts was determined. Surprisingly, the enzyme cuts at specific sites in the damaged strand on both sides of the lesion such that 7 undamaged bases are left on the 5' side and 3 or 4 undamaged bases on the 3' side of lesions including pyrimidine cyclobutane dimers, pyrimidine-cytosine adducts and psoralen adducts. Since this enzyme actually removes damaged regions of DNA by itself without the requirement of other proteins, the generally accepted model for nucleotide excision repair, in which a DNA polymerase removes the lesion, is incorrect and must be revised.

Experiments on the control of the genes (including the sequence determination of the control regions) indicate that promoters regulated by lexA and under SOS control are present in the uvrA and uvrB genes while such a promoter has not been observed for uvrC. While uvrA seems to have only one promoter, uvrB has a second promoter that is not regulated by lexA and also a third promoter whose in vivo function is unclear. The consequences of these various forms of regulation on the actual level of intracellular enzymatic activity are not yet clear and require further study.

0917 THE UVRD GENE OF E. COLI: MOLECULAR CLONING AND BIOCHEMICAL FUNCTIONS, Mutsuo Sekiguchi, Keiko Kumura, Kenji Oeda, Masahiro Akiyama and Takashi Horiuchi, Department of Biology, Kyushu University 33, Fukuoka 812, Japan

Mutations in the uvrD gene of E. coli K-12 result in multiple phenotypes, including increased sensitivity to UV light, alkylating agent and ionizing radiation, increased spontaneous mutation frequency and decreased rate of genetic recombination in certain genetic background. It has been shown, moreover, that double mutants having mutations in both uvrD and polA genes are conditionally lethal. To study the function of the uvrD gene, we have constructed hybrid phages and plasmids that carry the gene (1). Proteins produced by λuvrD$^+$ transducing phage were labeled with ^{14}C-amino acids and analyzed by SDS-polyacrylamide gel electrophoresis. The uvrD gene product was identified as a polypeptide of molecular weight 75,000 (2). A similar result was recently obtained with cells carrying uvrD$^+$ plasmid (3).

Biochemical analyses revealed that the uvrD protein possesses DNA-dependent ATPase activity. Cells carrying uvrD$^+$ plasmids or cells infected with λuvrD$^+$ contained about 15 times more ATPase than normal cells. Since wild type cells with uvrD$^+$ plasmids were more sensitive to UV than wild type cells without plasmid, the production of a large amount of the uvrD protein seems to be harmful to cell. The uvrD protein was purified by ammonium sulfate fractionation, several steps of column chromatography and glycerol gradient centrifugation. The ATPase activity was totally dependent on denatured DNA and was sensitive to N-ethylmaleimide. Furthermore, the purified protein was capable of unwinding double-stranded DNA at the expense of ATP. It was suggested that the uvrD protein may be identical to DNA-dependent ATPase I and DNA helicase II. Such enzyme activities may play vital roles in DNA replication, recombination and repair, and this would explaine the pleiotropic phenotypes of the uvrD mutants. Relationship between various mutations and enzyme activities will be discussed.

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0918 CLONING AND CHARACTERIZATION OF EXCISION REPAIR GENES FROM Saccharomyces cerevisiae. Errol C. Friedberg, Elizabeth W. Yang, William A. Weiss, Glenn A. Pure, and Louie Naumovski, Department of Pathology, Stanford University, Stanford, CA 94305.

We have previously described a screening protocol for the isolation of multicopy autonomously replicating plasmids containing yeast chromosomal DNA inserts that confer enhanced UV resistance to selected yeast rad mutants (1). Using this protocol we have isolated plasmids designated pNF1000, pNF2000, pNF3000, pNF4000, and pNF100, that respectively transform mutants defective at the RAD1, RAD2, RAD3, RAD4, and RAD10 loci to UV resistance. These five genes are known to be required for the incision of DNA in cells exposed to ultra-violet (UV) radiation (2,3). Genetic evidence indicates that plasmids pNF1000, pNF2000, and pNF3000 contain the structural RAD1, RAD2, and RAD3 genes respectively. Genetic analysis of pNF100 is in progress. Plasmid pNF4000 confers partial UV resistance to the rad4-3 mutant uniquely, and the properties of this plasmid will be presented elsewhere at this meeting. Restriction mapping has been carried out on all the plasmids, and it has been established that the RAD1, RAD2, and RAD3 genes do not contain SalI restriction sites. This has facilitated the subcloning of Sau3A partial digests of plasmids pNF2000 and pNF3000 into a unique BamHI site on a vector tailored to allow convenient retrieval of the subcloned inserts from flanking SalI sites. Mapping of the RAD2 and RAD3 mRNA transcripts to establish the size, location and orientation of these genes in the subclones is in progress. Data will also be presented on the subcloning and further characterization of the RAD1 gene. Evidence will be presented which indicates that an intact RAD3 gene is essential for viability of haploid cells regardless of exposure to DNA damage.

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The Repair of DNA II

0919 PHOTO-INDUCED CROSSLINKS AND MONOADDUCTS IN YEAST DNA: GENETIC CONTROL AND MOLECULAR STEPS INVOLVED IN THEIR REPAIR, E. Moustacchi, C. Cassier, R. Chanet, J.A.P. Henriques, N. Magana-Schwencke, and T. Saeki, Institut Curie-Biologie, Bat. 110, 91405 Orsay, France.

Photoaddition of bifunctional furocoumarins acts as stronger genotoxic agents in yeast than do monofunctional ones. This is supported by two lines of evidence. Firstly, the mutagenic and recombinogenic effects of bifunctional derivatives plus UVA are higher than those of monofunctional compounds including a new class of pyrido-psoralens (synthesized by E. Bisagni). Secondly, we demonstrate that cross-links are clearly genotoxic in wild type by a split dose protocol which allows for a constant number of lesions to increase the proportion of cross-links relative to monoadducts. This type of experiment also shows that a fraction of mono-adducts can be bypassed and the use of repair-deficient mutants (rad) demonstrates that the bypass process is genetically controlled. Taken together with findings on different test systems, we conclude that monofunctional furocoumarins plus UVA are less risky with regard to genetic damage.

The repair of photo-induced lesions by psoralens involves the three main pathways identified in yeast (excision, error-prone and recombinogenic pathways). New genes (PSO) initially identified by selection of mutants sensitive to psoralens photo-addition have been characterized. The PSO 2 gene is of particular interest since it is involved in both induced mutagenesis and recombination specifically following DNA cross-linking treatments.The interactions between PSO and RAD genes according to different types of induced lesions will be discussed.

Data on the kinetics of repair of monoadducts relative to cross-links showing that the former are the most rapidly eliminated will be presented, and the behaviour of repair-deficient mutants in this respect will be described.

0920 DROSOPHILA MELANOGASTER: A MODEL EUKARYOTE FOR THE STUDY OF DNA REPAIR, James B. Boyd, Department of Genetics, Univ. of California, Davis, CA 95616.

Mutants of Drosophila melanogaster which render larvae hypersensitive to mutagen treatment have been identified at 30 different genetic loci. Meiotic recombination is strongly reduced in mutants at four loci, and eight additional loci are represented by mutants which moderately disrupt meiosis. In addition, mutants at about one-half of the investigated loci exhibit somatic chromosome instability. This mutant collection, therefore, identifies genes which are potentially involved in DNA exchange as well as in the repair of spontaneous and induced DNA damage.[1]

Excision of pyrimidime dimers has been analyzed in organ or cell cultures of mutants at about one-half of the currently recognized loci. Mutants in two genes lack excision repair altogether, whereas five genes are associated with partial defects in that process. Alkaline elution has been employed to monitor DNA interruptions associated with excision repair. This approach has defined four different genetic blocks in incision and 3 blocks in post-incision steps of excision repair.[2]

Genetic analyses of the strongly excision-defective mutants at the mei-9 locus suggest that excision repair of alkylation damage is relatively error free in Drosophila.[1] These mutants also influence the mobility of insertion sequences[3] and the magnification of ribosomal DNA sequences[4]. In meiotic cells mei-9 mutants are postulated to inhibit DNA isomerization and mismatch correction but do not influence gene conversion.[5]

Mutants in four different complementation groups exhibit a strong deficiency in postreplication repair following UV treatment. Seven additional non-allelic mutants are somewhat less defective in that process. Among the strongly deficient mutants, two are excision proficient whereas the other two are deficient in both excision and post-replication repair.[6]

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0921 ANALYSIS OF MAMMALIAN CELL MUTAGENESIS AND DNA REPAIR USING IN VITRO SELECTED CHO CELL MUTANTS, L. H. Thompson, A. V. Carrano, and L.E. Dillehay, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

Our laboratory has undertaken a systematic effort to isolate and characterize mutants of Chinese hamster ovary (CHO) cells that are defective in DNA repair processes. One class of mutation was isolated on the basis of hypersensitivity to killing by UV radiation (1,2) and is represented by 5 genetic complementation groups (3). Mutants from each group show similar UV sensitivity, very low incision of DNA after UV exposure, and are hypersensitive to killing and mutation by chemicals that form bulky DNA adducts. Thus, by these criteria the mutants resemble cells from xeroderma pigmentosum (XP) individuals. However, mutants from CHO groups 2 and 4 are extremely sensitive to killing by DNA crosslinking agents (e.g. mitomycin C), a property that resembles Fanconi's anemia rather than XP. We are testing for complementation between the CHO mutants and 7 groups of XP by using cell fusion to produce viable hybrids with XP fibroblasts. Our results so far for mutants in groups 2 and 4 suggest that the human genome can provide the repair functions that are defective in these mutants.

Another class of mutation, which differs from the human genetic diseases, was isolated on the basis of 10-fold hypersensitivity to killing by ethyl methanesulfonate (1). The mutant strain EM9 has a 12-fold-elevated baseline frequency of SCE (sister-chromatid exchange), increased killing by alkylating agents and X rays, defective rejoining of DNA strand breaks, and retarded maturation of newly synthesized DNA when BrdUrd is in the template strand (4, 5). All these properties of EM9 are observed in normal cells treated with benzamide or 3-aminobenzamide, inhibitors of poly(ADP-ribose) polymerase. Thus, the defect in EM9 could lie in the poly(ADP-ribose) system, a DNA ligase, or other component. EM9 also is extremely sensitive to killing by CldUrd incorporation, which causes much higher SCE than BrdUrd. Using alkaline elution to analyze DNA strand breakage associated with BrdUrd or CldUrd incorporation in normal vs EM9 cells, with and without benzamide treatment, we find that SCE induction correlates with strand breakage (5). Thus, the high SCE frequencies in EM9 or in normal CHO cells treated with benzamide appear to result from longer lifetimes of strand breaks. (Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48 and supported by a grant from the U.S. Environmental Protection Agency, No. R808641-10).

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Adaptive Responses to Alkylation Damage

0922 REPAIR OF ALKYLATION DAMAGE IN E. COLI, Paul F. Schendel, Brent V. Edington, James G. McCarthy, and Michael L. Todd, Genetics and Cell Biology Section, The Biological Sciences Group, The University of Connecticut, Storrs, CT 06268

Alkylating agents like the alkyl-nitrosoguanidines are representative of a large group of mutagenic chemicals that alkylate the nitrogen and oxygen atoms of DNA. Nucleotide-excision repair, base-excision repair, or direct dealkylation repair systems are capable of processing the lesions produced by these chemicals. Since components of all of these systems are inducible, the cellular response to various alkylating agents differs and depends on the nature of the alkyl group transferred.

The adaptive response is the major cellular response to methylation damage[1,2]. This response results from the induction of proteins which are involved in the direct demethylation of some lesions[3] and the excision of others[4,5]. As the alkyl group gets bigger, the adaptive response is less effective, but nucleotide-excision repair, which has little effect on methylation damage, starts to play a more important role.

The mechanisms by which different alkylating agents cause mutations vary. Some methylating agents cause mutations via the umuCD mediated SOS response[6,7]. Others, such as methylnitrosoguanidine, produce mostly miscoding lesions which produce mutations without umuCD involvement[7]. Within the alkyl-nitrosoguanidine series, the involvement of umuCD mediated mutagenesis becomes greater as the size of the alkyl group increases. This increased role of umuCD parallels the increase in efficiency of nucleotide-excision repair to reduce the mutagenic effects of this alkylation. Thus the adaptive response and the SOS response act as complementary systems which affect different kinds of DNA damage. The interaction of these systems, their substrate specificities, and their control will be discussed.

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0923 MECHANISMS OF REPAIR OF ALKYLATION DAMAGE. Thomas Lindahl, Peter Karran, Bruce Demple, Barbara Sedgwick, Adrian Harris, and Peter Robins. Imperial Cancer Research Fund, Mill Hill Laboratories, London NW7 1AD, U.K.

The N-methylated purines 3-methyladenine, 3-methylguanine, and 7-methylguanine are removed from DNA by excision-repair, initiated by release of the methylated bases by specific DNA glycosylases. In contrast, o^6-methylguanine is repaired by a DNA methyltransferase. The latter enzyme has been purified to homogeneity from E. coli. A detailed kinetic study has shown that the enzyme undergoes suicide inactivation by transferring the methyl group from DNA to one of its own cysteine residues, the methylated enzyme accumulating as a dead-end complex. In addition to recognizing o^6-methylguanine, the methyltransferase acts on the analogous ehtyl derivative, and on adducts formed by clinically used halonitrosoureas such as CCNU and BCNU. Synthesis of the methyltransferase is under control of the ada+ gene in E. coli. The ada+ gene has been cloned in a multicopy plasmid; it encodes a protein different from the methyltransferase. An o^6MeG-DNA methyltransferase with very similar properties has been partly purified from human cells. It appears to be completely absent from repair-deficient cells of the Mex- (or Mer-) phenotype.

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0924 THE O^6-ALKYLGUANINE TRANSFERASE ACTIVITY OF RAT LIVER CHROMATIN, Walter G. Verly, biochimie, faculté des sciences, Université de Liège, Belgium.

Cell nuclei are isolated from rat liver and purified as in [1]. The nuclei suspension is incubated for 30 min at 37° with ethylnitrosourea (ENU). At the end of this treatment the O6-ethylguanine (O6-etG)/7-ethylguanine ratio in nuclear DNA is lower than that observed when naked DNA is treated with ENU; no free O6-etG is released in the incubation medium [2]. The extent of repair is similar to that obtained *in vivo* showing that all the necessary factors are in the nucleus.

Chromatin is prepared from the purified nuclei as in [1]; it is dissociated with heparin-Sepharose and the non-histones are extracted as in [3]. Incubation of DNA alkylated with ENU or MNU (methylnitrosourea) with these chromatin proteins is followed by the disappearance of O6-etG or O6-meG (O6-methylguanine) [3,4]. No release of free O6-etG or O6-meG, or of oligonucleotides enriched in these alkylated guanines, is observed; the elimination of O6-etG or O6-meG from DNA is thus not due to a DNA glycosylase or a team endonuclease-exonuclease. The alkyl group is transfered unto the cystein of an acceptor protein [5]; the same acceptor is used for ethyl and methyl [4].

The extent of repair obtained with the chromatin protein is of a level similar to that observed in whole liver. It is stimulated to the same extent by a pre-treatment with daily low doses of diethylnitrosamine [5].

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Inducible Responses to DNA Damage I

0925 GENETICS OF THE SOS RESPONSE OF *E. coli*, D. W. Mount, John W. Little, Ken Wertman, Ken Peterson, Gary Lyons, and H. Ginsberg, Departments of Molecular and Medical Microbiology and Biochemistry, University of Arizona, Tucson, Arizona 85724.

The SOS response is regulated by the products of two genes, *lexA* and *recA*. The *lexA* product, a repressor, limits transcription of at least 11 unlinked genes whose products are involved in a variety of metabolic processes (reviewed in ref.1). The *recA* product, in addition to possessing activities which promote interaction of homologous and heterologous DNA molecules (C. Radding and I. R. Lehman, this symposium), also has a specific protease function activated by DNA damage and directed towards *lexA* repressor in induced cells. This cleavage destroys repressor function and derepresses the *lexA* target genes.

Induction of the SOS response is prevented by mutations, denoted *lexA*(Ind$^-$) which make repressor resistant to cleavage. We are analyzing cleavage, repressor function and the DNA sequence change in a number of such mutants made by *in vivo* and *in vitro* mutagenesis. Another class of *lexA* mutants, *lexA*(Ts) formerly called *tsl* mutants, appears to make a temperature-sensitive repressor with varying affinities for its target operators. One such mutant gene has been cloned in order to study the product and determine the change in DNA sequence. Finally, mutants with reduced repressor function, *lexA*(Def) formerly called *spr*, lead to constitutive expression of the SOS functions.

Mutants in specific operator sequences have been obtained by us and by others (A. J. Clark and Roger Brent, personal communications). These operator constitutive mutants are useful for analyzing the role of individual SOS regulated genes in the overall SOS response. Of particular interest is the effects of mutations in the dual repressor binding sites ahead of *lexA* which are involved in autoregulation.

recA mutants with diminished or enhanced protease activity have also been studied by several laboratories (1).

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0926 CELLULAR COMPONENTS REQUIRED FOR MUTAGENESIS, Graham C. Walker, Karen L. Perry, Stephen J. Elledge, Biology Department, Massachusetts Institute of Technology, Cambridge, MA 02139

In Escherichia coli mutagenesis by UV and various chemicals is not a passive process. The isolation of umuC mutations (1) indicated that there was at least one cellular gene whose product was uniquely required for mutagenesis. The plasmid pKM101, which was derived from the clinically isolated plasmid R46 by in vivo means, increased the susceptibility of cells to mutagenesis in a recA$^+$lexA$^+$-dependent manner and was able to suppress the nonmutability and UV-sensitivity of umuC mutants. On the basis of these observations we had hypothesized that the muc locus of pKM101 which was responsible for these effects coded for an analog of the umuC product(s).

We have recently cloned the umuC locus of E. coli (2). A probe to the umuC region was derived from a umuC::Tn5 mutant and was used to screen a λ library of E. coli DNA. The umuC locus was then subcloned from λumuC$^+$ bacteriophage onto a low copy number plasmid. Through a combination of subcloning and Tn1000 mutagenesis, we have identified a region of 2.2 kb which contains the information necessary to complement umuC mutations. This region of DNA codes for two polypeptides with molecular weights of 16 and 45 kilodaltons. The genes for these proteins are organized in an operon that is repressed by the lexA protein. Complementation of previously isolated umuC mutations revealed that these two proteins correspond to two complementation groups, umuC which codes for the 45 kilodalton protein, and umuD which codes for the 16 kilodalton protein, and that therefore both proteins are essential for "error-prone" repair in E. coli.

In a similar series of experiments we have examined the muc locus of pKM101 (3). Like the umuC locus of E. coli, the muc locus of pKM101 consists of two genes, mucA and mucB, that code for two polypeptides with molecular weights of 16 and 45 kilodaltons respectively that are organized in an operon repressed by lexA. Complementation studies have indicated that both proteins are required for pKM101's effects on mutagenesis. Even though evidence from Southern blotting studies indicates that the nucleic acid sequences of the umu and muc genes have diverged it appears that pKM101 does in fact code for analogs of the umuC and umuD proteins.

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0927 REGULATION OF INDUCTION OF SOS FUNCTIONS, Jeffrey W. Roberts, Eric M. Phizicky, David G. Burbee, Patrice L. Moreau and Christine W. Roberts, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

The E. coli SOS genes are regulated by the LexA repressor. They are induced when disruption of cellular DNA replication, caused for example by radiation damage to DNA, invokes the protease activity of RecA protein to destroy LexA. Biochemical studies show that single-stranded DNA and a nucleoside triphosphate are required to activate the RecA protease in vitro, suggesting that induction of SOS genes occurs when disruption of replication allows RecA protein to bind single-stranded DNA.

We describe how this activation may occur in vivo, arguing from results of biochemical studies of mutationally altered RecA proteins and different repressor substrates.

0928 REGULATION OF THE CELLULAR RESPONSE TO DNA DAMAGE
Roger Brent, Department of Biochemistry and Molecular Biology, Harvard Universtity, Cambridge, Massachusetts 02138

The lexA gene product represses many genes in Escherichia coli. If the cell's DNA is damaged, lexA protein is inactivated and these genes are expressed. Recent genetic and biochemical studies suggest that the concentration of intact lexA protein after DNA damage may determine the detailed control of the SOS response.

Studies of the mutagenesis induced in E. coli after DNA damage may also be described.

Inducible Responses to DNA Damage II

0929 THE DNA DAMAGE INDUCED GENES OF YEAST. Jack W. Szostak, Stephanie W. Ruby, and Ann Lichens. Department of Biological Chemistry, and Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

The yeast Saccharomyces cerevisiae contains a set of genes that are induced in response to treatments that cause DNA damage. We have identified and cloned six DNA damage induced (din) genes as fusions to the E.coli lacZ gene. These were isolated from a pool of fusions of all yeast genes to lacZ, made by inserting random yeast DNA fragments in front of a truncated lacZ gene (derived from pMC1403, made by M.Casadaban). Plasmids in which a yeast gene is properly fused to lacZ cause the production of B-galactosidase in yeast, which will then turn blue on X-gal indicator plates. Since the B-galactosidase is produced from each fusion under the control of a yeast promoter, we screened for yeast transformants which made more B-galactosidase, and thus became more blue, after DNA damaging treatments. Five of the six din:lacZ fusions we isolated are induced after treatment with low levels of UV or gamma radiation, methylmethane sulfonate, 4-nitroquinoline-1-oxide, or mitomycin C, or after thymine starvation. One of the fusions appears to be induced by UV, but not by mms, or gamma radiation. Induction occurs at the mRNA level, and is detectable on Northern blots in less than 30 minutes. Heat and nutrient starvation do not result in significant induction.

We are studying the regulation of the yeast din genes by identifying unlinked regulatory genes. Preliminary experiments in which a strain carrying a chromosomally integrated din:lacZ fusion was mutagenized indicate that both constitutive and uninducible mutants can be recovered. The same strain was also transformed with a library of yeast DNA fragments cloned on a high copy vector. One plasmid was recovered which results in the constitutive expression of the din1:lacZ fusion. Additional genetic characterization of these mutants and plasmids will be presented.

0930 PERSISTENCE THROUGH MITOTIC DIVISIONS OF INDUCED RECOMBINATIONAL ABILITY IN YEAST, Francis Fabre, Institut Curie-Biologie, Bât. 110, Centre Universitaire, 91405 Orsay, France.

The rate of gene conversion in eucaryotic microorganisms is 10^3 to 10^4 lower in mitosis than in meiosis. Holliday proposed that it may be due to the repression in vegetative cells of a recombinational mechanism (1). The inducibility of the ability of mitotic cells to recombine was demonstrated in yeast by an indirect induction experiment (2): when haploids are irradiated and mated with diploids, gene conversion involving the chromosomes of the untreated diploids is induced. It is initiated by DNA lesions in the haploid nucleus and observed whether or not, the nuclei fuse in the zygote.

An important question, relative to the stability of vegetative cells in general, is to ask if the induced state persists through mitotic divisions. The inducible repair mechanisms in E. coli and in mammalian cells are repressed when the first post-irradiation cell division occurs (3,4). We have asked if the same is true for the yeast recombinational mechanism, by following the indirect induction of conversion as a function of the post-irradiation generations. The results indicate that after UV as well as after X-ray treatment, the cells retain the property to perform conversion at a higher rate than the control population, at least during the 11 generations that we could follow. The kinetics are interpreted to say that after a wave of derepression, a fraction of the population becomes progressively repressed, but the the cells of the other fraction remain derepressed and transmit this property to the daughter cells.

Experiments where the treated haploids were incubated before mating showed that, also in haploids, the recombinational ability is induced and persists after the disappearance of the initial signal that likely corresponds to the repair of DNA lesions. The data support a regulation model where a gene coding for a repressor of a recombinational gene is itself repressed by the recombinational protein. The irradiation of repressed cells would result in a cleavage of the bound repressor, as in the case of the recA protein in E. coli (5) or else in its displacement as in the case of the 32 proteins of T4 (6). After DNA repair, the two proteins are present, and the cells will fall upon division into the stable repressed or derepressed states according to which repressor first disappears.

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0931 MOLECULAR MECHANISMS OF SV40 DNA MUTAGENESIS INDUCED BY UV-LIGHT OR CHEMICALS, Alain Sarasin, Alain Gentil and François Bourre, Institut de Recherches Scientifiques sur le Cancer, B.P. 8, F-94802 - Villejuif, France.

The molecular mechanism of mutagenesis in mammalian cells treated with carcinogens has been studied using Simian Virus 40 (SV40) as a model system. SV40 is particularly relevant in carrying out this analysis because it is repaired and replicated by host-cell enzymes only, its replication mode being identical to the cellular DNA replication. Moreover, its molecular biology is very well known which allows us to easily analyse at the molecular level the type of mutation produced (1). The mutation assay we used, consists of a phenotypic reversion to wild-type growth of two different temperature-sensitive SV40 mutants (the early tsA58 mutant and the late tsB201 mutant).

Acetoxy-acetylaminofluorene (AAAF), UV-light or apurinic sites give rise to mutations on SV40 DNA after its replication in monkey kidney cells. The mutation frequency is proportional to the treatment dose, but the efficiency of mutagenesis varies greatly as a function of the type of lesions. Pretreatment of cells with UV-light, AAAF or mitomycin C strongly increases the mutation frequency in the virus progeny after infection with UV-irradiated SV40 virus but not after transfection with UV-irradiated SV40 DNA. We interpreted these differences in terms of different multiplicity of infection for the two experimental protocols (2,3).

The molecular analysis of UV-induced SV40 mutagenesis has been approached by analysing the DNA sequence of the reversion sites for several independantly-isolated SV40 revertants. These reversion sites have been mapped around the tsA58 mutation site using the marker-rescue technique. Results show that UV-induced mutations are single base-pair substitutions, always localized opposite a possible T-T dimer site within the T antigen gene and different from the original tsA58 mutation site still present. This result is the first demonstration of targeted mutagenesis in mammalian cells.

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Fidelity of DNA Replication

0932 CONTROLLING REPLICATION FIDELITY: THE PROPERTIES OF DNA POLYMERASE AND SUBSTRATE-TEMPLATE INTERACTIONS, Myron F. Goodman, Molecular Biology, University of Southern California, Los Angeles, California, 90089-1481

Enzyme-catalyzed reactions generally exhibit a high degree of substrate specificity. In the case of DNA synthesis, however, a high degree of specificity is already available from base-pairing interactions. A question then arises: does DNA polymerase reduce nucleotide misinsertion frequencies below that which can be attributed to free energy differences between matched and mismatched base pairs? Is there a source of free energy available in addition to base-pairing free energy differences which would allow the polymerase to adjust its active site conformation to reduce the rate of phosphodiester bond formation for potentially mispaired nucleotides? Using a model for DNA replication fidelity, the K_m Discrimination model (1,2), we will discuss free energy differences between matched and mismatched base pairs referring to the active site of the polymerase during DNA synthesis. The base-pairing free energy differences defined in the presence of the enzyme will be compared with those obtained from an analysis of thermal melting profiles using defined synthetic DNA. Data will be given for 2-aminopurine•thymine and 2-aminopurine•cytosine base pairs; these are the two mispaired intermediates in the A•T ⇄ G•C transition pathway induced by the base analogue 2-aminopurine (AP). A test of the postulated role of rare tautomers in base mispairs will be discussed. The measurement of thermal melting profiles of synthetic polymers containing AP•T and AP•C base pairs should permit a direct observation of the elusive imino tautomer of AP if the existence of this rare tautomer is indeed responsible for the formation of AP•C mispairs. A scheme which accounts for Pu•Py base mispairs without requiring the involvement of base tautomers will be mentioned briefly.

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0933 MECHANISMS OF CATALYSIS OF HOMOGENEOUS PREPARATIONS OF HUMAN DNA POLYMERASES α AND β, David Korn, Paul A. Fisher and Teresa Shu-Fong Wang, Stanford University School of Medicine, Stanford, California 94305

We have employed steady-state kinetics methodology, supplemented and corroborated by direct physical measurements, to illuminate some of the mechanisms of catalysis of homogeneous fractions of human DNA polymerases α and β. The interaction of pol α with its substrates obeys a rigidly ordered sequential ter mechanism, with obligate binding of template (single-stranded DNA) first, followed by primer stem, and finally, by dNTP. Although kinetically significant dNTP binding is absolutely dependent on antecedent primer binding, specification of which dNTP can add to the enzyme is strictly determined by template sequence. Primer binding requires a minimum length of 8 nucleotides, of which the terminal 3-5 nucleotides must be template-complementary. A single mispaired terminal primer nucleotide prevents correct primer binding as well as the subsequent step of dNTP addition to the enzyme. Primer binding, which is satisfied by 3'-terminal H or OH, but blocked by PO_4, occurs through the coordinated participation of 4 Mg^{+2}- primer-binding subsites that may serve as a "Mg^{+2}-shuttle" and thereby facilitate not only phosphodiester bond formation but polymerase translocation as well. Each catalytically active pol α molecule appears to possess 2 positively cooperative single-strand DNA binding sites, and very possibly, 2 complete active centers. We suggest pol α is a conformationally active protein that responds to signals generated by template sequence and transduced via template binding site(s) interactions. In sharp contrast, human pol β has only a weak affinity for single-stranded DNA that is not modulated by base sequence nor enhanced by the presence of potentially base-pairable 3'-OH termini. Pol β, like pol α, has no affinity for intact duplex DNA, but unlike α, pol β has extremely high affinity for nicked duplex DNA, with comparable avidity for nick termini bearing 3'-OH or 3'-PO_4 residues. Also in contrast to pol α, pol β is reactive with primers that contain 1-3 mispaired terminal residues, suggesting less stringent requirements by pol β for primer-stem binding. Although the mechanism of substrate addition to pol β is rigidly ordered and sequential (DNA, followed by dNTP), binding to template and primer may occur by a concerted mechanism. The primary signal for catalytically productive binding of pol β to DNA is a base-paired primer moiety that must be adjacent to a short length of (potentially) single-stranded template. Both the minimum length of required template, as well as the processivity of polymerization by pol β, are affected by the choice of divalent action: with Mg^{+2}, minimum template length is ≥ 5 nucleotides and polymerization is distributive; while with Mn^{+2}, minimum required template is but a single nucleotide, and the enzyme becomes modestly processive, inserting 4-6 nucleotides per binding cycle. There are thus fundamental differences between human pol α and pol β with respect to the nucleic acid signals that modulate their catalytic interactions with primer-templates; at least some of these signals may prove to be of physiological significance.

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0934 Fidelity of DNA Polymerases using Biologically Active Templates. Lawrence A. Loeb, Philip Liu, Thomas A. Kunkel, Roeland M. Schaaper and John Abbotts. The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195.

The error rates of DNA polymerases have been determined using the ΦX fidelity assays. Procaryotic DNA polymerases are highly accurate; the frequency of errors approaches that estimated from the spontaneous mutation rate. In contrast, all eucaryotic DNA polymerases so far examined are inaccurate; e.g., error frequencies from 1/3,000 to 1/40,000. This is greater than that extrapolated from their spontaneous mutation rate, 10^{-8} to 10^{-11}, implying that other factors in addition to polymerases function in accuracy. The ΦX assay should permit screening for these factors. Nevertheless, the error rates of these enzymes are less than non-enzymatic, template-directed polymerazation (1/200), indicating an active role in base selection by DNA polymerases in the absence of exonucleolytic proof reading.

The high specificity of aphidicolin as an inhibitor of DNA polymerase -α has provided a means to search for mutants. A Chinese hamster V79 cell mutant selected by resistance to aphicicolin, a specific inhibitor of DNA polymerase -α, has been defined (Somatic Cell Genetics, 7: 235-253, 1981). DNA polymerase -α was purified from mitochondria-free crude extracts by sequential column chromatography using DEAE-cellulose and phosphocellulose. DNA polymerase -α purified from the mutant is 10-fold more resistant to aphidicolin than the same enzyme purified from the parental cells. Moreover, the apparent Km for dCTP is 1.0 ± 0.4 μM for the mutant polymerase and 10 ± 4 μM for the parental enzyme. These differences observed are in accord with the known competition between aphidicolin and dCTP and provide a mechanism for the aphidicolin resistance of the mutant, i.e., the decrease in Km for dCTP. The elevated sponteneous and induced mutation rate exhibited by the mutant could be mediated by the alteration in DNA polymerase -α.

The comparative fidelity of DNA polymerase has also been assessed with respect to copying past non-instructive lesions. All DNA polymerases insert single base-substitutions when encountering apurinic sites on polynucleotides as well as natural DNA templates. The frequency of bypass correlates with the stringency of these enzymes in base-selection. Incorporation opposite apurinic sites is not random; deoxyadenosine is the predominant substitution in vitro using purified DNA polymerases and in vivo in SOS-induced spheroplasts. The frequency of bypass by eucaryotic DNA polymerases implies that apurinic sites may be highly mutagenic in these cells.

0935 A PROOFREADING MECHANISM FOR NUCLEOTIDE SELECTION IN DNA SYNTHESIS ASSOCIATED WITH PYROPHOSPHATE EXCHANGE. O.P.Doubleday (1), Ph.J.Lecomte (2) and M.Radman (2), Department of Molecular Biology, Universite Libre de Bruxelles, 1640 Rhode-St-Genese, Belgium. Present address: (1) MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, England; (2) Institut Jacques Monod, I.N.R.S., Tour 43, 2 Place Jussieu, 75251 Paris, France.

Analysis of pyrophosphate (PPi) exchange and incorporation of exogenous deoxyribonucleoside monophosphates (dNMP), which occur only during ongoing DNA synthesis, suggest the existence of an intermediate in the nucleotide incorporation step of DNA synthesis:

$$DNA_{(n)} + dNTP \rightleftharpoons [DNA_{(n)}.dNMP.PPi] \rightleftharpoons DNA_{(n+1)} + PPi$$

We shall present evidence suggesting that the $[DNA.dNMP.PPi]$ may be a key intermediate in nucleotide selection, subject to a proofreading reaction in the course of which an incorrect dNMP residue is preferentially aborted from the polymerisation reaction and released in the form of reconstituted free dNTP. This putative proofreading reaction, which can be monitored by PPi exchange in the presence of exogenous $[^{32}P]$ PPi and requires ongoing DNA synthesis, probably occurs before the establishment of the phosphodiester bond. Therefore we hypothesize that an energy-loaded DNA polymerase, involved in processive DNA synthesis, may be particularly well suited to perform this proofreading reaction.

On the basis of experiments demonstrating (1) an *in vitro* mutator effect of pyrophosphatase in a system with purified DNA polymerases and intact homopolymer templates, and (2) an efficient mutagenic "bypass" replication of apurinic/apyrimidinic (AP) sites in the presence of pyrophosphatase (mimicking SOS-dependent mutability of AP sites *in vivo*), we shall discuss the possible involvement of this proofreading reaction in the determination of spontaneous and induced mutation rates.

Mutagenesis

0936 MECHANISMS OF MUTAGENESIS FOR LAMBDA PHAGE, Franklin Hutchinson, Thomas R. Skopek and Richard D. Wood, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

We have been determining changes in DNA base sequence induced by specific mutagens in the cI repressor gene of lambda phage. Any sequence change which leads to a loss of function of the cI gene product can be detected and identified. The phage can be mutagenized as free particles, as isolated DNA in solution, in cells during lytic growth, or as prophage integrated into the host cell genome. When lambda lysogens are mutagenized, mutations which inactivate the phage repressor protein are detected by the appearance of clear-plaque-forming virus in the medium; thus, in this case the mutations in the prophage are representative of those in the *E. coli* genome.

Data on induced sequence changes, combined with other experimental results, allow firm conclusions to be drawn on some of the mechanisms by which certain agents cause mutations. The base analog bromouracil induces base transitions almost exclusively. In λ, these are A·T → G·C transitions which occur with marked sequence specificity at a number of "hotspots". (1). Various acridine derivatives induce single base-pair additions or deletions in runs of three or more identical base pairs. Runs of G·C base pairs are mutated at higher frequency than runs of A·T base pairs.

Ultraviolet light is a more complicated agent. Irradiated phage grown in irradiated host cells are selectively mutated to produce transitions at the 3' base of a pyrimidine pair (TC, TT, or CC). These are potential sites for formation of UV-induced photoproducts such as cyclobutane pyrimidine dimers or 6-4 pyrimidine-pyrimidine dimers. Unirradiated phage in heavily irradiated host cells are also mutated ("nontargeted mutagenesis"), producing mainly frameshifts in runs of identical base pairs.

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0937 MECHANISMS OF MUTAGENESIS IN *SACCHAROMYCES CEREVISIAE*, Chris Lawrence and Roshan Christensen, Department of Radiation Biology & Biophysics, University of Rochester, Rochester, NY 14642

Mutagenesis induced by radiations (1) and many chemical mutagens (2) requires the function of the *RAD6* gene, a locus that appears to regulate or be involved in a variety of processes that in aggregate are important for repair, recovery and growth (3). As others have shown, these include repair of single-strand breaks and post-replication gap closing, but not excision or recombination-dependent repair (see 4 for references and review).

Induced mutagenesis is associated with only some of these activities, however, and such error-prone processes are of little importance to repair and recovery (3). These mutagenic processes are capable of producing all kinds of mutagenic alterations, including frameshift changes as well as base-pair substitutions. Apart from *RAD6* itself, the functions of at least 10 genes are involved, but none, among those tested adequately, are concerned with the production of all kinds of mutations: each influences the reversion of a specific set of tester alleles. The basis for this specificity does not appear to be either the type of change involved or the surrounding nucleotide sequence; presumably, other features of chromatin structure are responsible and the same seems to be true in determining the sites for UV hotspots.

RAD6-dependent mutagenesis does not appear to require the induction of a factor which specifically reduces polymerase accuracy; mutation frequencies in unirradiated nuclei residing in the same cytoplasm as irradiated nuclei are not significantly higher than spontaneous levels. UV-irradiation does, however, reduce the accuracy of replication on undamaged templates (ie. leads to untargeted mutagenesis), though only when damage is located at other sites within the same nucleus. It does not seem to be necessary for this damage to be situated in the same chromosome, however. We suggest that these data are best explained by a *limited fidelity* model for mutagenesis (5). Untargeted mutations are thought to result from the limited capacity of those processes in yeast cells that are responsible for maintaining fidelity, which it is proposed are used during the replication of both irradiated and unirradiated DNA, leading to competition for the limited resource. Targeted mutations are thought to result from the limited, though far from negligible, capacity of lesions to form normal base-pairs. Yeast cells may possess processes designed to extract pairing information from distorted templates.

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0938 THE ROLE OF DNA SECONDARY STRUCTURE IN MUTAGENESIS, Lynn S. Ripley and Barry W. Glickman, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

We have proposed that DNA secondary structures specifically direct a variety of DNA sequence changes (1,2). The model suggests that the action of ordinary DNA metabolic events on the alternative DNA structures potentiated by palindromic or quasipalindromic DNA sequences are responsible for these changes. Mutations predicted by the model may be simple base substitutions and frameshifts or much larger addition and deletion mutations. The model has been particularly useful in explaining complex frameshift mutations associated with multiple base changes in yeast (1). The specificity of frameshift mutation in bacteriophage T4 also suggests a strong influence of DNA secondary structure on frameshift mutation frequencies (3). The specificity of sequenced base substitution, frameshift, addition and deletion mutations will be discussed in light of the role of DNA secondary structures in their formation.

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DNA Damage and Human Disease

0939 MECHANISM OF CARCINOGEN-MEDIATED GENE AMPLIFICATION. Sara Lavi, Yehudit Berko, Naama Kohn, Sara Etkin, Tamar Kleinberger and Bruria Shekel. Department of Virology, The Weizmann Institute of Science, Rehovot, Israel.

Exposure of SV40 transformed Chinese hamster cells (C060) to a variety of carcinogens induce the amplification of SV40 DNA (1). A functional origin of replication which consists of both the origin of replication sequences and an active A gene is required for the amplification phenomenon (2). A series of probes derived from the 30 Kb chromosome region surrounding the SV40 insert in C060 cell line was used to label DNA from control and carcinogen-treated cells. By comparing the intensity with which individual fragments were labeled in the two DNAs, the extent of their amplification was calculated. The level of amplification is maximal in a region which includes the SV40 origin of replication; amplification is decreased as the distance along the chromosome from the SV40 origin is increased. The amplified sequences are associated with chromosomal and extrachromosomal DNA. The amplification process is transient; the peak of amplification is reached 3-5 days following exposure to the carcinogenes, thereafter, there is a decrease in the amplified sequences and most of the amplified sequences disappear two weeks after the exposure to the carcinogen. The amplification of SV40 DNA is accompanied by an increase in SV40 specific RNA and proteins (3).

Carcinogen-mediated amplification of specific genomic sequences is a general phenomenon. Exposure of normal Chinese hamster embryo cells and CHO cells to a variety of carcinogens induced the amplification of dihydrofolate reductase sequences (DHFR) and the EJ bladder carcinoma sequences.

Following the exposure of CHO and C060 cells to carcinogens, a large fraction of the cells are arrested at the beginning of the S phase. The association between this arrest and the amplification phenomenon will be discussed.

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0940 DEFECTIVE REPAIR OF ALKYLATION DAMAGE IN CELLS FROM AN IMMUNODEFICIENT PATIENT Alan R. Lehmann, Michael R. James, Ian A. Teo, Leigh Henderson+ and Colin F. Arlett, MRC Cell Mutation Unit and +Sussex Centre for Medical Research, University of Sussex, Falmer, Brighton BN1 9QG, East Sussex, England.

The cell strain 46BR, derived from an immunodeficient individual (1) is hypersensitive to the lethal effects of a variety of DNA-damaging agents, this effect being particularly marked for monofunctional alkylation agents (2). We have not yet been able to detect any significant defect in DNA repair after UV- or gamma-irradiation. Following treatment with methylating agents, there is no reduction in either scheduled or unscheduled DNA synthesis when compared with normal cells, but the rate of disappearance of single-strand breaks from the DNA is slower in 46BR than in normal cells. At the cellular level 46BR is non-mutable by UV and ionizing radiation, but it shows an elevated level of sister-chromatid exchanges induced by UV-irradiation or by methylating agents.

46BR is also sensitive to the lethal effects of 3-aminobenzamide and other inhibitors of ADP-ribosyltransferase, an enzyme which is known to control the level of DNA ligase II (3) and thereby to reduce the steady-state level of breaks present during excision-repair of alkylation damage (4). Taken together these results have led us to the idea that 46BR may be defective in the activity of DNA ligase I. This could account for the repair deficiency, and it could result in a greater dependence on DNA ligase II and ADP-ribosyl transferase. In accordance with such a hypothesis we have found that the rate of ligation of "replication intermediates" during normal DNA replication is slower in 46BR than in normal cells.

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0941 HYPERSENSITIVITY TO DNA-DAMAGING AGENTS IN NEUROLOGICAL DEGENERATIONS, Jay H. Robbins, Dermatology Branch, National Cancer Institute, NIH, Bethesda, MD 20205

Inherited defects in DNA repair mechanisms render xeroderma pigmentosum (XP) cells hypersensitive to killing by the UV-type of DNA-damaging agents.[1] In addition to accelerated actinic degeneration of sun-exposed tissues, some XP patients develop a primary neuronal degeneration (PND).[2] Cultured fibroblast and lymphoblastoid lines from XP patients with the earliest onset of PND are the most sensitive to killing by UV, those from patients with later onset are less sensitive, and those from patients without PND are the least sensitive.[3] These findings led to the 'neuronal DNA integrity theory' which holds that when the integrity of neuronal DNA is sufficiently destroyed, e.g., by the accumulation of unrepaired DNA damaged spontaneously or by endogenous metabolites, the nerve cells will undergo a PND.[4] Cells from patients with ataxia telangiectasia, another PND, are hypersensitive to ionizing radiation[1] and to the radiomimetic chemical N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).[5] Hypersensitivity to the UV-type of DNA-damaging agents is present in cells from patients with Cockayne syndrome[1] (cachectic dwarfism, demyelination, and primary retinal degeneration) and from the 2 patients with both XP and Cockayne syndrome of XP complementation groups B[2] and H.[6] Using trypan blue to measure viability of X-irradiated lymphoblastoid lines, we found the lines tested from 4 patients with Huntington disease (a dominantly inherited PND) to be hypersensitive to X rays,[7] a finding confirmed with 10 patients in Australia[8] and 22 patients in the United Kingdom.[9] We have found hypersensitivity to X rays in lymphoblastoid lines from patients with other PNDs which are either genetic (familial dysautonomia, olivopontocerebellar atrophy with retinal degeneration)[10] or sporadically-occurring (idiopathic orthostatic hypotension,[11] multiple system atrophy,[11] Parkinson disease[12]). Lymphoblastoid lines from patients with Alzheimer disease[12] (a sporadically-occurring neurodegeneration), Usher syndrome[11] (retinitis pigmentosa and congenital deafness), Duchenne[13] and other muscular dystrophies[13] are also hypersensitive to X rays. Fibroblasts from these radiosensitive disorders are hypersensitive to MNNG[14,15] when tested by the method of Scudiero,[5] but neither the fibroblast nor lymphoblastoid lines are hypersensitive to the UV-type of DNA-damaging agents.[7,14] Death in vivo of excitable tissue in these radiosensitive diseases may result from unrepaired DNA. The hypersensitivity might provide the basis for presymptomatic and prenatal tests for these diseases and for elucidating their pathogenesis.

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DNA Damage/Excision Repair of Non-Alkylation Damage/DNA Repair Enzymes

0942 EFFECT OF DNA DAMAGES ON THE ACTIVITY OF PROKARYOTIC DNA TOPOISOMERASES. Antonia M. Pedrini, Nicoletta Politi, Giovanni Ciarrocchi. Istituto di Genetica Biochimica ed Evoluzionistica, C.N.R. Pavia, Italy.

Modifications of DNA structure by a number of DNA damaging agents result in the unwinding of DNA helix. We have studied the effect of DNA unwinding by UV photoproducts on the activity of prokaryotic DNA topoisomerases. The supercoiling activity of DNA topoisomerase II appeared not to be affected by UV damages. DNA topoisomerase I relaxing activity was instead altered by an unwinding of the substrate as small as the -14.3° produced by one pyrimidine dimer. When the activity was assayed in processive conditions an inhibition was observed. We have quantitated this inhibition by measuring the relative amount of substrate left in the RFI band. In distributive assay conditions instead a change in the mode of action became also apparent. In fact the Gaussian distribution of partially relaxed molecule became broader and more even, and more molecules were completely relaxed. The inhibition by UV photoproducts appeared different from the inhibition by single-stranded DNA and intercalating agents. The inhibition cannot be accounted for an alteration in the protein DNA complex formation. The results suggest that the inhibition by photoproducts is caused by changes in the conformation of the supercoil. Our findings suggest the possibility that DNA topoisomerase I may play a role in repair.

0943 CONTEXT EFFECTS IN AFLATOXIN B_1-DNA INTERACTIONS. M. Zafri Humayun, Kevin F. Muench and Ravi M. Misra, New Jersey Medical School, Dept. of Microbiology, Newark, NJ 07103
It has long been suspected that all target sites within an accessible domain of DNA are not equally reactive with carcinogens and that the nucleotide sequence context may be among the factors modulating the reactivity of carcinogens with DNA. We have examined this question with reference to aflatoxin B_1 (AFB1), a naturally occurring, potent carcinogenic mutagen. We report that the sequence environment has a strong and predictable influence on the extent of modification of G residues (the primary target) by AFB1, and that, a set of empirical "rules" that predict favorable and unfavorable sites in a given DNA sequence can be deduced. The experimental strategy is a simple extension of the Maxam-Gilbert sequencing technique and exploits the alkali-lability of G residues modified by AFB1. We show that (a) reactivity of AFB1 with G residues in double-stranded DNA is strongly and reproducibly influenced by the sequence context; in single-stranded DNA, the reactivity is both random and, surprisingly, suppressed when compared to duplex DNA; (b) residues flanked by A-T bp are poor targets; (c) certain, but not all, G residues in G-C bp clusters are highly favored in a manner predictable from the actual sequence, (d) the observed in vitro sequence specificity is independent of a number of tested parameters and appears to be an intrinsic property of AFB1-DNA interactions likely to occur in vivo; (e) in single-stranded DNA capable of forming hairpins, AFB1 reacts with base-paired G residues strongly and in accordance with the "rules", but weakly and randomly with non-base-paired G residues (thus, AFB1 can be used as a chemical probe to examine hairpins at the sequence level) and (f) there is evidence to suggest that a precovalent, sequence-specific association of AFB1 with DNA is the mechanism for the observed sequence specificity.

0944 ALKALINE LABILE UV-INDUCED DNA PHOTOPRODUCTS, William A. Franklin, Kwok Ming Lo, and William A. Haseltine, Sidney Farber Cancer Institute, Boston, Ma. 02115
We have studied the photochemistry of alkaline labile lesions produced in UV light irradiated DNA. These lesions are produced at the bipyrimidine sites T-C, C-C, and T-T, and are not a result of the formation of cyclobutane pyrimidine dimers. Studies with dinucleotides (dTpdC, dTpdT, dCpdC, and dCpdT) have indicated that the alkaline labile lesions produced are precursors to 6-4'-[pyrimidin-2'-one]-pyrimidine photoadducts that have been described previously. The most prevalent lesion occurs at a 5' T-C 3' site and is a precursor to the photoproduct Thy (6-4) Pyo. The (6-4) alkaline labile adducts are unusual in that their UV absorbance spectra are red-shifted relative to that of dinucleotides, and they are fluorescent. Indirect evidence has suggested that these lesions occurring a pyrimidine-cytosine sequences may be mutagenic. We are currently studying the repair of the adducts in E. Coli, specifically to determine if the adducts are excised by the uvrABC repair system.

0945 EVIDENCE FOR Z-FORM IN FORM V DNA, Bernard Malfoy[+], Pascale Rio[+], Marie-Claude Lang[++], Anne-Marie Freund[++] and Marc Leng[+],[+]Centre de Biophysique Moléculaire, 45045 Orléans, and [++]Institut de Biologie Moléculaire et Cellulaire, 67084 Strasbourg, France.
Since the discovery of left-handed DNA (Z-DNA) in crystals of oligo dG-dC, several studies have been devoted to the research of this form in natural DNA.
We have used immunological techniques to detect the Z-DNA. Antibodies specific to Z-DNA have been elicited in rabbit immunized by chlorodiethylenetriamino platinum(II) chloride modified poly(dG-dC).poly(dG-dC). Under physiological conditions, this polynucleotide is in Z-form.
In vitro we found no evidence for sequence in Z-form in naked DNA, while *in situ*, the presence of Z-DNA like conformation in chromosomes have been visualized by indirect immunofluorescence. These regions could be stabilized by several factors as proteins preferentially bound to the Z-form or topological constraints due to the organisation of the chromatine.
The effect of topological constraints can be studied *in vitro*. Annealing of covalently closed complementary single strands of a plasmid, lead to the formation of such a constraint structure (form V DNA). Radioimmunoassay and immuno electron microscopy studies show that there are several sequences in Z or Z-like form and they are distributed all over the form V DNA molecule. These studies suggest that in chromosome, some DNA sequences can be stabilized in Z conformation by topological constraints.

0946 IMMUNOCHEMICAL DETECTION OF RADIATION-INDUCED BASE LESIONS IN DNA, Susan S. Wallace, Robert J. Melamede, and Michael F. Laspia. New York Medical College, Valhalla, N.Y. 10595; and Raghavan Rajagopalan and Bernard F. Erlanger, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032.

Antibodies to thymine glycols and thymine dimers have been raised in rabbits using hapten-BSA conjugates as immunogens. Both antibodies specifically precipitate their respective haptens conjugated to RSA and react with thymine glycol- or thymine dimer-containing DNA as measured by the sensitive enzyme-linked immunoassay. The anti-thymine glycol antibody reacts with OsO_4-treated and x-irradiated calf thymus DNA by either competitive or direct measurements and femtomole levels of the damage can be detected. Hapten inhibition studies show the antigenic determinant in this reaction to be the thymine glycol monophosphate moiety. These procedures do not require radioactively labeled DNA; they are simple, quick and economical and provide a convenient way to measure DNA damage and repair.

These studies were supported by Contract DE-AC02-80EV10417 from the U.S. Department of Energy.

0947 THE USE OF A RADIOIMMUNOASSAY TO MEASURE DNA PHOTOPRODUCTS IN UV-IRRADIATED MAMMALIAN CELLS, David L. Mitchell and Judith M. Clarkson, The Univ. of Texas System Cancer Center, Science Park-Research Division P.O. Box 389, Smithville, Texas 78957

Antisera have been raised in rabbits against UV-irradiated DNA. The antibody was used to develop a radioimmunoassay (RIA) in which the competition between labelled UV-DNA and unlabelled sample DNA for antibody binding sites is monitored. The sensitivity of the assay has been optimized by using plasmid DNA(labelled to a high specific activity with ^{32}P) as competitive probe and is capable of detecting photoproducts in cellular DNA at doses as low as 1 $J \cdot m^{-2}$. Various polynucleotides and defined sequence oligonucleotides (linkers) have been used as competitive inhibitors in the RIA in order to elucidate the binding preference of the antibody. Whereas UV-irradiated poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) do not bind antibody, polyd(A)·polyd(T), polyd(G)·polyd(C) and polyd(C-T) are antigenic when irradiated. Using a standard curve it is possible to measure the removal of dimers from the DNA of UV-irradiated mammalian cells. The kinetics of this reaction resemble those for unscheduled DNA synthesis in both normal and UV-sensitive cell lines.

0948 DNA REPAIR SYNTHESIS AFTER PSORALEN/365-NM LIGHT INDUCED INTERSTRAND CROSSLINKING, Henry M. Jacocks III and Michael P. Hagan, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814.

Trimethoxy psoralen treatment combined with 365-nm light irradiation has been recently used to block semi-conservative DNA synthesis, thus permitting the assay of repair synthesis. Using cesium chloride density gradient analysis, we have partially characterized the DNA synthesized during this synthesis blockade. In addition, we have used an insult with cobalt-60 gamma radiation to examine DNA repair synthesis after cross-linking. "Background" radioactivity, representing DNA labeled after the trimethoxypsoralen/365-nm light treatment, was shifted to hybrid-density through the incorporation of BrdUrd. After exposure to a high dose of cobalt-60 gamma irradiation, BrdUrd incorporation produced no hybrid-density DNA. At intermediate doses of cobalt-60 gamma radiation, both density types were found. These results indentify a possible source of error in the trimethoxypsoralen/365-nm light technique for DNA repair studies and underscore the importance for characterization of DNA when multiple damaging agents are used.

0949 ANTIBODIES AGAINST O^6-ETHYLDEOXYGUANOSINE IN ALKYLATED DNA, Altaf A. Wani, R. Gibson-D'Ambrosio, C. Clay and S.M. D'Ambrosio, Ohio State University, Columbus, OH 43210
Alkylation at O^6-position of guanine in DNA after in vitro or in vivo exposure to alkylating carcinogens is a major promutational product. Poly- and mono-clonal antibodies were directed against O^6-ethyldeoxyguanosine (O^6-EtdGuo) in rabbits and mice. Two immunological methods [radioimmunoassay (RIA) using O^6-Et[^{3}H]dGuo as tracer antigen and enzyme-linked immunosorbant assay (ELISA) using hapten-BSA conjugate as antigen] were employed to detect O^6-EtdGua at femtomole levels, with practically similar results. The specificity of antibodies was demonstrated by the use of nucleoside analogues as inhibitors in RIA and ELISA. 50% inhibition of tracer antibody binding was observed with 0.1 picomoles of O^6-EtdGuo, followed by O^6-EtGuo and O^6-MeGuo. 17,000 and 44,000 as much 6-ClGuo and 7-EtdGuo respectively were required for same amount of inhibition. Inhibition by deoxyadenosine and deoxyguanosine was below 10% at 10 nmol of inhibitor concentration. 200 fmoles of O^6-EtdGuo in 100 µg of DNA hydrolysate obtained by an in vitro treatment with 10 µg ENU/ml of DNA, gave 25% inhibition of tracer-antibody binding. An in vivo dose (5 µg-100 µg ENU/g body weight) dependent response was observed giving 30% inhibition by 100 µg ENU/g body weight, corresponding to 100 fmoles of O^6-EtdGuo in 100 µg of liver DNA hydrolysate. This assay system for modified DNA components would be very useful in following the production, persistance and repair of these lesions in a variety of cells and tissues treated with a broad spectrum of alkylating carcinogens. (Supported by NIEHS Grant No. 1-R01-ES02388.)

0950. DNA DAMAGE IN HUMAN EPIDERMIS STUDIED BY IMMUNOHISTOCHEMICAL TECHNIQUES
by H. Krokan*, G. Eggset*+ and G. Volden+, *Institute of Medical Biology and +Department of Dermatology, University of Tromsø, 9000 Tromsø, Norway
Antiserum against UV-irradiated single-stranded DNA was raised in rabbits immunized with ss DNA complexed with methylated bovine serum albumin, and purified by affinity chromatography. Adsorbtion experiments indicated that the antibodies had a very strong preference for single stranded DNA and appeared to be directed mainly against pyrimidine dimers. These antibodies were used to study UV-induced DNA damage and subsequent repair in biopsies of human skin. Immunofluorescence and immunoperoxidase staining methods were used. In situ denaturation of DNA in cryostat sections strongly increased the specific staining without damaging the structure of epidermis. DNA damage could be detected after irradiation with less than 0.5 of the minimal erythemal dose (~200 J/m^2, wavelength maximum 310 nm). The immuno-staining was strongly reduced at 5 hr after irradiation and essentially abolished at 24 hr after irradiation. Light increased the rate of DNA repair, although the dark repair appeared to be quantitatively more significant.
G.E. is a fellow of the Norwegian Cancer Society (Landsforeningen mot Kreft).

0951 ISOLATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST HUMAN URACIL DNA GLYCOSYLASES, Pablo Arenaz and Michael A. Sirover, Fels Research Institute and the Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140
A series of hybridomas have been isolated which produce monoclonal antibodies against the uracil DNA glycosylase from human placenta. The anti-glycosylase activity of each hybridoma was quantitated by immunoprecipitation with second antibody. Of the four hybridomas examined in detail, glycosylase activity was inhibited 60-90% when compared to a spontaneous hybridoma used as a control. Cross reactivity was observed with glycosylase isolated from normal human skin fibroblasts and from a human TG cell tumor line; partial cross reactivity was observed with hamster glycosylase. No cross reactivity was noted with uracil DNA glycosylase isolated from yeast or from E. coli. The physical binding of the antibody to the human glycosylase was examined through glycerol gradient sedimentation analysis. Placental glycosylase was incubated with antibody from the control hybridoma or from the positive clones; layered on a 10-35% glycerol gradient; and sedimented at 40,000 rpm for 16 hr at 4°. Incubation of the glycosylase with the control antibody had no effect on the extent of enzyme activity nor on the position of the enzyme in the gradient. In contrast, glycerol gradient sedimentation of glycosylase incubated with antibody from positive clones resulted in two peaks of glycosylase activity, one sedimenting identical to that observed for untreated controls or for enzyme treated with control antibody but with diminished activity and a second peak sedimenting at a higher density with activity comparable to the loss observed in the first peak. These results suggest that the monoclonal antibody binds placental uracil DNA glycosylase but at a site which does not affect catalytic activity.

0952 UVRA, UVRB, AND UVRC DEPENDENT DNA REPAIR IN ESCHERICHIA COLI, Anthony T. Yeung, William B. Mattes, Euk Y. Oh, George H. Yoakum and Lawrence Grossman, The Johns Hopkins University, Baltimore, Md. 21205

The uvrA, B, and C genes of E. coli, which functions in the incision of DNA damage have been cloned on pBR322 in this laboratory. By using maxi-cell labeling and transposon inactivation of the cloned uvrA, uvrB, and uvrC genes, we have identified their gene products to be proteins of 114,000, 84,000, and 68,000 molecular weight respectively. The uvrA and uvrB proteins were amplified to 7% and 5% of total cellular protein respectively by putting the uvrA and uvrB genes under transcriptional control of Lambda PL promotor. uvrC has been amplified by cloning the uvrC gene onto a high copy number plasmid. We have purified these amplified proteins to homogeneity and their physical and kinetic properties are being studied. As part of the investigation dealing with the solution properties of these proteins it was found that uvrA protein undergoes sulfhydryl dependent polymerization and depolymerization. In vitro, the incision of UV irradiated RF-I DNA requires the presence of all three purified proteins and ATP. The rate of incision is stimulated by the presence of dithiothreitol, but is inhibited by the presence of unirradiated DNA. Binding studies of the uvrA, uvrB, and uvrC proteins to UV irradiated DNA is being conducted in order to better understand the stoichiometry and mechanism of these three proteins in the incision reaction. Alternative assays are being developed to facilitate mechanistic studies and to isolate the products and site of the incision reaction.

0953 PROPERTIES OF THE uvrABC ENDONUCLEASE, E. Seeberg, A-L. Steinum and S. Linn, Norwegian Defence Research Establishment, Division for Toxicology, N-2007 Kjeller Norway.

E. coli uvrABC endonuclease was reconstituted in vitro from purified components and shown to introduce breaks in DNA damaged by UV, psoralen plus light, benzo(a)pyrene diolepoxide, N-acetoxyacetylaminofluorene and platinum complexes (cis and trans). Analysis of the reaction between the uvrABC endonuclease and [^{14}C]-DNA carrying [^{3}H]-psoralen adducts revealed that the DNA is not only incised but the adducts excised by the action of the uvrABC endonuclease. The adducts are recovered in an ethanol-precipitable form indicating that the excision products are oligonucleotides and that the uvrABC endonuclease makes phosphodiesterbond cleavages at both sides of the damage. Complete excision repair in vitro is being observed by adding DNA polymerase I and polynucleotide ligase to the reaction mixtures. The mechanism of the uvrABC endonuclease reaction has been studied in more detail using UV-irradiated DNA as substrate. The reaction appears to be initiated by the uvrA protein which in the presence of ATP recognizes single stranded regions in the DNA but does not specifically bind to the lesions. Damage specific binding is being observed, however, when the uvrB protein is added together with the uvrA protein. The uvrB protein is not a DNA binding protein by itself but has affinity for the uvrA protein when this is attached to the DNA. The uvrAB proteins form a relatively stable complex with the DNA at the site of the lesion in an ATP-dependent reaction. This complex can be isolated by sucrose gradient sedimentation and addition of the uvrC protein then promotes the strand cleavages in an ATP-independent reaction.

0954 IN VIVO REGULATION OF THE E.coli K12 uvrA, B, C GENES. Claude Backendorf, Eva A. van den Berg, Jourica A. Brandsma, Cees A. van Sluis, Pieter van de Putte. Department of Molecular Genetics, State University of Leiden, Leiden, The Netherlands. In order to examine the response of the cloned uvrA, B, C genes to DNA damaging agents the regulatory region of these genes has been modified. Using a series of Ba131-induced deletions of the uvrA promoter fused to the galK gene, we established that the "-10 sequence" is able by itself to sustain a basic level of a LexA independent uvrA transcription. As the LexA binding site is overlapping with the uvrA "-35 sequence", SOS induction occurs by the exposure of the "-35 sequence", which results in a 3-4 fold increase in uvrA transcription. In addition, induced uvrA messenger RNAs are slightly shorter than consitutive transcripts. In vivo induction of the uvrB transcripts was observed using the S1-nuclease mapping technique. A direct influence of the "P2-region" on transcription from the P1 promoter has been demonstrated. These results were confirmed using uvrB-lacZ fusions. The physiological significance of this findings will be discussed.

The sequence of the cloned uvrC regulatory region was established by DNA sequencing and S1-nuclease mapping. The regulation of the uvrC gene as studied with uvrC-galK fusions, was dependent on the RecA-LexA system in a way different from the uvrA and uvrB genes. The characteristics of a filamentous uvrC mutant will be discussed with regard to the possible regulatory role of a 27 Kdal protein, encoded on DNA directly preceeding the uvrC 70 Kdal structural gene.

0955 IDENTIFICATION OF THE REGULATORY REGIONS OF THE uvrC GENE OF ESCHERICHIA COLI, Surendra Sharma and Robb E. Moses, Baylor College of Medicine, Houston TX 77030. Escherichia coli repairs ultraviolet radiation-induced pyrimidine dimers by an excision (uvr) system. Analysis of uvr mutants suggests that at least three unlinked genes, uvrA, uvrB, uvrC, control the excision repair. We have shown that the uvrC gene is located on a 1.9 Kb DNA fragment and codes for a protein of 66,000 monomer molecular weight. To identify the regulatory regions of the uvrC gene, we have done the following experiments: (1) Subcloning of the structural gene and segments of 5' flanking region; (2) Probing for the RNA polymerase binding sites in 5' flanking region; and (3) Nucleotide sequencing of the upstream regions from the structural gene. Our results indicate that there are three promoters preceding the uvrC gene. Plasmids lacking the distal promoter, but containing the region which encompasses two other promoters and the uvrC structural gene, fail to restore UV-resistance phenotype of uvrC$^-$ cells. Furthermore, the region containing the distal promoter exhibits the strongest binding of RNA polymerase. Analysis of protein synthesis encoded by a uvrC$^+$ plasmid containing an uninterrupted fragment which carries all three promoters and the structural gene suggests that there is another gene that lies between the distal promoter and the uvrC structural gene. The relation of this gene to uvrC function is not clear. (Supported by grants from USPHS, American Cancer Society and Robert D. Welch Foundation)

0956 CONSTRUCTION OF λ HYBRID PHAGES CONTAINING THE uvrA AND ssbA GENES OF E. COLI : UTILISATION TO STUDY THE REGULATION OF UVRA AND SSB PROTEINS, Roberd Alazard, Laboratoire de Pharmacologie et de Toxicologie Fondamentales 205 route de Narbonne 31400 Toulouse. A 9.3kb Eco RI fragment obtained by partial digestion of the plasmid pDR2000 and containing the uvrA and ssbA genes has been subcloned in the insertion vector λ gt4. Two hybrid bacteriophages carrying this fragment inserted in opposite orientations were isolated and used to lysogenize a uvrA and a ssbA mutant of Escherichia coli. Both phages confered to these host bacteria the ultraviolet resistance of the wild type parent indicating full complementation of the uvrA and of the ssbA defect. Two polypeptides corresponding to the molecular weight of the UVRA protein (115000 daltons) and of the SSB protein (18500 daltons) were synthesized and amplified after infection of a UV irradiated λind$^-$ lysogen with these two hybrid phages. The UVRA protein is no amplified after infection of a lex A3 host while SSB is still produced in large amount. These results establish that uvrA is repressed by lexA in vivo while ssbA is not.

0957 **INVESTIGATION OF E. coli SINGLE STRANDED (SS) AP ENDONUCLEASE AND ITS COMPARISON WITH uvrC PROTEIN IS FACILITATED BY A NEWLY-CONSTRUCTED STRAIN OF E. coli.** Roger Schultz*, Errol C. Friedberg*, Ronald E. Moses**, W. Dean Rupp†, Aziz Sancar†, and Surendra Sharma**, Department of Pathology, Stanford University, Stanford, CA 94305*, Departments of Therapeutic Radiology and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510†, and Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030**.
SS AP endonuclease of E. coli is an enzyme of Mr ~60,000, that selectively degrades single stranded deoxyribopolymers or single stranded DNA containing sites of base loss. E. coli uvrC protein has a similar molecular weight and indirect evidence suggests that it might have an AP endonuclease activity. In order to establish whether or not uvrC protein and SS AP endonuclease are the same protein, we constructed a strain of E. coli (RS 71) that is defective in both uracil-DNA glycosylase and exonuclease VII activities, both of which interfere with the measurement of SS AP endonuclease in crude extracts of wild-type strains. When this stain was transformed with plasmids known to overproduce uvrC protein, no increase in SS AP endonuclease was detected. A plasmid (pDR 3264) massively overproduces uvrC protein because the uvrC gene is regulated by an inducible lac promoter. This plasmid cannot be maintained in RS 71, however following transformation of E. coli RB3 with this plasmid and induction with IPTG, overexpression of uvrC protein is not correlated with increased SS AP endonuclease activity measured by partial purification. We conclude that SS AP endonuclease and uvrC gene product are distinct proteins.

0958 PHYSICAL, BIOCHEMICAL AND GENETIC CHARACTERIZATION OF THE UVRD GENE PRODUCT, Sidney R. Kushner, Valerie F. Maples, Alan Easton, Palanivelu Peramachi and Iain K. Farrance, University of Georgia, Athens, Georgia 30602

The uvrD gene of Escherichia coli K-12 has been cloned on a 2.9 kb PvuII fragment in the multicopy plasmid pBR325. Strains carrying this recombinant plasmid (pVMK45) overproduce a DNA dependent ATPase activity which is inhibited by antiserum directed against DNA helicase II of E. coli. The 76,000 dalton protein has been purified to greater than 90% homogeneity and possesses DNA unwinding activity. The current working hypothesis is that the uvrD locus is the structural gene for DNA helicase II. Strains containing a variety of mutations (uvrD3, uvrD3 uvr-257, recL152 and uvrE100) in the uvrD gene all possess levels of DNA dependent ATPase activity similar to those of wild type control strains. In addition, the 5' end of the uvrD gene has been sequenced and found to possess a canonical lexA binding site as well as a second degenerate lexA site. There is also evidence for a second promoter which is not under lexA control. Overproduction of DNA helicase II leads to increased sensitivity to ultraviolet light, mitomycin C, and methylmethane sulfonate. In addition, these strains are deficient in transductional and conjugal recombination and appear to have reduced spontaneous mutation frequencies. Overproduction of a mutationally altered uvrD protein causes similar phenotypic alterations. (This work was supported in part by grants GM27997 and GM28760 from the National Institutes of Health).

0959 AMPLIFICATION, PURIFICATION AND CHARACTERISATION OF THE uvrD GENE PRODUCT OF E. coli, Helen M. Arthur, David Bramhill, Ian D. Hickson and Peter T. Emmerson, The University, Newcastle upon Tyne, NE1 7RU, UK.

The uvrD gene has been cloned and the gene product identified as a 75 kd protein (Arthur et al., Gene 19, 285-295, 1982). Subcloning this gene into two different amplification vectors pPLc24 (Remaut et al., Gene 15, 81-83, 1981) and pMCR735 (kindly provided by A. Nakazawa and T. Miki, Yamaguchi University, Japan) results in elevated levels of the protein amounting to 2 to 3 per cent of total protein. These high levels permit a simplified purification procedure which yields a protein with DNA-dependent ATPase activity and ATP-dependent DNA unwinding activity. In addition to its role in DNA replication and repair, the uvrD gene plays a role in transposon excision; texB mutants isolated on the basis of increased precise transposon excision (Lundblad and Kleckner, in Molecular and Cellular Mechanisms of Mutagenesis, Lemontt and Generosa, Eds., Academic Press, 1982) are complemented by plasmids carrying uvrD$^+$ but not by those in which the uvrD gene is inactivated by insertion of Tn1000.

0960 THE INVOLVEMENT OF FOUR GENES, *uvsC, uvsD, uvsE* AND *mtcA* IN TWO PYRIMIDINE-DIMER EXCISION PATHWAYS IN *DEINOCOCCUS* (FORMERLY *MICROCOCCUS*) *RADIODURANS*, Bevan E. B. Moseley and David M. Evans, Department of Microbiology, University of Edinburgh, Edinburgh, EH9 3JG, Scotland.

Mutations in any one of three unlinked genes *uvsC, uvsD* or *uvsE* produce a U.V.-sensitive phenotype only in strains of *D. radiodurans* already mutant in the gene *mtcA*. These double mutants are excision deficient because they fail to incise their DNA after irradiation. Strains singly mutant in any one of the four genes are U.V.-resistant and are incision proficient although their patterns of dimer excision differ. These observations lead to the conclusion that there are two U.V. endonucleases in *D. radiodurans*. Both appear to be constitutive. Data obtained from alkaline sucrose gradients and from an *in vitro* assay of U.V. endonuclease activity suggests that the *uvsC, uvsD* and *uvsE* genes code for subunits of the same endonuclease.

The excision of pyrimidine dimers after incision in the wild type proceeds along two pathways, the particular route appearing to depend on the nature of the incision step that precedes it. This suggests that each endonuclease has an associated exonuclease activity (or is also an exonuclease). One of the pathways requires DNA degradation to be controlled by an induced product while the other does not.

The need for two independent mutations to block both pathways before the excisionless phenotype can be expressed offers an explanation for the many abortive attempts to isolate such strains.

0961 MECHANISMS OF DNA EXCISION REPAIR OF APURINIC/APYRIMIDINIC SITES, Dale W. Mosbaugh and Stuart Linn, Department of Biochemistry, University of California, Berkeley, California 94720

We have studied the ability of HeLa DNA polymerases to carry out DNA synthesis from incisions made by various endonucleases which recognize or form apurinic/apyrimidinic (AP) sites in DNA. The AP endonucleases used cleave either on the 3'-side of the AP site to produce 3'-sugar and 5'-phosphomonoester termini (Class I) or on the 5'-side of the AP site to yield 3'-hydroxyl nucleotide and pentose 5'-phosphate termini (Class II). HeLa DNA polymerase β was unable to carry out DNA synthesis from incisions made by Class I AP endonucleases. However, incisions made by Class II AP endonucleases support limited strand displacement DNA synthesis. The combined cleavage by a Class I and II AP endonuclease on depurinated PM2 DNA removes the baseless sugar phosphate from the DNA thus creating a one nucleotide gap. Short patch DNA excision repair of one nucleotide gaps was carried out in the presence of dGTP, DNA polymerase β and DNA ligase. Larger patches of DNA synthesis were produced by nick translation from Class II AP endonuclease incisions or one nucleotide gaps with HeLa DNA polymerase β plus HeLa DNase V. This bidirectional exonuclease will remove a terminal 3'-AP site thus activating the substrate to support DNA synthesis by the β-polymerase. HeLa DNA polymerase α was unreactive with all the incised DNA substrates. However, it acts at large gaps, produced by DNase V, but it could not completely fill them in unless the β-polymerase was added to the reaction. (Supported in part by the Department of Energy Contract DE-A703-76EV10190 and NIH Postdoctoral Fellowship AG05195 to D.W.M.)

0962 PURIFICATION AND CHARACTERIZATION OF MOUSE CORREXONUCLEASE, Samuel H. Wilson and S. Patricia Becerra, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205

Samples of mouse myeloma HD protein-1, highly purified by affinity chromatography on ssDNA-cellulose columns, contained an oligonucleotide-releasing DNA exonuclease activity. HD protein-1 in homogeneous form, however, is nuclease free (Planck and Wilson, J. Biol. Chem. 255, 11547). We found that this exonuclease was separated from HD protein-1 during a final step in HD protein-1 purification. It is likely that the co-chromatography of the exonuclease and HD protein-1 on ssDNA-cellulose is due to tight association between these two proteins, since the exonuclease in purified form does not bind to ssDNA-cellulose.

A polypeptide of M_r=41,000 is a main constituent of the purified exonuclease, and this polypeptide comigrated with the exonuclease activity during the final step of the purification, Sephacryl S-200 gel filtration, where the enzyme had a native M_r of 40,000. Overall purification of enzyme activity was approximately 20,000-fold. This exonuclease releases 5' oligonucleotides in a limited processive manner in both the 5'→3' and 3'→5' directions. Activity of this enzyme is resistant to 1 mM NEM, requires a divalent cation, has an alkaline pH optimum, and degrades ssDNA much faster than dsDNA or RNA. The predominant oligonucleotide product with uniformly labeled substrates is $(pdN)_2$. With 3' end labeled substrates >95% of the labeled products are $(pdN)_4$ and $(pdN)_5$; with 5' end labeled substrates the main labeled product is $(pdA)_2$. The rate of product release from 3' and 5' end labeled substrates is nearly identical at 37°C. A model on the mode of action of this enzyme and a comparison with human correxonuclease will be discussed.

0963 PURIFICATION AND PROPERTIES OF THE ESCHERICHIA COLI X-RAY ENDONUCLEASE, Harold L. Katcher and Susan S. Wallace, New York Medical College, Valhalla, N.Y. 10595.

An Escherichia coli enzyme, which recognizes two major radiolysis products of thymine, has been purified to apparent homogeneity using the criterion of SDS-polyacrylamide gel electrophoresis. The enzyme shows endonucleolytic activity against apurinic and apyrimidinic (AP) sites and a dose-dependent response to DNA that has been x-irradiated, UV-irradiated or treated with OsO_4. The endonuclease also nicks OsO_4-treated DNA that has been subsequently treated with alkali and DNA treated with potassium permanganate. The enzyme does not incise alkali-labile sites generated by x-irradiation in the presence of hydroxyl radical scavengers. The x-ray endonuclease has two distinct activities, an AP endonuclease which cleaves on the 3' side of the damage leaving a 3' OH and a 5' PO_4 and a DNA N-glycosylase which recognizes at least two substrates, thymine glycol residues and urea residues. The glycosylase activity is sensitive to N-ethylmaleimide while the AP endonuclease is not. The enzyme appears to contain activities ascribed to both endonuclease III and the urea N-glycosylase.

These studies were supported by CA33657 awarded by the NCI, U.S. DHHS and a contract from the United States Department of Energy.

0964 AP/UV SPECIFIC ENDONUCLEASE(S) OF *DICTYOSTELIUM DISCOIDEUM*, R. A. Deering and R. B. Guyer, Molecular & Cell Biology, Penn State University, University Park, PA 16802

Various aspects of DNA repair in the cellular slime mold D. discoideum have been studied in this laboratory. Recently we have turned our attention to the identification and characterization of damage-specific endonucleases from this organism. Cells of axenically grown D. discoideum NP-2 were lysed by sonication in 4 M NaCl (pH 8), followed by ultracentrifugation, phase partitioning of the supernatant in 6% polyethylene glycol and 4% dextran in 4 M NaCl, and dialysis of the top phase. Nicking activity was measured by agarose gel electrophoresis or a radial diffusion assay using undamaged, UV-irradiated, or apurinic (AP) ccc-DNA from PM2 as substrate. Fractions eluting from DEAE-cellulose in the range 0-0.1 M NaCl (pH 8) were further fractionated on CM-cellulose columns at pH 5.5. A peak of activity eluting in the range 0.3-0.4 M salt preferentially nicked AP or UV-irradiated PM2 DNA compared to undamaged DNA (pH 7; 0.05 M NaCl; 0.01 M EDTA). The activity on AP DNA was greater than on UV-irradiated DNA. This enzymatic activity is undergoing further characterization. (Supported by NIH GM 16620.)

0965 ENZYMATIC REPAIR OF IMIDAZOLE RING OPENED PURINES IN γ-RAY DAMAGED DNA, C. J. Chetsanga and C. Grigorian, University of Michigan, Dearborn, MI 48128

Ionizing radiation has been shown to cleave the imidazole rings of purine nucleosides and nucleotides. After ring cleavage, the resulting substituted pyrimidines are called formamidopyrimidines (FAPy). We have shown that adenine and guanine residues in DNA can be converted to FAPy by γ-irradiation. We found that FAPy-DNA glycosylase, known to remove methylated FAPy(ring opened 7-meGua) from DNA, does not excise these radiogenic FAPy residues.

We have identified a new activity that repairs the non-alkylated FAPy in DNA. The repair involves a reclosure of the C^8 to N^9 linkage in the opened imidazole ring of the adenine or guanine base still bound to the DNA. This reclosure of the imidazole ring of these damaged bases restores purine structure and effects a direct and prompt repair of DNA apparently without requiring the action of other enzymes generally known to participate in the excision mode of DNA repair. This activity that restores the C^8 to N^9 linkage in damaged purines has been named purine imidazole ring (PIR) linkase.

Supported by PHS grant No. CA 33025 awarded by NCI (DHHS).

0966 DNA POLYMERASES INVOLVED IN DNA EXCISION REPAIR IN HUMAN FIBROBLASTS. Steven L. Dresler and Michael W. Lieberman, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

Using several specific inhibitors, we have examined which DNA polymerases are involved in DNA excision repair in cultured human fibroblasts. The data indicate that DNA repair synthesis involves both DNA polymerase α and a non-α DNA polymerase, probably polymerase β. The portion of repair synthesis mediated by each of the polymerases depends on the DNA damaging agent, on the dose of damaging agent and possibly on whether the cells are growing or quiescent. In nondividing cells at low doses of DNA damage, DNA repair synthesis is mediated to a large extent by a non-α DNA polymerase. As the dose of damage increases, the fraction of DNA polymerase α involvement increases. At very high doses of damage, the fraction of repair synthesis involving DNA polymerase α reaches a maximum which varies for different damaging agents: for ultraviolet radiation and N-acetoxy-2-acetylaminofluorene, the maximal involvement of polymerase α is about 80%; for N-methyl-N-nitrosourea, it is about 70%; for bleomycin, it is about 40%. Our data are consistent with a simple model in which two repair synthesis systems each involving one of the polymerases compete for damaged sites in the DNA.

0967 RAPID SENSITIVE FLUORESCENCE ASSAYS FOR DNA REPAIR ENZYMES, A. Richard Morgan, David H. Evans and Bruce Futcher, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

Using the enhanced fluorescence of intercalated ethidium and the unique topological properties of CCC DNA, 10^{-15} g of DNase I can be readily detected. By appropriately modifying the CCC DNA substrate specific repair enzymes, e.g. AP endonucleases and various N-glycosylases can be rapidly and sensitively assayed. These assays should be particularly useful for the detection of mutant cell lines. With T4 UV endonuclease the two separate enzymic activities, $\hat{T}\hat{T}$ specific N-glycosylase and AP endonuclease, can be individually assayed on the same sample. The assays depend on the following: CCC DNA renatures in a pH 12 buffered ethidium assay solution after heating. The fluorescence is unaffected. On nicking relaxed CCC DNA (σ = o), there is a 100% increase in fluorescence due to removal of the topological constriction. In contrast an N-glycosylase acting on e.g. a uracil or thymine dimer in CCC DNA produces a CCC DNA with an AP site, with no increase in fluorescence. The OC DNA on denaturation in 50% DMSO at 60° at pH 8 loses all its fluorescence. In contrast CCC DNA with an AP site is unaffected. But in the pH 12 ethidium assay solution due to the alkali sensitivity of the AP site, AP CCC DNA is broken and denatured as is OC DNA. Substrate CCC DNAs are readily prepared for the various repair enzymes. As examples AP CCC DNA is obtained by depurination at pH 3.5, uracil containing DNA by bisulfite deamination of C and thymine dimers by 254 nm irradiation. The sensitivity is such that less than 100 ng of DNA is required and of the order of 10^3 - 10^4 cells can be assayed for enzyme activity.

0968 A MAJOR NUCLEASE IN NEUROSPORA CRASSA NUCLEI, Murray J. Fraser, McGill University Montreal, Quebec, CANADA H3G 1Y6

An existing method [J.A. Hautala et al. (1977) J. Bact. 130, 704-713] for purification of Neurospora nuclei in large quantities was modified to drastically reduce contamination by ungerminated conidia and pieces of broken mycelia and to reduce the amount of adsorbed nuclease released from other organelles to barely detectable levels. The purity of the nuclei was checked by fluorescence microscopy. Using an extraction method described for lymphocyte nuclei [J. Graw, E.J. Schlaeger and R. Knippers (1981) J. Biol. Chem. 256, 13207-13212], a tightly-bound nuclease activity was recovered from the purified nuclei which was similar in many respects to that recovered from lymphocyte nuclei. It appeared to be related enzymologically and immunochemically to Neurospora endo-exonuclease, an enzyme found in vacuoles and on the inner membrane of mitochondria and found in large amounts in an inactive (precursor?) form in the cytosol. The level of nuclease activity was 75-82% higher in nuclei purified from mycelia that had been pre-treated for 1 hr at 30°C with 4 µg/ml 4-nitroquinoline oxide (4-NQO), a mutagen which inhibited growth by 50% under the same conditions. An apparently ATP-dependent activity of the Neurospora enzyme seen only with double-strand (ds) DNA (and also found for the enzyme from lymphocyte nuclei) proved to be an artifact, a lowered pH stimulation of the ds-DNase activity. (Supported by a John Simon Guggenheim Foundation Fellowship and the Medical Research Council of Canada).

0969 HUMAN LYMPHOBLAST ENDONUCLEASE SPECIFIC FOR UV- OR X-IRRADIATED DNA HAS AN ASSOCIATED AP ENDONUCLEASE, Thomas P. Brent, Division of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38101

An endonuclease activity for UV- or X-irradiated DNA (termed UVX endonuclease) previously was partially purified from the human lymphoblast line CEM-CCRF [Biochim. Biophys. Acta 454: 172 (1976); Nucleic Acids Res. 4: 2445 (1977)]. Further characterization of this activity indicated it has an associated AP endonuclease that is distinct from the cells' major AP endonuclease. Differences were noted in 1) affinity for DNA-Sepharose, 2) Mg^{++} requirement, and 3) capacity of endonucleolytic nicks to act as primers for DNA Polymerase I. Further studies indicate that the characteristics for UVX endonuclease action on UV-irradiated DNA are the same as for its action depurinated DNA suggesting that AP sites are intermediates in the incision of UV-DNA. Current studies are aimed to identify such AP site intermediates and to demonstrate the UV-DNA glycosylase activity implied by this model. (This work was supported by NIH grant CA 14799 and by ALSAC.)

0970 ACTIVITY OF T4 ENDONUCLEASE V ON UV IRRADIATED OLIGODEOXYRIBOTHYMIDYLATE, Michel Philippe, Enzymology, I.R.S.C., Villejuif, France and C.A.Smith, Biological Sciences, Stanford Univeristy, Stanford, CA 94305.

Endonuclease V of bacteriophage T4 contains a DNA-glycosylase specific for pyrimidine dimers and an AP endonuclease. We have treated 5'-^{32}P-labeled oligo [dT] containing thymine dimers with T4 endo V and analyzed the reaction products on polyacrylamide sequencing gels. Analysis of the products without alkali treatment indicated sites at which both the glycosylase and the AP endo had acted, while alkali treatment of the products before analysis revealed any additional sites at which only the glycosylase had acted. We found that the glycosylase was more active than the AP endo on single stranded $[dT]_{10}$ or $[dT]_{17}$ oligomers containing dimers, and we could detect no AP endo activity on single stranded $[dA]_{16}$ containing AP sites. The glycosylase was more active on dimer sites in $[dT]_{17}$ than in $[dT]_{10}$ and appeared to discriminate against dimer sites near either end of the molecules. No activity was detected against dimers in the 4 sites nearest the 3' ends of $[dT]_{17}$ molecules and activity was reduced but not eliminated against dimers near the 5' ends. Measurements of dimer frequency vs UV dose suggested that this result was not due to a non-uniform distribution of dimers.

0971 PROPERTIES OF TWO MAMMALIAN DNA-REPAIR ENDONUCLEASES
Dag Helland, Rune Male, Ingolf F. Nes, Hans Krokan and Kjell Kleppe, Department of Biochemistry, University of Bergen, and Institute of Medical Biology, University of Tromsø, Tromsø, Norway.

The DNA-repair endonucleases from mouse plasmacytoma cells and calf thymus have been purified and characterized. Both enzymes possess molecular weights of approximately 30.000. They are active in the absence of divalent metal ions and are routinely assayed in the presence of EDTA. The enzymes act on a wide variety of DNA damages such as those caused by UV-light, OsO_4, X-rays and adducts of N-acetoxy-2-acetylamino fluoren. They do not appear to be active on psoralen treated DNAs. The substrate requirements for the enzyme from mouse plasmacytoma cells have been studied in more details in particular with regard to the effect of supercoiling in the DNA substrate. The enzyme is active on UV-light damages only in supercoiled DNA and not when the DNA is in the relaxed or linear form. Moreover, the enzyme also acts on UV-light damages in supercoiled SV40 minichromosomes.

It is suggested that these enzymes do not recognize the damages as such in the DNAs, but rather specific DNA structures introduced into the DNA by the modifying agents.

Helland, D., Nes, I.F. and Kleppe, K. (1982) FEBS Letters, 142, 121-124.

0972 INDUCTION AND REPAIR OF CLOSELY OPPOSED PYRIMIDINE DIMERS IN SACCHAROMYCES CEREVISIAE, Richard J. Reynolds, Harvard School of Public Health, Boston, MA 02115

An enzyme-sensitive site assay has been developed to investigate the induction and biological fate of closely opposed pyrimidine dimers in the DNA of UV-irradiated yeast. The assay utilizes pyrimidine dimer-DNA glycosylase activity from Micrococcus luteus to make single-strand incisions at pyrimidine dimers. Incisions at closely opposed dimers result in double-strand breaks and are detected by their effects on DNA double-strand molecular weight as estimated by velocity sedimentation through neutral sucrose gradients. With this assay closely opposed sites were found to be induced as a linear function of dose and at a frequency approximately 100 fold lower than that observed for total pyrimidine dimers.

Closely opposed sites were also found to be subject to both excision repair and enzymatic photoreactivation. Incubation of UV-irradiated excision-proficient cells in the dark or excision-deficient cells in the presence of photoreactivating light resulted in a time dependent loss of closely opposed sites. No reduction in the numbers of closely opposed sites were observed when excision-deficient cells were incubated in the absence of photoreactivating light. Under certain incubation conditions, isolated pyrimidine dimers were found to be repaired much more readily than were closely opposed dimers. Thus closely opposed pyrimidine dimers may create a particularly difficult problem for DNA repair processes in yeast.

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0973 THE ROLE OF DNA LIGASE IN DNA REPAIR IN SACCHAROMYCES, Shirley J. McCready, Joy M. Boyce and Brian S. Cox, Botany School, South Parks Road, Oxford, U.K.
We assay the repair of pyrimidine dimers in the form of UV-endonuclease sites in two classes of yeast DNA: chromosomal DNA and 2μm circular plasmids. We find that UV-endonuclease sites can be removed from 2μm plasmids and their covalently-closed structure retained in cells in which the CDC9-coded ligase is inactive. This is true up to relatively high doses of UV ($10 J.m^{-2}$ = 2.4 x 10^4 dimers per cell (McCready and Cox, Current Genetics 6, 29 (1982)). In contrast, we observe the accumulation of single-strand breaks in the chromosomal DNA of ligase-inactive cells after very low doses of UV ($1-2 J.m^{-2}$). These nicks are not apparent in ligase-active cells. They appear very rapidly, within 5 minutes of irradiation whereas assays of dimer excision in wild-type cells indicate that this is a slow process and may take up to 30 minutes to complete even at these low doses. Dimer removal from 2μm circles and chromosomes and the appearance of single-strand breaks in chromosomal DNA are dependent on the activity of RAD1, RAD2, RAD3 and RAD4 genes.

We conclude that 1) the CDC9-coded DNA ligase is not necessary for the (excision) repair of dimers in 2μm circles; 2) the single-strand breaks appearing in chromosomal DNA after UV are the consequence of a very early step in repair which is reversible by CDC9-ligase and 3) this step is by-passed in the repair of dimers in 2μm circles.

0974 MOLECULAR CLONING AND CHARACTERIZATION OF A DNA FRAGMENT FROM *Saccharomyces cerevisiae* THAT PARTIALLY COMPLEMENTS THE UV SENSITIVITY OF *rad4-3*, Glenn A. Pure and Errol C. Friedberg, Department of Pathology, Stanford University, Stanford, CA 94305.
We have isolated a multicopy autonomously replicating plasmid (pNF4000) that confers partial UV resistance uniquely to the mutant *rad4-3* and not to *rad4-2* or *rad4-4* mutants of *S. cerevisiae*. In contrast to other plasmids that confer essentially complete UV resistance to *rad1*, *rad2*, *rad3*, and *rad10* mutants, pNF4000 confers only a 10-fold increase in UV resistance to *rad4-3*. When pNF4000 (a multicopy YEp plasmid) is converted to an integrating plasmid, stable transformants (to URA^+) of *rad4-3* fail to show enhanced UV resistance. Southern blot analysis demonstrates that the 9.4 kb yeast chromosomal DNA fragment in pNF4000 contains repetitive DNA sequences and a comparison of the restriction map of pNF4000 with that of the yeast transposable element Ty1 demonstrates the presence of a 6.0 kb Ty1-like element. The enhanced UV resistance is somehow related to the presence of the Ty element since *rad4-3* transformed with a plasmid from which the Ty sequence is deleted, is no more UV resistant than the mutant transformed with the vector YEp24.

0975 ACCESSIBILITY OF PYRIMIDINE DIMERS TO EXOGENOUS UV-ENDONUCLEASE INCREASES DURING POST-UV INCUBATION OF DROSOPHILA CELLS. Paul V. Harris and James B. Boyd, University of California, Davis, CA 95616.
Excision-deficient cells derived from embryos carrying the *mei-9*^{a} mutation were irradiated with far- or mid-ultraviolet light at doses which produce 4-10 UV-endonuclease-sensitive sites (ESS) per 10^8 daltons. The cells were incubated for various periods and nuclei were isolated in buffer containing 0.5% triton X-100. These nuclei were incubated with UV-endonuclease from *Micrococcus luteus* and the number of ESS was determined by alkaline sucrose gradient sedimentation. In the absence of post-UV incubation of cells, the endonuclease produced nicks at an average of 16% of the total ESS present in purified DNA (mean of 10 experiments). In nuclei derived from cells incubated for 1-3 hr following irradiation, the accessibility of ESS increased by an average of 61 ± 8% (S.E., n-11) over the corresponding unincubated control. This increase did not occur in cells incubated with 1 mM novobiocin, suggesting that DNA-topoisomerase plays a role in modifying chromatin accessibility.
(Supported by PHS GM25562, GM22221, and DOE EV70210.)

0976 TWO MUTAGEN-SENSITIVE MUTANTS OF DROSOPHILA WHICH BLOCK EXCISION OF THYMINE DIMERS ARE INCAPABLE OF REPAIR REPLICATION IN RESPONSE TO MMS, EMS, UV AND X-RAYS, Ruth L. Dusenbery, Shannon C. McCormick and P. Dennis Smith, Emory University, Atlanta, GA 30322.

The mei-9 and mus(2)201 mutants of Drosophila melanogaster were identified as mutagen-sensitive mutants on the basis of larval hypersensitivity to methylmethanesulfonate and characterized as excision repair-deficient on the basis of a greatly reduced capacity to excise thymine dimers from cellular DNA. The high degree of larval cytotoxicity observed with a variety of other chemical and physical agents indicated that these mutants may be unable to excise other important classes of DNA adducts. We have measured the ability of the single mutants and the double mutant combination mei-9;mus(2)201 to perform the resynthesis step in excision repair by means of an autoradiographic analysis of unscheduled DNA synthesis (UDS) induced in a mixed population of primary cells in culture.

The three strains exhibit no detectable UDS activity in response to applied doses of 1.5-6.0mM methyl methanesulfonate, 1.0-4.5mM N-methyl-N-nitrosourea or 10-40 J/m^2 254nm U.V. light, dose ranges in which control cells exhibit a strong dose-dependent UDS response. The mei-9 and mei-9;mus(2)201 mutants also have no detectable UDS response to X-ray doses of 300-1800 rad, whereas the mus(2)201 mutant exhibits a reduced, but dose-dependent, response over this range. These data correlate well with the degree of larval hypersensitivity of the strains and suggest that mutations at both loci block the excision repair of a wide variety of DNA damage prior to the resynthesis step.

0977 DIMINISHED DNA REPAIR CAPACITY IN YOUNG AND OLD TURBATRIX ACETI, Harris S. Targovnik*, Sarah Locher & P.V. Hariharan, Roswell Park Mem. Inst. Buffalo, NY 14263
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Age related changes in the specific activity of numerous enzymes have been observed in nematodes, rodents and human fibroblasts. It is therefore reasonable that changes in DNA repair capacity may exist in aging organisms. DNA repair was measured by two methods: A) repair synthesis activity and B) direct measurement of base damage by chromatographic techniques following exposure to 254 nm UV light or ionizing radiation.

Equivalent yields of thymine-containing dimers were detected in the DNA of young or old T. aceti for far UV fluences up to 400 J/m^2. The levels of thymine-containing dimers removed during post-irradiation incubation are not significantly different, however higher levels of DNA degradation are observed in old T. aceti. This may indicate a loss of specificity in the repair of dimeric base damage with age.

The production and excision of base damage of the 5,6-dihydroxydihydrothymine type (t') was different in young and old T. aceti. The yield of t' lesions was two fold higher in young T. aceti than in old. Young T. aceti excise move t' type lesions than old T. aceti in the same time period.

X-ray and UV-induced repair synthesis activity is completed earlier in young nematodes than old. Whereas old nematodes are capable of repairing similar levels of DNA damage as young, they require longer repair times. The observed diminished DNA repair capacity is quite possibly due to an age related change in the specific activity of repair enzymes.

0978 EFFECT OF ULTRAVIOLET LIGHT ON DNA SYNTHESIS IN NORMAL AND REPAIR DEFICIENT MAMMALIAN CELLS, T.D. Griffiths, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115

Treatment of mammalian cells with UV light is known to produce lesions which temporarily block the rate of DNA chain growth. With time, however, the rate of DNA chain growth eventually recovers. Although some of this recovery appears to be due to excision of pyrimidine dimers other repair processes appear to be involved since this recovery process occurs faster than does the loss of endonuclease sensitive sites. In order to confirm that excision repair is important, the rate of recovery of DNA synthesis was examined in both normal (AA8) and excision repair deficient (UV-5) Chinese hamster cells. At various times following exposure to various fluences of UV light the rate of DNA synthesis was examined by conventional kinetic assays as well as by DNA fiber autoradiography. Immediately after exposure to UV light both cell lines exhibited a similar fluence-dependent decrease in the rate of ^{3}H-thymidine incorporation and extent of DNA chain growth. At later times, however, the two lines responded differently. For example, while the normal cells recovered synthetic rates rapidly, showing normal rates of DNA chain growth 5 hours after receiving 10 J·M^{-2}, the repair deficient lines showed virtually no recovery. The relation of this to excision repair and post-replication repair will be discussed. I wish to thank Dr. Larry Thompson for providing us with the AA8 and UV-5 cells. This work was supported by NCI grant 1 R01 CA32579.

0979 REPAIR OF N-ACETYLAMINOFLUORENE INDUCED DAMAGE IN UV SENSITIVE CHINESE HAMSTER OVARY CELLS, Moon-shong Tang, Rodney S. Nairn, Gerald M. Adair and Ronald M. Humphrey.
The UV-sensitive Chinese hamster ovary (CHO) cell lines - UVL-1 and UVL-10 - have been demonstrated to be deficient in removal of thymine dimers and in DNA repair synthesis. To study the relationship between repair of UV and chemically induced damage in DNA, we have treated UVL-1 and UVL-10 cells with N-acetoxy-2-acetylaminofluorene (NA-AAF) and found that for survival, these cells are 7-fold more sensitive to NA-AAF treatment than are parental cells. However, a somatic cell hybrid of UVL-1 and UVL-10 responds as the parental, repair-proficient cell line to both UV irradiation and NA-AAF treatment. To determine whether the specific acetylaminofluorene adduct induced by NA-AAF treatment is repaired in CHO cells by a pathway reflected in the UV hypersensitive phenotype, we have treated a cloned gene in vitro with NA-AAF or UV and assessed its potential to transform repair-proficient and deficient CHO cell lines. When a 3.5 kb Bam HI restriction fragment encoding the herpes simplex thymidine kinase gene was used to transfect thymidine kinase deficient (tk-) derivatives of UVL-10 and the wild-type parental cell lines, UVL-10 showed lower transfection frequencies than the parental cell line for UV-irradiated or NA-AAF treated 3.5 kb fragment. These results suggest that repair of UV and NA-AAF damage is through a common pathway in CHO cells.

Research supported by NIEHS grant ES 03124 and NCI grant CA 04484.

0980 LETHAL AND MUTAGENIC EFFECTS OF IONIZING RADIATION ON DIFFERENTIALLY SENSITIVE STRAINS OF MURINE LYMPHOMA CELLS, H.H. Evans, M.F. Horng, J. Mencl, E.C. Gregg, Case Western Reserve University, Cleveland, OH 44106, and J.Z. Beer, National Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD 20857.
Lethality and mutagenicity resulting from X-irradiation at two different dose rates (0.88 or 0.0004 Gy/min) were measured in two very closely related strains of mouse lymphoma cells (L5178Y-R and L5178Y-S) which differ in their sensitivity to ionizing radiation (Beer et al., Nature 199, 193, 1963). Lethality was determined by soft agar plating. The frequency of the induction of 6-thioguanine-resistant mutants was used as a measure of mutagenicity. LY-S cells were more sensitive than LY-R cells to the lethal effects of radiation delivered at either dose rate, but the difference in sensitivity was particularly evident after the low dose rate exposure, where the slope of the dose response curves between LY-R and LY-S differed by a factor of 7 at accumulated doses up to 2.5 Gy. For both strains the low dose rate exposure was less lethal than the high dose rate exposure. Thus, repair of lethal lesions evidently occurs in both strains, but is deficient in LY-S cells. LY-S cells, although more sensitive to the lethal effects, were less mutable than LY-R cells by X-irradiation. The frequency of mutations induced by 1.5 or 2.5 Gy of X-radiation in LY-R cells, but not LY-S cells, was decreased at the lower dose rate as compared to the higher dose rate. These results indicate that LY-S cells are deficient in (1) repair of lethal and mutagenic lesions and (2) a process involved in the production of radiation-induced mutagenic lesions.
(This research is supported by DOE contract DEAC0277EV04472 and NIH grant CA 15901)

0981 EXTREME SENSITIVITY TO KILLING IN CHO-CELL MUTANTS, WHICH MAY SPECIFICALLY REFLECT DNA-CROSS-LINK DAMAGE, C. Hoy, L.H. Thompson, and E. Salazar, Biomedical Sci. Div., Lawrence Livermore National Laboratory, Livermore, CA 94550
Five complementation groups of UV-sensitive CHO cell mutants were previously shown to be deficient both in the incision step of repair after exposure to 254 nm UV light and in the removal of large adducts, properties similar to those of xeroderma pigmentosum cells. The 5 groups fell into two classes, depending on whether or not they were highly (100-fold) or only slightly (3.5-fold) sensitive to killing by the DNA cross-linking agent mitomycin C. To determine if this differential sensitivity between classes is a general characteristic of cross-link damage, we tested 15 cross-linking chemicals in a rapid cytotoxicity assay. Dramatic hypersensitivity was always seen with group 2 mutants UV4 or UV20, ranging from 10-250 fold over that of normal cells; the strongest response was obtained with diepoxybutane. However, UV5 (a group 1 mutant) had only 1-3 fold sensitivity. With 8 monofunctional agents that form bulky adducts, UV4 and UV5 had similar sensitivity (2-8 fold). Decarbamoyl mitomycin C produced an unusually strong response in both UV5 and UV4 of 8- and 15-fold, respectively. In summary, the properties of the class 2 mutants suggest that there is a common component in the repair of cross-link and monoadduct damage. The relationship of these mutants to cells from Fanconi's anemia patients, which are also very sensitive to DNA cross-linking agents, is presently unclear. (Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48 and supported by a grant from the U.S. Environmental Protection Agency, No. R808641-10.)

0982 ISOLATION OF A CELL CYCLE-DEPENDENT GAMMA RAY-SENSITIVE CHINESE HAMSTER OVARY CELL, Thomas D. Stamato, Ronald Weinstein, Amato Giaccia, and Laurie Mackenzie, The Wistar Institute, Philadelphia, PA 19104

A technique for the isolation of gamma ray-sensitive Chinese hamster ovary (CHO) cell mutants is described, which uses nylon cloth replica plating and photography with dark-field illumination to directly monitor colonies for growth after gamma irradiation. Two gamma ray-sensitive mutants were isolated using this method. One of these cells (XR-1) had a two-slope survival curve: an initial steep slope and then a flattening of the curve at about 10% survival. Subsequently, it was found that this cell is sensitive to gamma irradiation in G-1, early S and late G-2 phases of the cell cycle, whereas in the resistant phase (late S phase) its survival approaches that of the parental cells. The D_{37} in the sensitive G-1 period is approximately 30 rads, compared with 300 rads of the parental cell. This mutant cell is also sensitive to killing by the DNA breaking agent, bleomycin, but relatively insensitive to UV light and ethyl methane sulfonate suggesting that the defect is specific for agents that produce DNA strand breakage.

0983 INDUCTION OF DNA DAMAGE AND REPAIR BY METAL COMPOUNDS, S.H. Robison O. Cantoni and M. Costa, University of Texas Medical School at Houston, Houston, TX 77025

A number of metal compounds have been shown to cause cell transformation. In order to further understand this process we have examined the ability of a number of metal compounds to induce DNA strand breaks and repair in Chinese Hamster Ovary Cells (CHO) and Syrian Hamster Embryo (SHE) cells. Analysis by nucleoid gradient sedimentation indicated that cadmium (Cd+2), chromium (CrO4-2), mercury (Hg+2) and nickel (Ni+2) all induced DNA strand breaks. Analysis of the effects of these metals revealled that Ni+2 induced the least amount of DNA damage, whereas Hg+2 induced the greatest amount of DNA damage. Mercury exhibited an effect after as little as one hour of treatment and chromium had an effect after 18hrs of treatment. DNA damage was induced by cadmium and nickel at 3hrs and 24hrs respectively. When purified nucleoids were used Hg+2 and CrO4-2 induced substantial amounts of DNA damage after as little as 15 min. of treatment. Purified nucleoids exposed to nickel and cadmium exhibited considerably less damage after a 15 min. treatment. Reformation experiments using mercury indicated that there was no DNA repair occuring. Cesium chloride equilibrium density sedimentation indicated that the other metals used induced DNA repair synthesis and mercury did not. Supported by Grants #R808048 (EPA), #06570(NCI), #CA 29581 (NCI), and # DEAS05-81ER60016 (DOE).

0984 THE CELLULAR AND MOLECULAR RESPONSE OF DNA REPAIR-DEFICIENT MUTANTS OF CHO CELLS TO CISDIAMINEDICHLOROPLATINUM, Raymond E. Meyn and Susan F. Jenkins, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

A battery of DNA repair-deficient mutants of Chinese hamster ovary (CHO) cells was examined with respect to sensitivity to the antitumor cross-linking agent, cis-diaminedichloroplatinum (Cis-DDP), at both the cellular and molecular levels. The variation in response of the mutant cell lines in terms of cell survival was surprisingly broad; particularly for a subset of mutants previously characterized as having a uniform hypersensitivity to ultraviolet light (UV) light. The dose modification factors (DMF), relative to their wild-type parent, ranged from 2.5 to 83. Assessment of the kinetics of DNA cross-link repair at the molecular level was performed using the technique of alkaline elution. The differential cellular sensitivities were reflected in the appearance and loss of DNA cross-links in the various mutants; and a reasonable correlation was obtained between the results derived from survival analysis and the relative amounts of DNA cross-linking remaining at 24 h. These data underscore the significance of DNA cross-links and their subsequent repair in the determination of cell killing by cis-DDP. In addition, the disparity in response of the subset of the UV-sensitive, repair-deficient mutants suggests that, although these cells may, in fact, share some common repair steps as a consequence of DNA damage induced by either cis-DDP or UV, repair of cis-DDP lesions may be a more complex process. (This investigation was supported by PHS grant number CA-23270 awarded by the National Cancer Institute, DHHS).

0985 MOLECULAR GENETIC ANALYSIS OF UV EXCISION REPAIR GENE TRANSFER IN CHINESE HAMSTER OVARY CELLS. Mark A. MacInnes and Gary F. Strniste, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

We have previously shown that a UV excision repair deficient mutant of CHO cells, UV-135 can integrate and express exogenous DNAs including a putative CHO UV resistance gene and a plasmid, pSV2gpt, which confers aminopterin resistance in HAT medium (M. MacInnes, et. al. in preparation). We now report tests of two genetic predictions; that UV resistance gene transfer is specific for normal (UV^r) CHO source DNA, and that cotransfer of UV^r and HAT^r phenotypes should be observed in $CaPO_4$ co-precipitations of normal CHO genomic and pSV2gpt DNAs. In all experiments, cells were treated with 40 µg genomic plus 10 µg pSV2gpt DNAs per 100 mm dish containing 2.5×10^6 UV-135 ($hprt^-$) cells (4 dishes per experiment). In 11 experiments, HAT^r colonies arose from normal genomic + pSV2gpt DNA transfers at an average frequency of 5×10^{-4} and cotransferants were found for 4 of 15,050 HAT^r colonies tested (a cotransfer frequency of 1.3×10^{-1}). This value was ~10% of the net frequency for UV^r colonies after treatments with normal genomic DNA alone. Eight parallel experiments with UV 135 genomic DNA + pSV2gpt gave no cotransferants per 13,100 HAT^r colonies tested. All cotransferants were stably resistant to UV for more than 50 generations in the absence of HAT^r selection. Gel hybridizations of Eco R_1 digested transferant DNA with ^{32}P-labeled pSV2 probe showed multiple copies of plasmid sequences within their genomes. The transferants also regained excision repair capacity as assessed by filter alkaline elution assay. We are doing restriction enzyme sensitivity tests with the UV resistance gene transfer for cloning purposes. (Supported by US DOE)

0986 EFFECTS OF HYPERTHERMIA ON THE REPAIR OF X-RAY INDUCED DNA DOUBLE STRAND BREAKS, Ian Radford, Biological Research Unit, Cancer Institute, Melbourne, Australia.

The effect of a prior heat treatment on the ability of mouse L-cells to repair DNA double strand breaks produced by subsequent X-irradiation, has been investigated using the neutral filter elution technique. In untreated cells, DNA double strand break repair kinetics showed two first order components; a rapidly-repaired component with a half-life of 2-3 minutes and slowly-repaired component with a half-life of 30 minutes. After the non-lethal hyperthermic treatment of 43°C for 0.5 h the slow repair component was inhibited, whilst after the moderately lethal hyperthermic treatment of 43°C for 1 h both components were inhibited.

It was also found that hyperthermic treatment could enhance the level of DNA double strand breakage produced by subsequent X-irradiation.

This ability of hyperthermia to inhibit DNA double strand break repair may explain its synergistic effect with X-irradiation on cell survival.

0987 INCREASED UNSCHEDULED DNA SYNTHESIS IN ISOLATED RAT LYMPHOCYTES, M. J. Skinner, S. E. Irwin and C. A. Schreiner, Biochemistry Department, Mobil Environmental and Health Science Laboratory, Princeton, NJ 08540

We have previously shown that the detection of induced unscheduled DNA synthesis (UDS) in circulating lymphocytes is possible by in vivo/in vitro techniques. Blood taken via cardiac puncture from rats treated with DNA damaging agents is maintained in the presence of ^3H-thymidine to detect UDS by radioautographic techniques. Although the induced UDS is dose related and significantly higher than control cultures, the level of induction has not been as great as would be expected considering the potency of the mutagens. The amount of repair activity may be limited by other blood elements (e.g. platelets or serum); parallel cultures of lymphocytes (whole blood and isolated lymphocytes) from animals dosed with methyl methane sulfonate were established to determine if there is an increase in the level of UDS when lymphocytes are segregated from whole blood elements. Our data indicate that at doses below cytotoxicity there is a significant increase in UDS in isolated lymphocytes. Therefore, a component of blood elements may either inhibit or suppress repair in lymphocytes treated in vivo with mutagens.

0988 OPERATION OF A HUMAN CELL DNA EXCISION REPAIR PATHWAY IN VITRO, William K. Kaufmann, University of North Carolina, Chapel Hill, NC 27514

This section of the Workshop on DNA Repair in Normal and Repair-Defective Human Cells will review aspects of DNA excision repair as it occurs in open cells or isolated nuclei after ultraviolet (UV) irradiation. The promise of identification of multiple genetic mutants with deficiencies in repair of pyrimidine dimers, the xeroderma pigmentosum cells, was the possibility of identifying the gene products that are needed for this repair by in vitro complementation analysis. DNA excision repair involves several steps: strand incision and excision of damaged DNA; template-directed, gap-filling DNA synthesis; and strand ligation. The damaged chromatin-DNA substrate also appears to undergo structural transitions at repairing sites. All of these steps in DNA excision repair have now been examined in cell-free systems derived from UV-irradiated human cells. Strand incision and excision of damaged DNA have been observed in sonicated or permeable fibroblasts. Incision in permeable cells was found to occur at only a small fraction of the rate seen in intact cells. Accordingly, open cells activate reparative gap-filling after irradiation to only 10-25% of the level achieved by intact cells. Gap-filling DNA synthesis after UV irradiation has been extensively studied in isolated fibroblast nuclei, and lysed or permeabilized fibroblasts, lymphocytes and HeLa cells. Ligation of repair patches appears to be efficiently performed in permeable fibroblasts as judged by the insensitivity of newly synthesized DNA to digestion by exonuclease III. Apparently contradictory results concerning nucleosome reassembly in permeable cells and artifacts caused by non-specific nuclease activity in cell nuclei will also be discussed.

0989 REPAIR OF UV DAMAGE IN HELA CELLS IS INHIBITED BY APHIDICOLIN, Andrew R.S. Collins and Robert T. Johnson, Cancer Research Campaign Mammalian Cell DNA Repair Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK.

When repair DNA synthesis is measured as incorporation of [^{3}H]thymidine after UV irradiation, diploid human cells are susceptible to inhibition by aphidicolin while HeLa cells appear resistant. However, repair in isolated HeLa nuclei is blocked by the drug. We set out to resolve these discrepancies, using two independent assays which do not rely on incorporation of thymidine as a measure of repair. First, brief incubation of HeLa cells with aphidicolin after UV irradiation results in accumulation of DNA breaks representing cellular incision at damage sites with incomplete subsequent polymerization; a higher concentration of the drug is required for this effect in HeLa than in normal human cells. Second, using the in vitro UV endonuclease assay for UV-induced pyrimidine dimers modified to permit detection of damage inflicted by doses as low as 1 Jm^{-2}, we find that aphidicolin prevents removal of most of this damage in HeLa cells incubated for 1 day. Inhibition of repair by aphidicolin is consistent with involvement of DNA polymerase α in this process. We offer a possible explanation of the contradictory reports.

0990 NASCENT ANALYSIS OF UV EXCISION REPAIR ON HELA AND HUMAN DIPLOID CELLS, C. Stephen Downes and Robert T. Johnson, CRC Mammalian Cell DNA Repair Group, University of Cambridge, England.

We have tested the efficiency with which hydroxyurea (HU) and cytosine arabinoside (ara C) convert repair sites to strand breaks, using the nascent analysis method in which repair-labelled DNA at nascent sites adjacent to strand breaks is selectively denatured in alkali, and separated from repair-labelled DNA at completed, denaturation-resistant sites by hydroxyapatite chromatography. Normal human diploid cells are known to form more strand breaks when given HU and ara C after UV than tumor-derived transformed cells do[1]; but the efficiency of break formation at repair sites is not constant. In G_1 HeLa, efficiency is low ($\simeq$ 20%) at < 1 Jm^{-2} and rises to 50% at high doses; this accounts for the apparent sigmoid dose-response of break formation[1]. In G_o diploid cells, efficiency at high doses is similar to HeLa; so the difference in UV repair between tumor-derived and normal cells is probably real. Also, when HeLa are converted to a quiescent, "detransformed" state by sodium butyrate, break formation and nascent distribution still closely resemble proliferating HeLa, though repair synthesis becomes HU/ara C-sensitive as in G_o diploids and not as in proliferating HeLa. So the transformation-related decrease in excision repair is independent of growth state; furthermore, excision repair is not affected by extensive butyrate-induced histone acetylation.

Reference 1; Squires, S., Collins, A.R.S. and Johnson, R.T. Mutat. Res. 95, 389-404, 1982.

0991 FORMATION AND REPAIR OF DNA LESIONS INDUCED BY IONIZING IRRADIATION OF HUMAN LYMPHOBLASTS IN THE PRESENCE AND ABSENCE OF MISONIDAZOLE, Patricia Hentosh, Richard J. Reynolds, and John B. Little, Harvard School of Public Health, Boston, MA 02115.

The formation and repair of DNA lesions induced by ionizing radiation in the presence of Misonidazole (Miso), an hypoxic cell radiosensitizer, was investigated in human lymphoblasts (TK6 cells). Two classes of damage have been studied by alkaline sucrose gradient techniques: strand breaks (SSBs) and base damage which is recognized by a crude extract of M. luteus (referred to as endonuclease sensitive sites, ENSS). A six fold increase in SSBs was detected in cells irradiated under aerobic conditions as compared to hypoxic conditions (OER=6.0). Miso (15mm) also sensitized hypoxic cells to SSB damage for a sensitizer enhancement ratio (SER) equal to 4.7. However, no increase in the number of ENSS was observed when cells were irradiated in O_2 as compared to N_2. In addition, no difference in ENSS yield was detected if Miso was present during the hypoxic radiation. An OER for ENSS of 1.1 was obtained; the SER was 0.8. To explore the possibility of a qualitative change in DNA lesions, the rates of repair were compared after cells were irradiated with 25K rad plus O_2, N_2, or N_2 + Miso. The repair kinetics of SSBs were similar and very rapid after all three conditions with 80-90% of the SSBs repaired within one hour. ENSS repair was much slower than SSB repair with 50-60% of the sites remaining in the DNA after eight hours. Once again, however, there were no marked differences in the kinetics of repair after the three irradiations. Thus, it appears that in TK6 cells, neither O_2 nor N_2 + Miso irradiation environments cause any detectable quantitative or qualitative differences in ENSS induction. (Supported by NIH grant No. CA 09078).

Excision Repair (DNA Sequence and Conformation Effects)/Repair of Alkylation Damage/ DNA Repair and Human Disease

0992 REPAIR OF AFLATOXIN-DNA ADDUCTS IN α DNA OF MONKEY CELLS, Steven A. Leadon and Philip C. Hanawalt, Biological Sciences, Stanford University, Stanford, CA 94305

Repair of covalent aflatoxin B_1 (AFB_1)-DNA adducts was studied in African green monkey cells. Repair synthesis elicited by AFB_1 adducts was deficient in the 172 bp highly repeated α DNA sequence compared with that in the bulk of the DNA. Analysis of the adducts by HPLC showed that the initial level of modification was the same for α and bulk DNA. However, the primary initial adduct, AFB_1-N^7-Gua, was removed more slowly from α than bulk DNA. The kinetics of this slow removal resemble those previously shown in repair-deficient human XPA cells and probably represent the spontaneous loss of the AFB_1 moiety directly, or with concomitant loss of the guanine to yield an AP site, rather than adduct recognition by a repair endonuclease. The formation of the more chemically stable, secondary product, AFB_1-formamidopyrimidine, was found to occur more rapidly and to a greater extent in α DNA than in bulk DNA. The repair patch size for excision repair of AFB_1 adducts in α DNA was found to be about 10 nucleotides compared with 20 nucleotides for excision repair of UV damage in α DNA or for repair of AFB_1 adducts in bulk DNA. This suggests that the removal of AFB_1 adducts by excision repair initiated by endonucleolytic action at the AFB_1-N^7-Gua is deficient in α DNA while the repair pathway involving an AP endonuclease is proficient. Irradiation of cells with low doses of UV_{254} immediately after treatment with AFB_1 increased the rate and extent of removal of AFB_1 adducts from α DNA to the levels found in the bulk of the DNA. This increase in removal of AFB_1 was shown to be UV dose dependent.

0993 PERTURBATIONS OF ENZYMIC URACIL EXCISION DUE TO GUANINE MODIFICATIONS AND CONFORMATIONAL CHANGES IN DNA, Nahum J. Duker, Temple University School of Medicine, Philadelphia, PA 19140.

Phage PBS-2 DNA, which contains uracil in place of thymine, was used as substrate for B. subtilis uracil-DNA glycosylase. Reaction of this DNA with N-acetoxy-N-2-acetylaminofluorene resulted in guanine arylamidation at the C-8 position. The enzymic V_{max} was decreased by 33% for DNA containing 2.6% modified guanines. DNA challenge experiments, where excess unmodified PBS-2 DNA was added after reaction with the arylamidated substrate, showed delayed enzymic release from the modified DNA. The effects of conformational changes on glycosylase activity were examined using poly(dG-m^5dC)·poly(dG-m^5dC) in which 10% of the guanines were arylamidated. This caused conformation transition to the Z form as determined by circular dichroism. After enzyme incubation with this modified polynucleotide, addition of PBS-2 DNA resulted in a 66% reduction of uracil release. The same experiment performed on unmodified polynucleotide in 5mM $MgCl_2$, which also introduces the Z conformation, caused no reduction. Enzyme incubation with arylamidated polynucleotide in 5 mM $MgCl_2$ prior to addition of PBS-2 DNA did not substantially reduce the rate of uracil release. Neither the reaction rate nor processivity of uracil-DNA glycosylase were changed in 5 mM $MgCl_2$. These results suggest that the binding of this glycosylase to N-(deoxyguanosin-8-yl)-acetylaminofluorene in DNA is altered by the ionic environment, even at conditions where the enzyme's reaction with its own specific substrate is invariant. Thus the ionic environment constitutes a factor influencing the simultaneous interactions of two separate types of DNA damage, possibly affecting enzymic initiation of DNA excision-repair in living cells.

0994 ANALYSIS OF THE DISTRIBUTION OF UV INDUCED REPAIR PATCHES IN THE DNA-NUCLEAR MATRIX COMPLEX FROM HUMAN CELLS, L.H.F. Mullenders, A.A. van Zeeland and A.T. Natarajan, University of Leiden, The Netherlands

The distribution of UV induced repair patches along DNA loops attached to the nuclear matrix, was investigated by digestion with DNA degrading enzymes and neutral sucrose gradient centrifugation. When DNA was gradually removed by DNase 1, pulse label incorporated by UV-irradiated cells during 10 min in the presence of hydroxyurea showed similar degradation kinetics as prelabelled DNA. No preferential association of pulse label with the nuclear matrix was observed, neither within 30 min after irradiation, nor 13 hours after irradiation. When the pulse label was incorporated by replicative synthesis under the same conditions, a preferential associated of newly synthesized DNA with the nuclear matrix was observed. Single strand specific digestion with nuclease S_1 of nuclear lysates from UV-irradiated cells, pulse labelled in the presence of hydroxyurea and arabinosylcytosine, caused a release of about 70% of the prelabelled DNA and 90% of the pulse labelled DNA from the rapidly sedimenting material in sucrose gradients. The results suggest no specific involvement of the nuclear matrix in repair synthesis, a random distribution of repair patches along the DNA loops and simultaneously multiple incision events per DNA loop.

0995 THEORETICAL LIMITATIONS OF ANALYSIS OF REPAIR ENDONUCLEASE ACTIVITIES ON SUPERCOILED, CIRCULAR DNA. W. Clark Lambert and Muriel W. Lambert, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ.

A major technique for analysis of DNA endonuclease activity is to use a small, uniform supercoiled circular (Form I) phage DNA as substrate. Cleavage on one strand at a single site allows the supercoiling to relax, converting the DNA molecule into relaxed, circular (Form II) DNA; cleavage of both strands converts the DNA molecule into linear, duplex (Form III) DNA. These three forms of phage DNA then may be separated by gel electrophoresis, allowing quantitation of the assay. The number of cleavages of either type per DNA molecule, n, is then estimated, based on the Poisson distribution, using the expression: (1) $n = \ln I_B - \ln I = \ln \frac{I_B}{I}$

where I_B = the proportion of the DNA existing as Form I following incubation of the substrate without enzyme, and I = the proportion of the DNA existing as Form I following enzymic incubation. A critical, but usually unstated assumption of this computation, however, is that the enzyme may cleave with equal proficiency any of an infinite number of sites on each substrate molecule. Many endonucleases may recognize and cleave only a small number, N, of sites per DNA molecule in this type of system, however, and the number of cleavages they produce is grossly overestimated by (1). For these enzymes, a more accurate estimate of n is given by

(2) $n = \frac{N(I_B^{1/N} - I^{1/N})}{I_B^{1/N}}$

This in turn leads to expressions for analysis of more complex applications of supercoiled, circular DNAs used to assay endonucleases active in DNA repair processes. These equations will be presented and the derivatives and implications of all of these expressions will be discussed.

0996 DISTINGUISHING REPAIR FROM DEGRADATION WHEN CONFORMATION CHANGES ARE THE MAJOR MEASURE OF DNA DAMAGE, C.S. Lange, SUNY Downstate Med. Ctr., Brooklyn, N.Y. 11203

Strand-breakage in straight-chain random-coil DNA molecules can be reliably measured by gel electrophoresis (up to 50kbp), sedimentation (up to 10^9 or 10^{10} M_r) and viscoelastometry (10^7 - 10^{11} + M_r). However, in higher order structures, e.g., superhelical nucleoids, strand breakage causes the relaxation and/or cleavage of domains with the consequent increase in molecular volume. Thus, for condensed DNA, damage can be expressed as an increase in τ_{11} (viscoelastic retardation time) or a decrease in S (due to increased solvent friction (drag) on the extended molecule). Such data exist for E. coli and mammalian cells. Return of the τ_{11} (or S) value to that of the original population can come about by either REPAIR or DEGRADATION of the extended structure (e.g., E. coli domain/loop). Degradation must not be confused with repair. The E. coli nucleoid model shows that if the nucleoid minus the relaxed loop has a net contour length < that of the intact nucleoid, the viscoelastic recoil ($\Gamma_{11,r=0}$) for intact nucleoids will be < the preirradiation value and the difference will yield the number of molecules degraded. If the effective contour length is indistinguishable from the original, in spite of the loss of one or more domains, then repair and/or degradation will yield similar results (τ, Γ, S). However, protease treatment to release constrained conformations may yield detectable genome (or subunit) fragments if degradation occurred instead of repair. Supported by DOE Contract No. AC0280EV10503.

0997 DEFICIENT REPAIR OF PSORALEN ADDUCTS IN α DNA OF MONKEY CELLS, Miriam E. Zolan and Charles Allen Smith, Biological Sciences, Stanford University, Stanford, CA 94305.

We are studying DNA damage and repair in the highly repeated 172 bp α DNA component of cultured African green monkey cells. We have shown (Cell 28, 613) that the level of DNA damage and the rate and extent of repair synthesis were the same in α DNA and the bulk of the genome after treatment of cells with 254 nm ultraviolet light (UV_{254}). In contrast, repair in α DNA was only about 30% of that in bulk DNA after treatment of cells with 4' aminomethyl trimethylpsoralen (AMT) plus 360 nm light (UVA). Studies with isotopically labeled drug showed that the initial levels of AMT damage were the same in α DNA and bulk DNA, and that the deficient repair synthesis in α DNA reflected deficient removal of AMT adducts from α DNA. The patch size for excision repair was the same (about 20 nucleotides) in α DNA and bulk DNA after AMT damage.

We are examining this phenomenon further by analyzing the repair of a psoralen derivative, 4'hydroxymethyl trimethylpsoralen (HMT), for which the chemical structures of all the adducts to DNA have been determined by others (JACS 103, 2347). Repair synthesis in α DNA was about 20% of that in bulk DNA in cells treated with HMT and UVA and incubated for 4-48 hours. Removal of isotopically labeled HMT from α DNA was also about 20% of that from bulk DNA over 48 hours of repair. Unlike the result for aflatoxin B_1 (see abstract by Leadon and Hanawalt), the removal of HMT adducts from α DNA was not enhanced by a low dose of UV_{254}. Therefore, since HMT forms several structurally distinct adducts with DNA, it is possible that the sequence or chromatin structure of α DNA favors the formation of HMT adducts that are refractory to repair.

0998 NUCLEOSOME REARRANGEMENT FOLLOWING DNA REPAIR IN HUMAN CELLS TREATED WITH SODIUM BUTYRATE OR HYDROXYUREA, Michael J. Smerdon, Washington State University, Pullman, WA 99164-4630

The rate and extent of redistribution of repair-incorporated nucleotides within chromatin during very early times (10-45 min) after UV irradiation was examined in normal human fibroblasts treated with 20 mM sodium butyrate or 2-10 mM hydroxyurea. Under these conditions, the amount of DNA replicative synthesis is reduced to very low levels in each case. Furthermore, in the sodium butyrate-treated cells, the core histones are maximally hyperacetylated. Using methods previously described by us, it was found that treatment of cells with sodium butyrate had little or no effect on the rate and extent of redistribution of repair-incorporated nucleotides. On the other hand, in cells treated with hydroxyurea, the rate of redistribution during this early time period was less than half the rate observed in either control cells or sodium butyrate-treated cells. However, the extent of redistribution was unchanged in the hydroxyurea-treated cells. Since hydroxyurea has been shown to decrease the rate of completion of "repair patches" in mammalian cells, these results indicate that nucleosome rearrangement in newly repaired regions of DNA does not occur until the final stages of the nucleotide excision repair process are completed.

0999 DISTRIBUTION WITHIN CHROMATIN OF NEWLY REPAIRED DNA IN HUMAN CELLS FOLLOWING DAMAGE BY METHYL METHANESULFONATE OR METHYLNITROSOUREA, Khalifah Sidik and Michael J. Smerdon, Washington State University, Pullman, WA 99164-4630

The distribution of repair synthesis within chromatin following damage by methyl methanesulfonate (MMS) and methylnitrosourea was examined in cultured human fibroblasts. Initially, we examined the contribution of DNA replicative synthesis to the levels of total nucleotides incorporated during repair using the BrdUrd density-shift method. These studies indicated that immediately after damage by low, nontoxic doses of MMS, DNA replicative synthesis is suppressed. However, during later times (i.e., >9 hrs) the MMS-treated cells yielded a higher level of DNA replicative synthesis than untreated cells. Indeed, by 15 hrs after damage, we observed an ∿3 fold increase in DNA replicative synthesis in the MMS-treated cells over that of the untreated cells. When these contributions were corrected for, it was found that initially repair incorporated nucleotides are staphylococcal nuclease sensitive and are underrepresented in nucleosome core DNA. These characteristics were observed for repair synthesis occurring immediately after damage (0-1 hr) or during later times after damage (i.e., 6-7 hrs and 12-13 hrs). Furthermore, we observed nucleosome rearrangement in the newly repaired regions of DNA with a rate and extent similar to that observed in UV irradiated cells. Thus, the repair of these two alkylating agents is associated with the same overall chromatin distribution characteristics as the repair induced by UV radiation and bulky chemical carcinogens.

1000 SALT-INDUCED FORMATION OF NUCLEOSOMES IN NEWLY REPAIRED REGIONS OF DNA IN VITRO, John F. Watkins and Michael J. Smerdon, Washington State University, Pullman, WA 99164-4630

Nuclei were prepared from normal human fibroblasts which were irradiated with UV light and labeled for 30-40 min during repair synthesis in the presence of 2 mM hydroxyurea. Under these conditions, 65-75% of the newly repaired DNA lacks a nucleosome conformation. Treatment of these nuclei with 0.6 M KCl for 1 hr at 37°C resulted in a decrease in the average nucleosome repeat length from ∿193 base pairs (control nuclei) to ∿146 base pairs. There was a concomitant increase in the amount of repair-incorporated nucleotides, relative to bulk DNA, associated with isolated nucleosome "core particles." Approximately half of the newly repaired DNA that was not initially in a nucleosome conformation was converted to a "nucleosome-like" structure; the remaining newly repaired regions were resistant to the salt-induced formation of nucleosome-like structures. Similar results were obtained for cells not treated with hydroxyurea, as well as nuclei depleted of histone H1 prior to salt treatment. Thus, under the conditions used in these studies, some, but not all, of the newly repaired regions of DNA acquired a salt-induced, nucleosome-like structure even though these regions had not yet undergone nucleosome rearrangement in the intact cell.

1001 DISTRIBUTION OF UV-INDUCED DNA REPAIR BETWEEN DIFFERENT NUCLEOSOME SPECIES. Klaus Erixon, Research Institute of Scripps Clinic, La Jolla, Calif.92037. Confluent human fibroblasts were UV irradiated and repair labeled in the presence of hydroxyurea. Nuclei were digested with Micrococcal nuclease. Intact nucleosomes, i.e. DNA associated with histone and non-histone proteins, were extracted and analysed in agarose-polyacrylamid gels. In these nucleosome gels, four different forms of mononucleosomes could be distinguished, i.e. the core and nucleosomes in association with histone H1 and proteins HMG 14/17 in different combinations.

In cells repair labeled (H-3) for one hour, the distribution of label was indistinguishable from bulk DNA (C-14) , i.e. no preferential repair of any particular nucleosome species was observed. In an attempt to study a more transient repair state in chromatin, cells were repair labeled in the presence of hydroxyurea and cytosine-arabinoside, conditions which cause accumulation of the intermediate single-strand breaks in excision repair. Under these conditions incorporation of repair label is reduced, but located at the nicked, repairing sites, as judged by the local denaturation in weak alkali. Repair label was now digested faster by the nuclease. Some differences were observed relative to bulk DNA in the nucleosome gel. A relative enrichment for the core nucleosome, a possible depletion of H1 containing nucleosomes, different mobility of dinucleosomes and a subnucleosome species could be inferred from the H-3/C-14 ratio in the sliced gel, indicating a possible conformational change in repairing chromatin.

1002 CHROMATIN FACTORS AFFECTING DNA REPAIR IN MAMMALIAN CELL NUCLEI, Julie Harless, Walter Hittleman, Raymond Meyn, and Roger Hewitt, University of Texas at Houston Graduate School of Biomedical Sciences and M.D. Anderson Hospital, Houston, Texas 77030.

We are investigating chromatin factors that participate in the incision step of DNA repair in eukaryotic cells. Localization of repair activity within nuclei, the stability and extractability of activity, the specificity for recognizing damage in chromatin or purified DNA as substrates are of interest in this investigation of human cells, CHO cells and their radiation sensitive mutants. We have developed procedures that provide nuclei in which their DNA can be proven to behave as a collection of covalently closed circular molecules. The integrity of the DNA in human nuclei can be maintained during incubation in appropriate buffers for as long as 60 minutes. When cells or nuclei are exposed to UV light prior to incubation, incisions presumably associated with DNA repair can be demonstrated. Incision activity is stable to prior extraction of nuclei with 0.6M NaCl, which removes most non histone chromosomal proteins. Our studies to date are consistent with an hypothesis that factors responsible for initiating DNA repair are localized in the nuclear matrix.

1003 THE RECONSTITUTION OF HIGHER-ORDER DNA STRUCTURE AFTER X-IRRADIATION OF MAMMALIAN CELLS, Michael R. Mattern, Leonard A. Zwelling, Donna J. Kerrigan and Kurt W. Kohn, National Cancer Institute, NIH, Bethesda, MD 20205

Two highly sensitive methods were utilized concurrently to study the restoration of secondary and tertiary structure to the DNA of mouse leukaemia (L-1210) cells exposed to biological doses (150 or 400 R) of x-radiation -- the alkaline elution technique, which measures the number of DNA single strand breaks; and nucleoid sedimentation, which is a measure of the amount of compaction of intact, supercoiled DNA. DNA repair took place in two distinct phases. The first was rapid ($t_{1/2}$ less than 10 min); during this phase, more than 90 percent of the DNA strand breaks were sealed and some compaction of nucleoid DNA occurred. After a lag of 1-2 hours, a second phase of repair took place, during which there was further compaction of nucleoid DNA but little or no DNA strand ligation. The first phase was delayed by 3-aminobenzamide, an inhibitor of poly ADP-ribose biosynthesis; the second was blocked by novobiocin, an inhibitor of DNA topoisomerases.

The two phases of nucleoid reconstitution following x-irradiation are thus distinguishable by their times of occurrence and their sensitivities to two inhibitors of enzymes involved in DNA metabolism. These results suggest that a sequential process of strand ligation followed by the generation of higher-order DNA structure is intrinsic to the repair of DNA damaged by ionizing radiation and, possibly, to DNA repair in general.

1004 EFFECT OF 3-AMINOBENZAMIDE, AN INHIBITOR OF POLY (ADP-RIBOSE) TRANSFERASE, ON EXCISION REPAIR IN HUMAN CELL LINES, Ian G. Walker, John P.H. Th'ng, Thomas W. Norry and Paula Nizankovsky, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1

3-aminobenzamide (3-AB) caused a 3-fold enhancement of repair replication in T98G cells treated with DMS but no enhancement of repair replication induced by UV-light. T98G cells were derived from a glioblastoma. When KB cells (HeLa) were used the repair replication induced by DMS, 4NQO or UV-light was not enhanced by 3-AB. The repair of single-strand breaks (alkali labile lesions) in the DNA from KB cells treated with DMS, MMS or 4NQO was not inhibited by 3-AB nor was the repair of 4NQO damage in T98G cells. A remarkable finding was that T98G cells would not repair single-strand breaks induced by DMS treatment alone, but did so when they were also treated with 3-AB. The fall in NAD levels induced in T98G and KB cells by DMS treatment was prevented by 3-AB treatment. Thus, 3-AB was entering the cells and exerting its expected biological effect.

1005 REGULATION OF A DAMAGE-SPECIFIC DNA BINDING PROTEIN BY POLY(ADP-RIBOSE), Urs Kuhnlein and Siu S. Tsang, Environmental Carcinogenesis Unit, British Columbia Cancer Research Centre, Vancouver, B.C., Canada

Poly(ADP-ribosyl)ation of proteins in mammalian cells has been implicated in DNA repair. It has been suggested that poly(ADP-ribosyl)ation may change the chromatin structure to a form which renders DNA damage more accessible to repair and/or regulate the activity of enzymes involved in the repair process. We have recently identified a DNA binding protein from human fibroblasts which preferentially binds to supercoiled DNA damaged by a variety of different types of agents. DNA supercoiling is required for the induction of binding sites on the substrate DNA. There are at least 10^5 DNA binding proteins per cell, indicating that it is a major protein. Two observations indicate that the activity of this DNA binding protein is controlled by poly(ADP-ribosyl)ation: (1) The binding activity is sensitive to snake venom phosphodiesterase but not to alkaline phosphatase or RNase; (2) Cells incubated with dimethyl sulfate have a several-fold higher level of binding protein than cells incubated with dimethyl sulfate and 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase.

1006 POLY(ADP-RIBOSE) SYNTHESIS IN PERMEABILIZED HELA CELLS, Miyoko Ikejima[1], Yosuke Sawada[2], An Quoc Dang[2], Robert J. Suhadolnik[2] and D. Michael Gill[1], Department of Molecular Biology and Microbiology, Tufts University School of Medicine[1], Boston, 02111 and Department of Biochemistry, Temple University School of Medicine[2], Philadelphia, 19122.

Poly(ADP-ribose) polymerase has a high affinity for DNA breaks and absolutely needs broken DNA for its activity. Flush-ended double-stranded DNA is the best activator known. In intact eukaryotic cells, more poly(ADP-ribose) is synthesized after DNA damage caused by alkylating agents or irradiation. Poly(ADP-ribose) polymerase inhibitors delay DNA rejoining. Those facts strongly suggest that poly(ADP-ribose) synthesis is involved in DNA repair, but it is most unclear how.

We measure poly(ADP-ribose) synthesis by incubating permeabilized HeLa cells with [^{32}P]NAD in the presence of DNase I, which causes a pulse of DNA breaks. By analysis on SDS polyacrylamide gels we find an increased amount of poly(ADP-ribose) on the polymerase molecules themselves, in free polymer and in core histones. We have asked which of these is made first, how an elevated poly(ADP-ribose) synthesis increased the ADP-ribosylation of core histones and whether ADP-ribosylation of core histones is involved in DNA repair or not. Our experimental data show that poly(ADP-ribose) polymerase is the first acceptor of poly(ADP-ribose) synthesis in DNA-damaged cells and synthesis proceeds via new ADP-ribose addition at the proximal end of preformed polymer. Polymerase-bound chains form rapidly when DNA breaks are present, but are soon degraded when the breaks are removed, suggesting that they are the most relevant to DNA repair events. The modification of histones appears to be a secondary, and possibly inconsequential event.

1007 CLONING OF GENES FOR 3-METHYLADENINE DNA GLYCOSYLASES I AND II, N. Clarke, G. Evensen, I. Kaasen and E. Seeberg, Norwegian Defence Research Establishment, Division for Toxicology, N-2007 Kjeller, Norway

E. coli has two different 3-methyladenine DNA glycosylases which participate in DNA alkylation repair in vivo. One is constitutively expressed and encoded by tagA, while the other is inducible and controlled by ada and alk. Mutants deficient in one or the other of these enzymes are only slightly or moderately sensitive to alkylating agents, while double mutants (tagA ada and tagA alk) show extreme sensitivity. This extreme sensitivity makes the double mutant very powerful as a probe to select for recombinant plasmids which confer alkylation resistance. Two plasmids have been isolated which both increase the viability of a tagA ada double mutant on methylmethanesulfonate plates by a factor of more than 10^5. One of the plasmids results in 15-fold overproduction of TagI and carries the tagA$^+$ gene. The other plasmid results in fairly high constitutive expression of TagII. However, alkylation induction increases the amount of TagII 10-15 fold, to a level 3 fold higher than in induced cells not carrying the plasmid. The induction is ada$^+$-dependent. We conclude that the second plasmid carries the structural gene for TagII (tagB) including its control region responding to adaptation. Further characterization of this region should should help to elucidate the molecular basis for adaptation to alkylating agents in E. coli.

1008 *THE GENES INVOLVED IN THE REPAIR OF ALKYLATED DNA IN E.coli K12*, *Yoko Yamamoto**, Hiroko Kataoka and Mutsuo Sekiguchi, Kyushu University, Fukuoka 812, Japan

To elucidate the repair mechanism for alkylated DNA in E.coli, we isolated several mutants which exhibited increased sensitivity to methyl methanesulfonate (MMS) and decreased host cell reactivation (HCR) capacity for MMS-treated λ phage but normal sensitivity to UV light. The mutations were mapped at two different loci, alkA (45min) and alkB (47min).

We constructed a recombinant plasmid, pYY100, which consisted of an E.coli K12 EcoRI DNA fragment and the pBR322 vector. The alkA mutant carrying pYY100 showed almost normal sensitivity to MMS and its crude extracts prepared from adapted or unadapted cells showed very high activity of 3-methyladenine DNA glycosylase II (TagII). Furthermore cells carrying pYY100 synthesized a polypeptide of molecular weight 27,000, which is the same as that of TagII. One of the γδ-inserted pYY100 derivatives did not show any of these three effects. These results suggest that the alkA may be the structure gene of TagII.

The alkB mutant possessed normal levels of both constitutive and inducible 3-methyladenine DNA glycosylase and exhibited normal adaptive response to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The mutant was specifically sensitive to MMS and high frequency of mutation was induced when it was exposed to MMS but not to MNNG. The alkB gene product may be involved in the repair of some lethal and premutagenic DNA damage(s) which is produced by MMS rather than by MNNG. * Present address; Department of Radiation Biophysics, Kobe University School of Medicine, Chuoku, Kobe, Japan

1009 STRUCTURE AND MECHANISM OF E. coli O^6-METHYLGUANINE-DNA METHYL TRANSFERASE, A SUICIDE ENZYME Bruce Demple, Tomas Lindahl and Michael Waterfield, Imperial Cancer Research Fund, London NW7 1AD, England.

The adaptive response in E. coli includes the marked induction of an activity which repairs the highly mutagenic lesion O^6-methylguanine in DNA. We have purified this activity to physical homogeneity and have shown that it is a novel type of DNA repair enzyme which acts both as a methyltransferase and acceptor, the O^6-methylguanine methyl group captured as an S-methyl cysteine on the protein. As no other change in the polypeptide has been observed, this covalent blockage accounts for the suicide behaviour of this enzyme. Because of this unique mechanism of O^6-methylguanine correction, its importance in alkylation mutagenesis and carcinogenesis, and the similarity of the bacterial and the human activities, we are determining the primary structure of E. coli O^6-methylguanine DNA methyltransferase. Amino acid analysis revealed the presence of 4 cysteine residues per enzyme polypeptide, and 3 have been identified by amino acid sequencing at positions 18, 23 and 33 from the N. terminus; preliminary evidence suggests that the fourth cysteine residue is close to residue 37. Each methyltransferase captures only one methyl group. The position of the acceptor cysteine residue therefore is being determined by amino acid sequencing of the self-methylated protein. Fragments containing the cysteine residues are being purified to test for their methyltransferase or acceptor activity. A complete sequence determination is also underway, which may prove useful in locating the E. coli methyl transferase structural gene. Knowledge of the methyltransferase amino acid sequence should also allow secondary structure and perhaps mechanistic predictions.

1010 DNA REPAIR AND REPLICATION IN E. COLI CELLS INFECTED BY ALKYLATED T7 BACTERIOPHAGE, Margaret D. Mamet-Bratley, Guy Czaika, Jean-François Racine and Barbara Karska-Wysocki, Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7

T7 phage, treated with methyl methanesulfonate, is subject to host-cell reactivation. Analysis of infected host cells showed that only a fraction of the cells produced infectious particles. However, the phage burst for those cells was not decreased because of alkylation. This suggested that DNA repair, for phages which survived alkylation, must be efficient. To better understand this repair, we have begun to characterize events in DNA metabolism, from the time of DNA injection to the time of phage production. An early step is degradation, by phage-specific enzymes, of host DNA to provide precursors for phage DNA synthesis. We measured the rate of host DNA degradation, using a T7 mutant which degrades host DNA but synthesizes no phage DNA. We also determined the kinetics of DNA synthesis by pulse-labelling infected cells. In addition, using gel electrophoresis, we analyzed the production of phage-specific proteins in infected cells. Results show that phage alkylation caused a delay in both host DNA breakdown and phage DNA synthesis. However, analysis of phage-specific protein synthesis showed that T7 DNA polymerase (gene 5 product) was synthesized well before the time that DNA synthesis occurred. Thus, the delay in DNA synthesis appears to be due to a deficiency of DNA precursors (created by delayed host DNA breakdown) rather than to the absence of phage DNA polymerase. Presumably, it is during this delay that DNA excision repair can occur.

1011 REPAIR OF ALKYLATION DAMAGES IN M. LUTEUS. S.Riazuddin, A. Ather and Z. Ahmed, Nuclear Institute for Agriculture and Biology,Faisalabad,Pakistan.

Wild type M. luteus cells are highly sensitive to the killing effects of N-methyl-N-nitro-N'-nitrosoguanidine (MNNG). However, pretreatment of the cells with sub-lethal concentrations of the drug induces an adaptive response. The adapted cells exhibit 2.5 fold higher resistance and improved repair of O^6-G, N7-G, N3-G and N7-A when compared with the wild type parent after a challenge dose of 5 ug/ml of MNNG The adapted cell-extract has been resolved to isolate a demethylase and two DNA N-glycosylases. The demethylase corrects O^6 methyl guanine in the presence of 1 mM EDTA and in the process gets stably alkylated. The demethylation process does not require cofactors or divalent cations. The protein is heat sensitive being completely inactivated in 5 min at 50°.

The two DNA N-glycosylases having specificities for N-3 and N-7 alkyl purines, are being purified to compare their catalytic properties.

Wild type M. luteus cells were given a step-wise treatment with MNNG to generate mutants showing increased resistance to the drug. One of these mutants appears to repair a DNA base modification which shows chromatographic properties similar to O^4-T. This mutant is being further studied.

1012 ADAPTIVE RESPONSE IN BACILLUS SUBTILIS, Sankar Mitra, Robert S. Foote and Charles T. Hadden, Biology Division, Oak Ridge National Laboratory and University of Tennessee Graduate School of Biomedical Sciences, Oak Ridge, TN 37830

Bacillus subtilis after exposure to 5 μg/ml N-methyl-N'-nitro-N -nitrosoguanidine (MNNG) for 2 h shows increased resistance to a challenge dose (100 μg/ml) of MNNG with regard to cell killing and decreased mutagenesis. This adaptive response is accompanied by induction of O^6-methylguanine-DNA methyltransferase from a basal level of about 250 molecules to about 3000 molecules per cell as a result of de novo synthesis of the enzyme. The majority of MNNG-induced mutations in B. subtilis are of the guanine-to-adenine transition type. A UVR$^-$ mutant of B. subtilis showed increased lethality and mutagenesis by MNNG in spite of an increase in the methyltransferase level, following adaptation treatment. (Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation, and the National Cancer Institute under Grant No. 1 ROl CA31721-01).

1013 O^6-METHYLGUANINE-DNA METHYLTRANSFERASE IN MAMMALIAN CELLS, Robert S. Foote, Abraham W. Hsie, Bimal C. Pal and Sankar Mitra, University of Tennessee Grad. Sch. of Biomed. Sci. and Biol. Div., Oak Ridge National Laboratory, Oak Ridge, TN 37830.

O^6-Methylguanine-DNA methyltransferase activity was assayed in extracts of mammalian cells which are proficient (Mer$^+$) or deficient (Mer$^-$) in the repair of DNA methylation damage. The Mer$^+$ HeLa CCL2 cell strain was found to contain approximately 100,000 molecules/cell of methyltransferase, assuming that each molecule can demethylate only one m^6G residue, whereas a Mer$^-$ HeLa S3 strain contained no detectable activity. Constitutive methyltransferase activity was also lacking in extracts of CHO and V79 cells. No induction of methyltransferase activity was observed in CHO cells which had been treated with N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) according to a protocol reported to render the cells resistant to alkylation-induced sister chromatid exchanges. Preliminary experiments indicate that treatment of HeLa CCL2 cells with subtoxic doses of MNNG results in reduced levels of methyltransferase for up to 24 hours following treatment, rather than an adaptive increase in activity. (Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation and the National Cancer Institute under Grant No. 1 ROl CA31721-01.)

1014 REPAIR OF O^6-METHYL GUANINE IN DNA IN EXTRACTS OF HUMAN CELLS AND TISSUES

by H. Krokan*, B. Myrnes* and K.E. Giercksky$^+$
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University of Tromsø, 9001 Tromsø, Norway.

HeLa cells, human liver, ventricle, small intestines and colon contain activities that remove methyl from O^6-methyl-guanine-containing DNA. In HeLa cells and human liver the methylgroup is transferred to a cysteine residue of an acceptor protein of size 23.000-24.000 dalton, as shown by SDS gelelectrophoresis. In small intestines, the mechanism for removal of methyl may be different because a large fraction of the radioactivity eluted from the amino acid analyzer much earlier than did S-methyl-cysteine. A smaller fraction coeluted with S-methyl-cysteine.

The level of activity in extracts varied several fold in different individuals (n=6-8) and between different organs. O^6-methyl-guanine repair was highest in liver (0.3-1.7 pmol/mg protein) and lowest in ventricle (lower than 0.15 pmol/mg protein). With the exception of small intestines, uracil DNA glycosylase activity did not correlate with O^6-methyl-guanine methyl transferase activity, indicating that these DNA repair enzymes are controlled differently.

B.M. is a fellow of the Norwegian Society for Fighting Cancer (Norsk Forening til Kreftens Bekjempelse).

1015 CHARACTERIZATION OF RAT LIVER O^6-METHYLGUANINE-DNA TRANSMETHYLASE, Anthony E. Pegg, Dept. Physiology, Hershey Med. Cntr., Penn State Univ., Hershey, PA 17033.
A protein fraction catalyzing the removal of O^6-methylguanine from methylated DNA was purified 7000-fold from regenerating rat liver. This protein catalyzed transfer of the methyl group from O^6-methylguanine in DNA to a cysteine residue and formed guanine in the DNA. There was a stoichiometric relationship between the production of S-methylcysteine, the formation of guanine and the loss of O^6-methylguanine from the DNA. A rapid assay of the O^6-methylguanine DNA transmethylase using antibodies specific for O^6-methylguanine to isolate this base facilitated the purification. The mammalian O^6-methylguanine-DNA transmethylase was also able to remove ethyl groups from O^6-ethylguanine in DNA forming S-ethylcysteine but this occurred at a much slower rate. It did not attack any of the other methylated bases present in DNA methylated by reaction with N-methyl-N-nitrosourea. The protein demethylated O^6-methylguanine in synthetic polymers containing poly(dC,dG,m^6dG) and in partially depurinated DNA but no demethylation of O^6-methylguanosine in RNA or of the free nucleoside or deoxynucleoside could be detected. The reaction with methylated single stranded DNA was considerably slower than with native double-stranded DNA. The protein became inactive after incubation with methylated DNA presumably because of the lack of regeneration of the cysteine which acts as a acceptor of the methyl group but was also inactivated by reaction with 5 mM N-methyl-N-nitrosourea. Both of these reactions could contribute to the apparent saturation of DNA repair when mammalian cells are exposed to alkylating agents.

1016 CELL AND SPECIES SPECIFICITY IN O^6-ALKYLGUANINE REPAIR, James A. Swenberg, Mary A. Bedell, Kathryn C. Billings and Charles Lindamood III, Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.
Persistence of O^6-alkylguanine (O^6AG) in DNA of tissues shows a high correlation with target site specificity for carcinogenesis following exposure to alkylating agents. A prominent exception to this correlation is rodent liver, a tissue that rapidly removes O^6AG. We have previously shown that the target cell for dimethylhydrazine (SDMH), the hepatic nonparenchymal cell (NPC), accumulates O^6AG during continuous exposure, while the non-target hepatocyte removes this promutagenic product with increasing efficiency (Bedell, et al, Cancer Res. 42:3079, 1982). These cell specific differences in promutagenic alkylation are the result of differences in O^6AG alkyl acceptor protein (AAP) activity (Swenberg, et al, P.N.A.S. 79:5499, 1982). When C3H mice were exposed to dimethylnitrosamine (DMN), the hepatocytes removed O^6AG more efficiently than NPC, but O^6AG concentrations remained relatively constant over the time course of exposure (Lindamood, et al, Cancer Res. 42:4153, 1982). Following exposure to 10 ppm DMN, AAP activity was examined in hepatocytes of C57Bl and C3H mice, strains having a low or high incidence of spontaneous liver tumors, respectively. Initial hepatocyte AAP activity was similar in both strains and decreased to approximately half this amount after 32 days exposure to DMN. This is in marked contrast to the rat, where a 2-3 fold enhancement of AAP activity follows continuous exposure to SDMH. Thus, even the presence of O^6AG in the DNA and a 3-fold increase in de novo DNA synthesis, mouse hepatocytes do not exhibit enhanced AAP activity.

1017 O^6- BUT NOT N^7-METHYLGUANINE LEVELS CORRELATE WITH TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS INDUCED BY METHYLATING CARCINOGENS, J. Doniger, R. Day and J.A. DiPaolo, Laboratories of Biology and Molecular Carcinogenesis, NCI, Bethesda, MD 20205.
Syrian hamster embryo cells (HEC) exposed for 1 hr to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylnitrosourea (MNU), or methyl methanesulphonate (MMS) were analyzed for excision repair of methylated bases, lethality, host cell reactivation of adenovirus, and neoplastic transformation. Transformation and survival were analyzed in a 7 day colony assay. DNA, isolated from confluent cultures treated with ^{3}H-methyl carcinogen, was acid hydrolized. ^{3}H-methylated bases and total guanine were analyzed using reverse phase high pressure liquid chromotography. No transformation was observed in non-treated cultures. Equitoxic doses (90-95% survival) of MNNG (.25 µg/ml), MNU (10 µg/ml) or MMS (110 µg/ml) induced similar transformation frequencies (1.2 -1.5%) and also yielded the same level of O^6-methylguanine adducts (4-5 µmol/mol guanine). Moreover, MNNG and MNU resulted in 55-65 µmol N^7-methylguanine mol guanine, whereas MMS induced 1900. The levels of both $N^7$and O^6-methylguanine per mol guanine were reduced by about one half 24 hr after MNNG, MNU, or MMS incubation. Thus, HEC have a limited capacity to repair both of these adducts. The survival of MNNG treated adenovirus plaqued on confluent non-treated HEC monolayers was intermediate compared to survival on mer$^+$ or mer$^-$ human cells. Mer- cells lack the ability to repair O^6-methylguanine. O^6-methylguanine levels but not those of N^7- correlate with the quantitative transformation results. Furthermore, transformation is accompanied with limited removal of O^6- lesions. Therefore, these data support the hypothesis that O^6- is the critical lesion for initiation of carcinogenesis by methylating agents.

1018 ADAPTATIVE DNA REPAIR PROCESS IN CHO CELLS. Françoise LAVAL and Sylviane MICHEL, Institut Gustave Roussy, 94805 Villefuif, France.

Exposure of E.coli to several non-toxic doses of an alkylating agent induces an adaptative repair pathway which allows the bacteria to resist the killing and mutagenic effect of further alkylation damage (Samson,L. & Cairns, J. Nature, 267, 281, 1977). When CHO cells are exposed to low doses of N-methyl-N'-nitro-nitrosoguanidine (MNNG), they become more resistant to the killing effect of the same agent (Samson,L. & Schwartz,J. Nature,287,861,1982)

We now show that CHO cells can be adapted by chronic exposure to either MNNG,methyl-nitroso urea (MNU) or methyl methane sulfonate (MMS). Exposure to low doses of MNNG, MNU or MMS increases the survival of cells challenged with the same agent. Furthermore, when CHO cells are adapted with MMS, they become more resistant to the killing effect of MMS and also of MNNG.

The mutagenicity of the alkylating agents was measured in adapted cells. The number of mutants (6-thioguanine resistant cells) decreases in cells adapted with MNNG and challenged with MNNG or MMS. However this number does not change in cells adapted with MMS and challenged with either MMS or MNNG.

The number of SH goups, the duration of the cell cycle, and the activity of the 7-methyl guanine and 3-methyl adenine glycosylases were not significantly different in adapted cells from those measured in control cells.

1019 REMOVAL OF O^6-METHYL GUANINE IN 9L CELLS SENSITIVE AND RESISTANT TO BCNU, W.J. Bodell and M.R. Rosenblum, Brain Tumor Research Center, University of California 94143

The development of cellular resistance to the cytotoxic effects of 1,3,bis(chloroethyl)nitrosourea (BCNU) appears to be a major factor for the failure of clinical chemotherapy. The purpose of our investigations is to identify the molecular mechanisms involved in cellular resistance to the cytotoxic effects of BCNU. 9L-2 is a resistant gliosarcoma cell line derived from the sensitive 9L cells. 9L-2 is approximately 7 fold more resistant to the cytotoxic effects of BCNU than 9L as measured by a colony forming assay. Treatment of 9L, and 9L-2 with 2 μM BCNU results in the induction of 90, and 20 sister chromatid exchanges (SCE's) respectively. Recent work by Erickson et al., Nature 288(727) 1980, suggests that a increased capacity to repair O^6-methyl guanine (O^6-MeG) in DNA during a one hour treatment with methyl nitrosourea (MNU) may be related to resistance to the cytotoxic effects of BCNU. To extend these observations 9L and 9L-2 were treated with 0.1 mM ^{3}H-MNU for one hour, and then frozen. The DNA was isolated, and the alkylation products N-7-methyl guanine, (7-MeG) and O^6-MeG were quantitated. The results of these experiments were that 1) the cellular DNA of 9L and 9L-2 were alkylated to the same extent by MNU, and 2) the ratio of O^6-MeG/7-MeG was 0.12 for 9L, and 0.10 for 9L-2. These results indicate that although there are large differences in the cytotoxic response, and the induction of SCE's by BCNU in 9L and 9L-2 cells their capacity to remove O^6-MeG from their DNA during a 1 hr. treatment with MNU is the same. Supported by grant CA-13525-11.

1020 CONTROL OF O^6-METHYLGUANINE REMOVAL IN HUMAN LYMPHOBLASTOID CELLS, Bernard S. Strauss and Robert M. Sklar*, University of Chicago, Chicago, Illinois 60637

Epstein-Barr-virus-transformed human lymphoblastoid cells can be divided into two major classes on the basis of their ability to remove O^6-methylguanine [O^6-MeG] from their DNA. Mex^+ cells remove this alkylation adduct rapidly by transfer of the methyl to an acceptor protein. Mex^- cells are deficient in this transfer activity. At least two types of stable Mex^- lines have been isolated: a) absolutely deficient, and b) with about 25% of the removal activity of Mex^+ lines. The activity of whole cells is mirrored by the activity of cell extracts; non-alkylated Mex^- cells yield extracts with diminished or missing O^6-MeG acceptor activity. No evidence for acceptor inducibility has been obtained. We have recently isolated Mex^+ and Mex^- lines by EBV transformation of a single sample of blood from the same male donor. The lines differ not only in O^6-MeG removal activity but also in sensitivity to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Both lines have a modal chromosome number of 46 and a single Y chromosome. We conclude that neither X-inactivation nor a change in chromosome number is responsible for the Mex^- characteristic. Crosses of Mex^+ X Mex^- lines indicate that the characteristic is autonomous, that is, the removal capacity per cell is the same in hybrids as in Mex^+ cells. 3-methyladenine removal is normal in Mex^- cells. Although Mex^- cells are sensitive to MNNG inactivation, there is not a quantitative correlation between removal and sensitivity in all cases implying that different reactions are involved. We suggest that the Mex^- characteristic is due to a change in an element which controls more than one reaction. [Supported by grants from the NCI and the DOE.]

*Senior author

1021 O^6-METHYLGUANINE METHYLTRANSFERASE IS LACKING IN MER$^-$ HUMAN TUMOR CELLS. Daniel B. Yarosh[1], R.S. Foote[2], S. Mitra[2], and Rufus S. Day III[1], [1]National Cancer Institute N.I.H., Bethesda, Md. 20205 and [2]Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830

The ability of human tumor cell extracts from twenty strains to demethylate O^6-MeG in DNA was assayed in two ways: [1] using as substrate the DNA polymer poly(dCdG[8-^{3}H]m^{6}dG) and [2] measuring the transfer of methyl groups from [^{3}H-CH_3]O^6-MeG in DNA to protein. Cell strains proficient in repair of O^6-MeG *in vivo* (Mer$^+$ phenotype) contained a methyltransferase activity while repair deficient strains hypersensitive to alkylation cell killing (Mer$^-$ phenotype) had little or no activity. (Both Mer$^+$ and Mer$^-$ strains contained MNU-damage-specific endonuclease activity.) Mixing extracts of different Mer$^-$ strains did not result in activity. A hybrid formed from two Mer$^-$ strains had post-MNNG survival similar to that of either parent. Extracts of Mer$^-$ cells did not inhibit the methyltransferase activity in extracts of Mer$^+$ cells. Adenovirus 5 virions were treated with ^{3}H-MNNG and both the number of O^6-MeG bases per genome and the survival of the virus on three Mer$^-$ strains were determined. 2 ± 1 O^6-MeG bases per Ad5 genome correlated with one lethal hit. This number of O^6-MeG bases per Ad5 genome correlates with little or no virus killing upon infection of Mer$^+$ cell strains. We suggest that a deficiency in the basal level of methyltransferase in Mer$^-$ cells is the fundamental reason for their hypersensitivity to the cytotoxic effects of DNA alkylation. Methyltransferase activity has been paritally purified from a Mer$^+$ human colon carcinoma cell strain and from human placenta. Its kinetics suggest that the enzyme is inactivated during repair of DNA.

1022 RESPONSE OF HUMAN CELLS TO MNNG, MMS, UV, BCNU, ENNG, AND HYDROXYETHYL-CNU: INFLUENCE OF MER$^-$ and REM$^-$ PHENOTYPES. Rufus S. Day, III, Dominic A. Scudiero, and Michael R. Mattern, N.I.H., Bethesda, Md. 20205, and FCRC, Frederick, Md. 21701. We have analysed the response of MNNG-sensitive human cell strains (Mer$^-$; D_0=0.14 uM), MNNG-resistant human cell strains (Mer$^+$ Rem$^+$; D_0=3.1 uM), and human cell strains with intermediate sensitivity (Mer$^+$ Rem$^-$; D_0=1.0 uM) to inactivation by other DNA-damaging agents in terms of post-treatment colony forming ability: (Relative slope is relative to actual slope obtained with Mer$^+$ Rem$^+$ strains; Table summarizes 378 survival curves on 22 strains.)

	Mer$^+$ Rem$^+$			Mer$^+$ Rem$^-$		Mer$^-$ (Rem?)	
	actual slope	relative slope	extrap. number	relative slope	extrap. number	relative slope	extrap. number
MNNG	0.32/uM	1.0±0.2	1.4±0.4	2.9±0.8	1.1±0.5	21±14	0.7±0.2
ENNG	0.056/uM	1.0±0.1	1.1±0.2	2.0±1.1	1.2±0.3	3.2±0.5	1.0±0.4
HECNU	0.023/uM	1.0±0.4	8.6±4.7	0.8±0.2	4.6±3.5	5.7±2.6	4.3±5.2
BCNU	0.087/uM	1.0±0.4	19±15	0.9±0.5	16±23	1.7±0.6	1.7±0.5
MMS	2.9/mM	1.0±0.3	8.6±6.1	0.8±0.2	7.2±3.6	2.6±1.5	1.2±0.3
UV	0.44/J/m^2	1.0±0.2	3.0±1.5	1.2±0.3	4.6±1.0	1.0±0.2	2.8±1.1

Strains within a group showed sensitivity differences. As a group Rem$^-$ strains were only clearly sensitive to MNNG, while Mer$^-$, also sensitive to MNNG, were sensitive to HECNU and ENNG (slope difference) and MMS and BCNS (extrap. no. difference). Although survival differences were paralleled by differences in DNA repair as assayed by nucleoid sedimentation, adenovirus survival after MMS or BCNU treatment was independent of Mer or Rem phenotype.

1023 THE REM$^-$ PHENOTYPE: DEFECTIVE REPAIR OF MNNG-PRODUCED DNA DAMAGE IN HUMAN TUMOR CELLS CAPABLE OF HOST-CELL REACTIVATION OF MNNG-TREATED ADENOVIRUSES (MER$^+$); Dominic A. Scudiero, Rufus S. Day, III, and Michael R. Mattern; FCRF, Frederick Md., 21701, and N.I.H., Bethesda, Md. 20205. We have identified a group of 5 human tumor cell strains which are Mer$^+$ (perform levels of host-cell reactivation of MNNG-treated adenovirus 5 like those of normal human fibroblasts; and, after treatment with a 5 uM MNNG dose remove at least half of the O^6-methylguanine produced in their DNA) but which are sensitive to MNNG-produced reduction in colony forming ability. These strains (called Rem$^-$ for resistance to MNNG negative) have a D_0 of about 1 uM/lethal hit compared to 3.1 for Rem$^+$ strains, and 0.14 for Mer$^-$ strains. During attempted repair following MNNG-treatment, Rem$^-$ strains do not incorporate ^{3}H-TdR at the level of the other Mer$^+$ (Rem$^+$) strains, but perform Mer$^-$ levels of either DNA-repair synthesis (as defined by the Scudiero and Strauss BND-cellulose assay) or DNA-repair replication (as defined by the Pettijohn and Hanawalt CsCl assay). Moreover, after treatment for 1 hour with increasing amounts of unlabeled MNNG, Rem$^-$ strains become less able than Rem$^+$ strains to remove O^6-methylguanine due to a 1 hour dose of ^{3}H-MNNG given immediately afterwards. In addition, MNNG-pretreatment reduced the capacity of Rem$^-$ Mer$^+$ strains more than that of Rem$^+$ Mer$^+$ strains to support the growth of MNNG-treated adenovirus 5. It was found by the use of the Cook and Brazell nucleoid sedimentation assay *that Mer$^+$ Rem$^-$ strains restored DNA tertiary structure to the control level more slowly* than did MNNG-treated Mer$^+$ Rem$^+$ cells. We believe, therefore, that the Rem$^-$ phenotype may be due to a defect in the regulation of the level of O^6-methylguanine repair protein(s).

1024 MNNG PRETREATMENT OF NORMAL HUMAN FIBROBLASTS (MER$^+$) ALLOWS THE FORMATION OF PREVIOUSLY UNOBSERVED DNA INTERSTRAND CROSSLINKING BY CHLOROETHYLNITROSOUREAS, Chana Zlotogorski and Leonard C. Erickson, Natl. Cancer Inst., NIH, Bethesda, MD.

Human cells differ in their capacity to repair the O^6-methyl-guanine lesion. Cells that repair these lesions are designated Mer$^+$ and deficient cells Mer$^-$. Chloroethylnitrosoureas (CNU) are antitumor agents which produce DNA interstrand crosslinks. In a recent report (Erickson et al., Nature 288: 727, 1980), we showed a clear correlation between CNU-induced DNA interstrand crosslinking and the Mer phenotype. Mer$^-$ cells produced consistently higher levels of interstrand crosslinks than did Mer$^+$ cells. We have proposed that crosslinks in DNA form via monoadduct formation at the guanine-O^6 position, followed by a delayed reaction with the opposite DNA strand. Guanine-O^6 alkyl transferase is known to remove methyl and ethyl groups from the O^6 position of guanine and transfer them to a thiol in a protein acceptor molecule. Treatment of cells with low doses of MNNG has been shown to induce the repair of O^6-methyl-guanine in CHO and Hela cells; however, single high dose exposures of those cells to MNNG decreased the repair of this lesion. In the present study we have measured the CNU induced DNA interstrand crosslinking in normal human fibroblasts (Mer$^+$) and SV-40 transformed human cells (Mer$^-$) following pretreatment with MNNG. Cells were treated for 1 hr with MNNG, then for an additional hr with CNU. Comparable levels of CNU induced DNA interstrand crosslinks were observed in both cell lines. This crosslinking has been previously undetected in the Mer$^+$ cells. These data are consistent with the hypothesis that in Mer$^+$ cells guanine-O^6-alkyltransferase may remove chloroethyl monoadducts before the lethal DNA interstrand crosslinks can form.

1025 VARIATIONS IN DNA REPAIR AMONG PEOPLE, R. B. Setlow, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

A number of human genetic diseases, often cancer prone, are known (or at least suspected) to involve deficiencies in DNA repair. XP is the most extensively studied and best understood of these diseases because the etiologic agent is known to be sunlight exposure and because cells from individuals with the disease are known to be defective in the repair of UV damage to their DNA. The repair deficiencies in XP are not absolute. Such deficiencies, on the average, are associated with a 10^4-fold enhancement in the susceptibility to sunlight induced skin cancer. Hence it is reasonable to suppose that relatively small variations (10-20%) in DNA repair among "normal" people would result in large variations in cancer risk among the apparently normal population. Three general techniques have been used to assess the DNA repair capacity of normal cells: 1) measurements of repair replication or unscheduled DNA synthesis; 2) the determination of the cytotoxic effects of x-rays or MNNG on cultured fibroblasts; and 3) the estimation of the activity in extracts able to remove from DNA, O^6-methylguanine. The standard deviations of the responses of fibroblast cultures were about 20%. The deviations in leukocyte or lymphocyte cultures were greater and the extremes of sensitivity differed by 5 to 10-fold. The observations of large variations in DNA repair among cells from normal individuals supports the hypothesis that such variations could have a large impact on the cancer susceptibility of subgroups at the extremes of the normal distribution and indicates the need for further studies, of both laboratory and epidemiological nature, on this subject.

1026 GENETIC ANALYSIS OF UV REPAIR-DEFICIENT HUMAN MUTANTS, Rebecca D. Goldfarb, Daniel J. Sussman, Nam D. Huh, Inger Sandlie, Segio Ohi, Gregory Milman and Lawrence Grossman, THE JOHNS HOPKINS UNIVERSITY, Baltimore, Md. 21205

The E. coli uvrA, B and C genes carry out the incision step in excision repair. There is a close analogy between the defect in E. coli uvrA, B, C mutants and cells derived from patients with xeroderma pigmentosum (XP). Our goal is to introduce the E. coli uvrA, B, and C genes into SV40-transformed XP cells to determine if these genes can genetically complement the repair deficiency in XP cells. To facilitate these experiments, we transformed XP cells of complementation groups A, C, D, E, and the variant (V) by transfection with an origin defective linear SV40 DNA (1). The transformed cell lines retained their sensitivity to ultraviolet light.

The uvrA, B and C genes were cloned into composite pPR322, SV40 and Ecogpt vectors (2) in which the uvr gene and the gpt gene are in tandem and flanked by SV40 regulatory elements. We have also constructed gpt containing vectors in which each E. coli uvr gene possesses individual SV40 regulatory elements. These vectors were introduced into the transformed XP lines by the calcium phosphate procedure (3). Colonies selected for gpt$^+$ were isolated and cloned (4). The presence of specific uvr mRNA, DNA and gene products is being determined and correlated with phenotypic reversion to repair proficiency in these cells.

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1027 TRANSFECTION OF XERODERMA PIGMENTOSUM CELLS WITH CLONED DNA, Miroslava Protić-Sabljić, David B. Whyte, John Fagan, and Kenneth H. Kraemer, N.C.I., Bethesda, MD 20205
Cells from patients with xeroderma pigmentosum (XP) are hypersensitive to killing by ultraviolet (UV) radiation. As a first step in attempting to clone the gene(s) responsible for the XP cells' UV hypersensitivity, we chose the model system described by Mulligan and Berg (Proc Natl Acad Sci 78:2072, 1981) utilizing the cloned bacterial gene xanthine-guanine phosphoribosyl transferase (XGPRT) (in a plasmid containing pBR322 and SV_{40} sequences) to assess transfection ability. Reconstruction experiments utilizing a 6-thioguanine (6TG) resistant ($HGPRT^-$) subline of the FB virus transformed lymphoblastoid cell line (LCL) XP2Os demonstrated the ability to detect $HGPRT^+$ cells at a frequency of 10^{-5} to 10^{-6} in mass culture using HAT (hypoxanthine, aminopterin, thymidine) selection. Treatment of the $HGPRT^-$ LCL with cloned DNA using modifications of the calcium phosphate precipitation technique of Graham and Van Der Eb (Virology 52:456, 1973) failed to produce a detectable frequency of $HGPRT^+$ cells. Similarly, treatment of XP fibroblast lines XP6Be and XP2Ne with cloned XGPRT DNA using MAAT-X (mycophenolic acid, adenine, aminopterin, thymidine, xanthine) selection failed to produce detectable colonies. In contrast, treatment of the SV_{40} transformed cell lines XP2Os(SV_{40}), XP12T703(SV_{40}), XP12Be(SV_{40}), XP6Be(SV_{40}) and XP2Ne(SV_{40}) with cloned DNA, using MAAT-X selection, all produced detectable numbers of colonies. Transfected subclones of XP2Os(SV_{40}), and XP12Be(SV_{40}) have been established that grow well in selective or non-selective medium for at least 3 months. SV_{40} transformation, whether by selection or by alteration of cellular properties, appears to facilitate uptake and/or expression of cloned DNA sequences in XP cells.

1028 DIMER EXCISION FROM CHROMATIN IN XERODERMA PIGMENTOSUM CELLS. Yoshisada Fujiwara and Yoshio Kano, Kobe University School of Medicine, Kobe 650, Japan.
Various complementation goups XP cells lack the early activity of nucleotide excision repair of UV-induced pyrimidine dimers to various extents. However, little is known about the repair enzymes in human cells. Mortelmans et al.(PNAS 73: 2757,'76) first suggested elegantly that XP cells were defective in factors regulating dimer excision from crude cell sonicates. Here we will report a successful repetition and further extentions. We used the crude nuclear sonicates, isolated chromatins, partially deproteinized chromatin and purified DNA from ^{3}H-dThd prelabeled, heavily UV-irradiated normal and XP (groups A, C, D and G) cells as substrates, and crude extracts as enzyme sources, to see to what extents dimers are excised in different extract/substrate combinations. In normal cells, their extract excised dimers rapidly from sonicate and chromatin. However, Groups A, C and G extracts were unable to escise dimers from the corresponding sonicates and isolated chromatins, but able speicifically from chromatin preparations partially deprived of non-histone proteins and from purified DNA, on the background of only little non-specific degradation of DNA from such different preparations under the assay conditions. In group-D XP6BE cells, their extract uniquely removed dimers from sonicate, chromatin and purified DNA at a similar rate, suggesting that the group-D factor is sensitive to sonication. Thus, our results support the previous basic data from Friedberg's Lab., and further indicate that chromatin-associated factors releasable into the non-histone protein fraction may regulate excision repair, in which XP cells are defective. Further, different extract/substrate combinations proved the in vitro complementations

1029 Mapping of repair genes and biochemical characterization of repair proteins deficient in xeroderma pigmentosum. J.H.J. Hoeijmakers, A. Westerveld, W. Keijzer, A.J.R. de Jonge, W. Vermeulen and D. Bootsma. Dept. of Genetics, Erasmus University, P.O.Box 1738, Rotterdam, The Netherlands.

Cytoplasts from human cells or proliferating Chinese hamster x human hybrids induce (in contrast to cytoplasts from Chinese hamster cells alone) fast complementation of the repair defect when fused with cells from xeroderma pigmentosum complementation group A (XP-A). *This property was used for the chromosomal localization of a human gene involved in XP-A complementation.* Using a panel of Chinese hamster x human hybrids with different human chromosomes a strong correlation was found between the presence or absence of chromosome 1 and the presence or absence of fast complementation. Regional mapping studies indicated this gene to be located on the distal part of 1q. It is of interest to note that cytoplasts from a Chinese hamster x XP-A hybrid also gave fast complementation after fusion with XP-A fibroblasts. This may be due to an activator/effector interaction between Chinese hamster and human repair proteins in the proliferating hybrid.
Using microinjection of crude extracts from repair proficient cells as a means to provide living XP-cells with the deficient repair factor we have been able to restore UDS of cells belonging to XP complementation groups A, G, and the new group H. The XP-A correcting factor, which was studied in detail, is a protein, stable on storage and abundantly present in repair proficient cells. It acts rapidly after injection and is probably not involved in a rate limiting step in the repair pathway.

1030 ON THE ROLE OF HUMAN APURINIC ENDONUCLEASES IN THE REPAIR OF ALKYLATION DAMAGED DNA, Leona Samson and Stuart Linn, University of California, Berkeley, California 94720.
The classical genetic and biochemical approaches to the assignment of various enzymes to the repair of DNA damage has proved extremely difficult in human cells despite extensive studies on the UV sensitive Xeroderma Pigmentosum (XP) cell lines. It was shown in 1976 (Kuhnlein et al., PNAS 73, 1169), that the apurinic (AP) endonuclease activity in crude extracts of XP cells of complementation groups A and D is qualitatively, and for group D quantitatively, different from that of normal cells and of four other XP complementation groups. Furthermore, XP group D cells were subsequently found to be totally deficient in one of the two human AP endonuclease species, namely AP endonuclease I (Kuhnlein et al., 1978, Nucleic Acids Res., 5, 95; Mosbaugh and Linn, 1980, J. Biol. Chem., 255, 11713). The existence of human DNA glycosylases that are specific for various alkylated bases, and the fact that alkylation damage induces excision repair in human cells, suggests that AP endonucleases might be involved in the repair of alkylation damage via a base excision repair pathway. We therefore set out to do the following:- (1) To confirm that our particular XP group D cells are, indeed, deficient in AP endonuclease I. (2) To determine whether XP group A cells are also deficient in AP endonuclease I as suggested by the altered activity seen in crude extracts. (3) To determine whether these qualitative and quantitative differences in AP endonuclease activity have any effect on the efficiency of alkylation induced excision repair. (4) To determine whether our XP group A and group D cells are more or less sensitive to alkylation induced cell killing. (Contradictory results have been reported for XP group A cells; Simon et al. 1981, Carcinogenesis 2, 570; Teo and Arlett, 1982, Carcinogenesis 3, 33.) So far we have confirmed that XP group D cells are totally deficient in AP endonuclease I. However, the induction of excision repair by alkylating agents, as measured by CsCl density gradient analysis and by auto-radiography, turns out to be at least as efficient in XP group D cells and slightly more efficient in XP group A cells.

1031 DEFECTIVE DNA ENDONUCLEASE ACTIVITIES ON DNA DAMAGED BY INTERCALATING DRUGS IN XERODERMA PIGMENTOSUM CELLS, M.W. Lambert, W.C. Lambert and D.E. Lee, Department of Pathology, *UMDNJ - New Jersey Medical School, Newark, NJ.*
Nuclear DNA endonuclease activities (Endos) from normal human (GM 1989) (HL) and xeroderma pigmentosum (complementation group A) (GM 2345) (XP) lymphoblastoid and Cloudman mouse melanoma (MM) cells on DNA treated with the intercalating antitumor antibiotics adriamycin (ADM), daunomycin (DM) and actinomycin D (ACT) were compared. Non-histone chromatin-associated (CA) and nucleoplasmic (NP) proteins were isolated from all three cell lines, subjected to isoelectric focusing (IF), and assayed for Endo using calf thymus DNA as substrate. Pooled peaks of Endo were assayed for activity on native and drug treated PM2 phage DNA and analyzed by electrophoresis on 0.9% agarose gels. A marked increase in activity against 25 uM ADM treated DNA was found in HL cells in a CA Endo fraction with pI 4.6. On the other hand, the major activity against 10 uM DM and 1 uM ACT DNA was found in HL cells in a CA Endo with pI 3.9. In MM cells, moderately increased activity was found on all three drug treated DNAs in CA Endos with pIs 3.9, 4.6 and 5.4. In both HL and MM cells, activity of one NP Endo with pI 4.6 was slightly increased on all of the drug treated DNAs. XP cells, on the other hand, were deficient in any increased Endo on all three drug treated DNAs. By contrast, we have previously shown a CA Endo, pI 4.6, in HL cells only, which is active on anthramycin treated DNA and a principal CA Endo, pI 9.8, for apurinic DNA in HL cells which is partially reduced in XP and MM cells.
Supported by Basil O'Connor Grant No. 5-287 from the March of Dimes Birth Defects Foundation and the Foundation of UMDNJ.

1032 DOMAIN-LIMITED REPAIR OF DNA IN ULTRAVIOLET IRRADIATED FIBROBLASTS FROM XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP C, Jonathan N. Mansbridge and Philip C. Hanawalt, Int. Psoriasis Res. Foundation, P.O.Box V and Biological Sciences, Stanford Univ., CA 94305.
Cell lines from xeroderma pigmentosum patients of complementation group C exhibit only 10-40% of normal levels of DNA repair synthesis following ultraviolet (UV) irradiation. Does this partial deficiency result from reduced amounts of essential repair enzymes or limited accessibility of different domains of the genome to repair enzymes? We have addressed this question by determining the distribution of remaining pyrimidine dimers in the DNA during the time course of repair in UV irradiated fibroblasts. Regions of the genome undergoing repair were labeled with ^{3}H-thymidine and BrdUrd. The single-strand molecular weight distribution of repair-labeled DNA (isolated on CsCl gradients) was compared to that of the bulk (^{14}C labeled) DNA after treatment with the dimer-specific endonuclease V of bacteriophage T4. Normal human fibroblasts exhibited the same distribution of dimers in the regions undergoing repair as in the bulk labeled DNA at any time after irradiation as demonstrated by congruence of the ^{3}H and ^{14}C sedimentation profiles in alkaline sucrose density gradients. In contrast, the fibroblasts from XP-C patients showed a significant disparity between ^{3}H and ^{14}C profiles with the bulk of the DNA sedimenting more slowly than those DNA molecules containing repair patches. The possibility of cellular population heterogeneity was ruled out by autoradiographic determination of unscheduled DNA synthesis. We conclude that the limited repair in cells of XP-C takes place in localized segments of the genome and that many domains of their chromatin are inaccessible to repair enzymes.

1033 ABNORMAL RESPONSE OF XERODERMA PIGMENTOSUM FIBROBLASTS TO BLEOMYCIN, Myra M. Hurt, Arthur L. Beaudet and Robb E. Moses, Baylor College of Medicine, Houston, TX 77030

The response of human fibroblasts to the DNA-damaging drug, bleomycin, was examined in whole cells isolated from patients having the disease xeroderma pigmentosum (XP). The appearance of low molecular weight DNA was observed in the presence of increasing amounts of the drug in normal fibroblasts. The studies in XP fibroblasts produced results which differed from those obtained in normal cells in at least two ways. (1) Prelabeled XP cells from most complementation groups contained more low molecular weight DNA than observed in the other human fibroblasts examined. (This was not observed in XP-G cells or a variant cell strain). (2) When XP strains were exposed to low doses of bleomycin, the low molecular weight DNA disappeared rather than showing a dose-dependent increase as did all other cell strains examined. If the XP cells were exposed to bleomycin in the presence of hydroxyurea and arabinofuranosylcytosine, the disappearance of low molecular weight DNA was not observed. Instead, we obtained a normal dose response to the drug. These data indicate that the DNA in XP cells carries single-strand breaks or alkaline-sensitive sites greater in number than in other cells even under unchallenged conditions. Introduction of BLM into XP cells induces a repair response which is blocked by hydroxyurea and arabinofuranosylcytosine. The presence or absence of this process in normal cells is unknown. This response of XP cells to BLM may demonstrate a process similar to the induction of recA observed in bacteria.

1034 N-ACETOXY-2-ACETYLAMINOFLUORENE-INDUCED REPAIR SYNTHESIS IN REPLICATING AND NON-REPLICATING REGIONS OF DNA IN XERODERMA PIGMENTOSUM VARIANT CELLS, Kouichi Tatsumi[1], Masao Inoue[2], Takashi Yagi[1] and Hiraku Takebe[1] Radiation Biology Center, Kyoto University, Kyoto 606[1] and Central Laboratory, Kanazawa Medical University, Ishikawa 920-02[2]

Benzoylated naphthoylated DEAE-cellulose(BNDc) columns can be used to separate DNA growing point regions from the bulk of the cellular DNA. In order to test the hypothesis that the defective "post-replication repair" in xeroderma pigmentosum(XP) variant cells is ascribed to inefficient excision of the small (but biologically significant) fraction of lesions at replication forks, the repair synthesis in replicating and non-replicating regions of cellular DNA was estimated by the combined use of the BNDc columns and the repair replication technique. Repair synthesis induced by N-acetoxy-2-acetylaminofluorene(AAAF) occurred to a slightly greater extent in growing point regions than in non-replicating regions for both XP variant and normal lymphoblastoid cells. The ratio of specific repair activity (^{3}H-dT cpm / μg DNA) at the growing point and that in the bulk DNA was indistinguishable between two types of cells. AAAF-induced repair synthesis in XP variant cells was inhibited by 2mM of caffeine to an equal extent in growing point and non-replicating regions of the DNA, indicating that the hypothesis seemed unlikely. (Supported by Grant-in-Aid from the Ministry of Education, Science and Culture)

1035 A NEW PATIENT WITH BOTH XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME COMPRISES THE NEW XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP H, A.N. Moshell, M.B. Ganges, M.A. Lutzner, H.G. Coon, S.F. Barrett, J.-M. Dupuy, and J.H. Robbins. Dermatology Branch and Lab. of Cell Biol., NCI, Arthritis, Musculoskeletal and Skin Diseases Program, NIADDK, NIH, Bethesda, MD, Inst. Pasteur, Paris, France, and Inst. Armand-Frappier, Univ. of Quebec, Quebec, Canada.

The first patient (XP11BE) reported to have both xeroderma pigmentosum (XP) and Cockayne syndrome (CS) had the unique XP DNA excision repair defect of complementation group B.[1] We have confirmed the diagnosis of a second patient (XP-SC-8) reported with both XP and CS.[2] He had the skin manifestations of XP (including a skin tumor by 2 years of age) and the following features of CS: characteristic facies, personality, and gait; cachectic dwarfism with microcephaly; skeletal abnormalities; 'salt and pepper' pigmentary retinal degeneration; spasticity and hyperactive reflexes in the lower extremities. Skin fibroblasts (GM 3248) from XP-SC-8 were fused with inactivated Sendai virus to cells from each of the known XP complementation groups A-G. The cultures were then irradiated with 254-nm UV light, incubated at 37° with tritiated thymidine, and autoradiographs were then prepared. The XP-SC-8 cells had 30% of the normal rate of DNA excision repair as determined by their UV-induced unscheduled DNA synthesis (UDS). Fusing XP-SC-8 cells with each of the other cell lines resulted in complementation, since there was obtained in each case a population of multinucleate cells which had a normal amount of UV-induced UDS. Since XP-SC-8 cells complement all the currently known XP complementation groups, patient XP-SC-8 is in a new group which we designate group H.

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2. Lafforet D and Dupuy J.-M. Arch Fr Pediatr 35:65, 1978.

1036 CORRELATION BETWEEN SURVIVAL AND THE RATE OF EXCISION OF DNA-CARCINOGEN RESIDUES,* John H. Miller, Pacific Northwest Laboratory, Richland, WA 99352 and Robert H. Heflich, National Center for Toxicological Research, Jefferson, AR 72079.
A model that correlates survival of cloning ability with the rate of excision of carcinogen residues bound to DNA was developed on the following assumptions: (1) cellular inactivation involves an expression time during which potentially lethal damage (PLD) can be removed by excision repair and (2) the amount of PLD is proportional to the number of carcinogen residues bound to DNA. (Miller and Heflich, Chem.-Bio. Interactions, 39 (1982) 45-55). Application of this model to survival of normal human fibroblasts (NF) and fibroblasts from xeroderma pigmentosum (XP) patients from complementation groups C and E led the conclusion that XPE and XPC cells excise residues of N-acetoxy-2-acetylaminofluorene (N-AcO-AAF) with a rate that is 60% and 40% of the normal rate respectively. To test this prediction, we measured the rate of excision of residues of N-AcO-AAF by NF, XPE and XPC cells and found that the rate of excision by XPE and XPC is about 50% and 10% of normal respectively. When the same arguments are applied to damage induced by ultra-violet (UV) light, we again find that the survival of XPC is greater than expected on the basis of the rate of removal of UV-endonuclease-susceptible sites. Possible explanations for this enhanced survival of XPC fibroblasts will be discussed.

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1037 FAR-UV INDUCED BIFILAR ENZYME-SENSITIVE SITES
Lun H. Lam, John B. Little and Richard J. Reynolds
Harvard School of Public Health, Boston, MA 02115
Irradiation of DNA with far-UV light induces lesions that can be converted to double-strand breaks by incubation with enzymatic activities from Micrococcus luteus. Velocity sedimentation through neutral sucrose density gradients can then be used to detect and quantitate these enzymatically-induced double-strand breaks. Closely-opposed pyrimidine dimers and UV-induced DNA-DNA interstrand cross-links are the most likely sources of these bifilar enzyme-sensitive sites. A variety of experimental approaches have been used to determine which, if either, of these two models is correct. Results accumulated to date indicate that the bifilar sites are most likely closely-opposed pyrimidine dimers. However, the bifilar sites are induced as a linear function of dose and this result is not readily explainable in terms of closely opposed dimers. We have found that approximately 27 pyrimidine dimers and 0.27 bifilar sites are induced per 10^6 bp per J/m^2 at 254nm.
We have also examined the fate of these far-UV induced bifilar sites in a variety of normal and UV-hypersensitive human fibroblast strains. Preliminary results with cells from individuals afflicted with Cockayne's syndrome and dyskeratosis congentia indicate that these cell strains are proficient in the removal of bifilar sites. One cell strain from an individual exhibiting extreme sun-sensitivity was found to be deficient in the repair of both pyrimidine dimers and bifilar sites. These results indicate that excision repair functions are necessary for the repair of bifilar sites in human diploid fibroblasts. (Supported by NIH grant No. CA 09078)

1038 INVOLVEMENT OF α POLYMERASE IN REPAIR OF POTENTIALLY LETHAL DAMAGE INDUCED BY ULTRAVIOLET RADIATION IN HUMAN SKIN FIBROBLASTS, Rex M. Tyrrell, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.
Aphidicolin, a specific inhibitor of the eucaryotic α polymerase, has been employed to study the role of this enzyme in repair of potentially lethal damage (PLD) induced by far-ultraviolet (UV, 254 nm) radiation in normal and repair defective primary human skin fibroblasts. Toxicity to untreated cells was minimised by exposing them to aphidicolin under low serum conditions for limited periods. However, there is a strong concentration dependent specific toxicity to cells treated with a fluence of 6 Jm^{-2} of far-UV radiation and incubated with aphidicolin for 2 days over the concentration range 0.0025 - 2.5 μg/ml. A similar effect is seen with a Xeroderma pigmentosum (XP) variant (excision proficient) strain but there is no selective toxicity to excision deficient XP cells of complementation group A. Inactivation of irradiated normal fibroblasts is rapid over the first 6 hours aphidicolin (1 μg/ml) exposure, but takes 2 days to complete after a low fluence of 1.5 Jm^{-2} and longer after doses of 6 Jm^{-2} and higher. Previous studies have shown that strand breaks accumulate in UV treated normal fibroblasts (1,2) but not in excision deficient fibroblasts (1) during aphidicolin treatment and that excision of pyrimidine dimers is inhibited (2). Our results demonstrate that this apparent uncoupling of excision repair prevents repair of PLD and is lethal to the cells. From survival curves, we estimate that the α polymerase is involved in repair of at least 60 percent of all UV-induced PLD. (1) Waters, R., Carcinogenesis 2, 795-797 (1980)
(2) Snyder, R.D. and J.D. Regan, BBRC 99, 1088-1094 (1981).

1039 EFFECTS OF CHRYSOTILE B ASBESTOS ON NORMAL AND REPAIR DEFICIENT HUMAN FIBROBLAST CELL LINES, R.C. Johnson, L. Bishop, M. Massenberg, and R.A. Vincent Jr., Dept. BCIM, Medical Univ. S.C., Charleston,S.C. 29425.
Our previous studies have raised the possibility that chrysotile B could damage DNA at concentrations that demonstrate little disruption to human fibroblast growth in vitro. We have examined here the effect of chrysotile B on DNA size from cells exposed to doses showing no obvious effect on cell growth. Growth of human skin and lung fibroblast was affected at 100µg/ml, but not at 10 or 1 µg/ml. However, DNA from cells exposed for 24 hours to 10µg/ml and 1µg/ml of chrysotile B sedimented more slowly in alkaline sucrose gradients. It is interesting that extensive single strand DNA breakage may occur in chrysotile B - treated cells in the absence of diminished cell growth. DNA repair deficient skin fibroblasts showed little or no greater sensitivity to chrysotile B than normal cells.

1040 DEFECTIVE REPAIR OF MITOMYCIN CROSSLINKS IN FANCONI'S ANEMIA, CONFLUENT NORMAL HUMAN AND XERODERMA PIGMENTOSUM CELLS. Yoshisada Fujiwara, Kobe University, Kobe, Japan.
Fanconi's anemia (FA) manifests chromosomal instability and predisposition to cancers. FA cells have been shown to be supersensitive to killing, chromosome aberration and SCE by crosslinking agents. However, the results thusfar obtained for repair defect in FA are contradictory among strains and crosslinking agents. I have studied mitomycin C (MC)-induced interstrand crosslinking and its repair in 4 typical unrelated FA strains by hydroxylapatite column chromatography, S1-nuclease digestion and alkaline sucrose centrifugation. Such three methods demonstrated unequivocally that crosslinking occurred at a rate of ~0.13 crosslinks/10^8 daltons per 1 µg/ml (1 h treatment) and the first half-excision progressed in the rapid first-order kinetics of 2 h half-life in exponentially growing normal, XP group-A and transformed WI38CT-1 cells. But, the first half-excision was completely defective during log-growth phase up to 24 h tested in three FA strains that exhibited a 30-fold supersensitivity to MC killing. One FA strain retained only a residual repair activity, which showed only a 5-fold more MC-killing. All the FA strains were, however, only twice more sensitive to psoralen-light killing, suggesting that interthymine crosslink may be repaired differently from interguanine crosslink. Furthermore, confluent, otherwise repair-proficient, normal human and XP group-A cells lost the ability of the first half-excision of MC crosslinks. Although its relation to a defective FA factor is not clear at present, these results strongly suggest that the enzyme or regulatory factor responsible for half-excision, which differs from those for constitutively active nucleotide excision repair in confluent cells, is lost at confluence, but induced only in cycling cells.

1041 RESPONSES OF HUMAN CELLS TO DNA MONOADDUCTS AND CROSSLINKS, Dieter C. Gruenert and James E. Cleaver, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143
The photoactive furocoumarin 8-methoxypsoralen (8MOP) will form DNA monoadducts and interstrand crosslinks following absorption of one or two photon of near-UV (NUV, 320-380 nm) light, respectively. Another furocoumarin analogue, angelicin (ANG), was previously thought to form only DNA monoadducts in human cells following NUV exposure. We have shown that ANG will form crosslinks in human cells under certain conditions. Comparison of binding and crosslink levels in human normal, xeroderma pigmentosum (XP) group A, and Fanconi's anemia (FA) fibroblasts indicated no apparent difference between these cell lines. However, repair measurements showed that normal and FA cells repaired monoadducts while XP fibroblasts did not, and that XP and FA cells were deficient in crosslink repair. Further analysis also indicated that DNA interstrand crosslinks have the capacity to inhibit the repair of monoadducts. Measurements of DNA synthesis inhibition suggested that monoadducts rather than interstrand crosslinks were responsible for blocks to replication fork displacement. Our results suggest that 1) although ANG will produce interstrand crosslinks in human cells, it may be used as a monofunctional control at low concentrations and NUV doses, 2) that the endonucleolytic step of crosslink repair may involve more than one enzyme, and 3) furocoumarin monoadducts may not be readily repaired when in close proximity to an interstrand crosslink.

1042 ACTIVATION OF POLY(ADP-RIBOSE) POLYMERASE MEDIATES THE SUICIDE RESPONSE TO MASSIVE DNA DAMAGE: STUDIES IN NORMAL AND FANCONI'S ANEMIA CELLS, N.A. Berger, J.L. Sims, D.M. Catino and S.J. Berger, Washington University School of Medicine and The Jewish Hospital of St. Louis, St. Louis, MO 63110

When cells are treated with sublethal doses of DNA damaging agents, they alter their metabolic and synthetic processes to repair the damage and restore cellular functions. When cells are treated with massive doses of DNA damaging agents, they undergo rapid reductions in glycolysis and macromolecular synthesis followed by cell death. This rapid cell death occurs before the genetic effects of DNA damage become manifest and before DNA repair can be completed. Such a suicide mechanism may be beneficial to multicellular organisms, since it might be preferrable for cells with severely damaged DNA to die quickly rather than risk repair with a high level of infidelity. Our studies indicate that the activation of poly(ADP-ribose) polymerase by DNA damage and the consequent depletion of cellular NAD^+ and ATP levels is the primary pathway mediating this suicide response to massive DNA damage. Our studies also show that cells from patients with Fanconi's Anemia have lower than normal NAD^+ levels. When cells from these patients are treated with DNA damaging agents, their NAD^+ levels are depleted to lower levels than those that occur in cells from normal donors. This impaired ability to maintain nucleotide pools and energy dependent functions may be etiologically related to the abnormalities that are characteristic of Fanconi's Anemia.

1043 STUDIES ON THE SUSCEPTIBILITY OF FANCONI'S ANEMIA CELLS TO CROSSLINKING AGENTS. Fré Arwert and Everdina H.A. Poll, Free University, Amsterdam, The Netherlands.

Clonogenic survival experiments with mitomycin-C (mitC) and with Trimethylpsoralen + UVA (TMP-UVA) show that Fanconi's anemia (FA) fibroblasts are highly sensitive to mitC, whereas the sensitivity TMP-UVA is only moderate. Fibroblasts from the parents of a patient with Fanconi's anemia exhibited the same sensitivity as normal controls. Studies on the cytogenetic toxicity of platinum compounds cisPtII, cisPtIV and transPtIV show that FA lymphocytes have a greatly increased sensitivity to the cisPt compounds. The cytogenic data suggest that the specific sensitivity of FA cells to cisPtII and cisPtIV is due to the fact that these compounds unlike transPtIV, introduce DNA-DNA interstrand crosslinks. Next, our experiments support the notion that FA cells are more sensitive to a Gua-Gua than to a Thy-Thy interstrand crosslink.

1044 INFLUENCE OF THE INTERVAL BETWEEN PLATING AND UV-IRRADIATION ON THE SURVIVAL OF HUMAN FIBROBLASTS, Patricia Henson, Zeda F. Rosenberg, Christopher Sarhanis and John B. Little, Harvard School of Public Health, Boston, MA 02115.

We have found that 18 hours after trypsinization and replating, cells enter S phase in a synchronous wave. Since sensitivity of human fibroblasts to UV depends on cell cycle position, we have measured the cytotoxicity of UV irradiation at 3 hour intervals for a total of 30 hours after trypsinization. Passage into S phase was simultaneously monitored by measuring uptake of ^{3}H-thymidine. We found that two strains of normal fibroblasts were initially very resistant to UV, but showed a rapid increase in sensitivity as the cells progressed to the G_1/S boundary. The cells were 5- to 8-fold more sensitive 18 hours after plating than they were initially. The cells regained some resistance as they continued to progress through the cycle. A similar variation in cytotoxic response was obtained with fibroblasts from patients with Gardner's syndrome, familial polyposis and the variant form of xeroderma pigmentosum. The effect was much less marked in a strain of Cockayne's syndrome fibroblasts, probably because these cells were less well synchronized by the trypsinization. The cells from all these genetic disorders show some degree of hypersensitivity to UV, yet all are capable of excision repair of pyrimidine dimers. Two separate strains of xeroderma pigmentosum complementation group A fibroblasts, however, performed no excision repair and showed no increase in sensitivity as they progressed towards the G_1/S boundary. The initial resistance probably reflects the amount of repair the cells can perform before entering S phase. The magnitude of the effect reported here is much larger than the differences in hypersensitivity reported between some cell strains. We suggest that such an effect might explain some of the discrepancies in the results reported from different laboratories.

1045 EFFECTS OF VARIOUS INHIBITORS AND OF NAD ON THE RECOVERY OF NUCLEIC ACID SYNTHESIS AFTER UV-IRRADIATION OF NORMAL AND COCKAYNE FIBROBLASTS, Lynne V. Mayne and Alan R. Lehmann, MRC Cell Mutation Unit, Brighton, England.

UV-irradiation of both normal and Cockayne fibroblasts causes a rapid reduction in the levels of RNA and DNA synthesis. Nucleic acid synthesis returns to the level in unirradiated cells in normal but not in Cockayne fibroblasts (4 jm^{-2}) (A.R.Lehmann, S. Kirk-Bell and L.Mayne, Can.Res. 39:4237-4241 (1979), L.V. Mayne and A.R. Lehmann, Can.Res. 42: 1473-1478 (1982). In contrast to a previous report (Y.Fujiwara, K.Goto and U.Kano, Exp.Cell Res. 139:207-215 (1982), the failure to recover normal rates of RNA/DNA synthesis in Cockayne fibroblasts was not relieved by the exogenous addition of NAD in our experiments. In normal fibroblasts, cytosine arabinoside in conjunction with hydroxyurea, and aphidicolin prevented the recovery of RNA synthesis both in stationary and growing cells. 3-Aminobenzamide, an inhibitor of ADP-ribosyl transferase did not affect the recovery of DNA synthesis in normal cells.

1046 REGULATION OF DNA REPAIR IN HUMAN CELLS, Pawan K. Gupta and Michael A. Sirover, Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140

Recent results from this laboratory demonstrated that normal human cells stimulate DNA repair pathways prior to the induction of DNA replication during cell proliferation. We suggested that this temporal enhancement of excision repair pathways may serve as a protective mechanism by prescreening DNA to eliminate critical DNA adducts before DNA replication. This capacity of normal human cells to regulate excision repair genes suggests the possibility that cells from individuals who are cancer prone might exhibit defects in regulatory mechanisms of DNA repair. We have thus quantitated the regulation of DNA repair in synchronously growing cells from cancer prone individuals as follows: (1) Unscheduled DNA synthesis or repair replication after exposure to either MMS or to UV irradiation at various intervals during cell proliferation; and (2) the induction of uracil DNA glycosylase using cell free extracts from undamaged cells in parallel cultures. We report that: (1) xeroderma pigmentosum cells (complementation groups A, C and D) failed to induce nucleotide excision repair after UV irradiation at any interval of the cell cycle; (2) Bloom's syndrome cells which have increased spontaneous mutation rate fail to enhance base excision repair, nucleotide excision repair and fail to induce uracil DNA glycosylase activity prior to the onset of DNA replication during cell proliferation; and (3) ataxia telangiectasia cells which are not hypersensitive to either UV light or to MMS are proficient in the regulation of both excision repair pathways prior to DNA replication. These results suggest that individuals with genetic disorders predisposing to malignancy might be characterized by alterations in regulatory mechanisms of DNA repair.

1047 CELL CYCLE PROGRESSION OF ATAXIA TELANGIECTASIA CELLS FOLLOWING TREATMENT WITH RADIATION AND BLEOMYCIN, Nancy L. Oleinick, Helen H. Evans, Libby R. Friedman, and Nancy M. Sokany, Case Western Reserve University, Cleveland, OH 44106

We have determined the effects of ionizing radiation, bleomycin, and UV radiation on mitosis and on DNA synthesis in Ataxia telangiectasia fibroblasts (AT5BI) as compared to normal human fibroblasts (GM 3440). The AT cells were less sensitive than the normal cells to ionizing radiation and to bleomycin with respect to delays in both S and G2, suggesting that the same mechanism is responsible for cell cycle delay in both phases, and that the mechanism is affected by the AT mutation. In contrast, the relative response of the two strains to UV radiation was dose-dependent and more complex. Thus, after treatment with 5 J/m^2, the mitotic index of the normal cells fell nearly to zero and then recovered after a 2.8 hour delay, measured at the 50% level. The mitotic index of the AT5BI cells decreased to a lesser extent than that of the normal cells, but the delay was longer, amounting to 6.8 hours at the 50% level. ^{3}H-Thymidine incorporation into DNA was affected to a lesser extent in AT cells than in normal cells following treatment with 5 J/m^2, but the two strains were affected similarly following irradiation with 10 J/m^2 or higher. The relative response to low and high doses of UV radiation was observed for labeling periods as long as 4 hr and as short as 15 min, conducted either immediately or up to 4 hrs after irradiation. Further investigation of the UV dose-response characteristics for S and G2 effects in these two strains is in progress and may provide important information concerning the relationship of cell cycle delay in S and G2 and the relationship of cell cycle delay to repair and lethality effected by DNA damaging agents. Supported by NIH Grant CA-15378 and DOE Contract DEAC0277EV04472.

1048 IONIZING RADIATION DAMAGE, DNA REPLICATION AND THE CELL CYCLE, Martin F. Lavin, Miriam Ford and Jane Houldsworth, Department of Biochemistry, University of Queensland, Brisbane, Australia

Ionizing radiation has been shown to inhibit DNA replication and interfere with the progression of mammalian cells through the cell cycle. Irradiation of cells in G1 or S phase generally leads to a delay in progression through these phases as well as an additional delay in G2 phase. The lesion causing G2 blockage has not yet been described but it has been assumed to be due to incomplete repair of chromosomal damage.

Recent results from this laboratory have demonstrated less inhibition of DNA replication in ataxia telangiectasia cells but a considerably greater degree of blockage of these cells in G2 after irradiation. AT cells showed not only a greater degree of blockage but also a slower rate of recovery from the block. The greater decrease in viable cell numbers for AT cells over this period after irradiation suggests that a large number of these cells die in G2. The greater degree of blockage in AT cells is in keeping with data for other radiosensitive cells. The continuation of normal levels of DNA synthesis and the subsequent marked delay in G2 phase after irradiation of AT cells may be due to a deficit in DNA repair or an abnormality in replication.

1049 ALTERED CELL SURFACE PROTEINS IN ATAXIA-TELANGIECTASIA, John P. Murnane and Robert B. Painter, Laboratory of Radiobiology, Univ. of Calif., San Francisco, CA 94143

Abnormalities in regulation of cell cycle and DNA synthesis following X irradiation has led to the proposal that ataxia-telangiectasia cells may have altered structural proteins influencing chromosome conformation (Painter and Young, P.N.A.S.,U.S.A. 77: 7315, 1980; Jaspers and Bootsma, P.N.A.S, U.S.A. 79: 2641, 1982). Protein content and production in both A-T skin fibroblasts and lymphoblastoid strains were therefore compared with their normal cell counterparts. Although most A-T proteins were nearly identical to those in normal cells, several major differences were noted in secreted and cell surface proteins both in the skin fibroblast and lymphoblastoid cells. In A-T fibroblasts this involves overproduction of fibronectin, procollagen and proteoglycans, all components of the extracellular matrix; while A-T lymphoblastoid cells secrete large amounts of three unidentified proteins of 80, 75, and 30 Kdaltons. A deficiency in a 130 Kdalton surface glycoprotein in some strains of A-T fibroblast and lymphoblastoid cells is currently under investigation. Abnormalities in the cell surface of A-T cells could explain several aspects of this disease, such as the reduced response of lymphocytes to mitogens (Cohen and Simpson, in Ataxia-Telangiectasia, pp. 203, John Wiley and Sons, New York, 1982) and the incomplete tissue differentiation suggested to be one of the primary aspects of A-T (Petersen et al., Am.J.Med. 41: 342, 1966; Waldemann and McIntire, Lancet 2: 1112, 1972). The possible influence of these changes on nuclear functions may be a further indication of the close interactions between the extracellular and nuclear architectures (Penman et al., Cold Spring Harbor Symp. Quant. Biol., 46: 1013, 1981).

1050 REPAIR OF O^6-METHYLGUANINE BY CELL AND TISSUE EXTRACTS FROM VARIOUS MAMMALIAN SPECIES Janet Hall, Henriette Brésil and Ruggero Montesano, International Agency for Research on Cancer, Lyon, France.

DNA alkylation damages have been shown to be repaired by specific DNA repair processes and the persistance in DNA of O^6-methylguanine (O^6-meG) correlates in many animal models with the carcinogenic potential of these compounds. Both bacterial and mammalian cells, and more recently, human liver (Pegg, et al., PNAS, 79, 5162, 1982) have been shown to be able to remove the methyl group at the O^6-position of guanine transferring it to a cysteine residue in the receptor protein forming S-methylcysteine, restoring the original guanine. We have measured the O^6-meG removing capacity in cell extracts from Ataxia Telangiectasia (AT) lymphoblastoid cell lines. The amount of enzyme present is measured by the ability of the extracts to remove 3-H labelled methyl groups from in vitro labelled DNA. All AT lines studied possess such an activity which is specific for O^6-meG and the amount of removal is proportional to the amount of protein added; but the activity varies in different cell lines. Experiments have also been carried out to measure the proportion of methyl groups transferred to cysteine residues in cell and tissue extracts originating from various mammalian species.

Work reported in this abstract was undertaken during the tenure of a Research Training Fellowship awarded to J.H. by the International Agency for Research on Cancer.

1051 COMPLIMENTATION AND ANALYSIS OF GENE DOSAGE EFFECTS IN ATAXIA TELANGIECTASIA BY CELL FUSION. Philip Chen, Paula Imray and Chev Kidson. Queensland Institute of Medical Research, Brisbane, Australia. 4006.

Complementation amoung 7 ataxia telangiectasia (AT) lymphoblastoid cell lines from unrelated individuals was assayed by the frequency of ionizing radiation induced chromosome aberrations in hybrids formed by cell fusion following treatment with polyethylene glycol. Chromosomes of one cell line were distinguished by incorporation of 5-bromodeoxyuridine (BUdR) for 40 hours before fusion. The mixture of unfused cells, homokaryons and heterokaryons was irradiated 2 hours after fusion and incubated for 28 hours before the cells were harvested. Chromosome preparations were made and differentially stained. Chromosome aberrations were scored in hybrids containing one set of BUdR-substituted chromosomes and one unlabeled set. Our series of AT mutants fell into 4 complementation groups.

When complementing pairs of AT lines were fused the frequency of chromosome aberrations induced by radiation was restored to the normal level. This result was surprising in view of the intermediate radiosensitivity of AT heterozygotes from these same families.

Fusions between normal, AT heterozygote and AT homozygote cells yield hybrids with varying ratios of normal and AT alleles. Hybrids formed by fusion of a normal cell with an AT homozygote cell were normally radioresistant. These data have important implications for identifying the nature of AT mutations.

1052 ABNORMAL CELL CYCLE REGULATION IN GAMMA-IRRADIATED ATAXIA TELANGIECTASIA CELLS AND ULTRAVIOLET DAMAGED XERODERMA PIGMENTOSUM VARIANT CELLS DETECTED BY DNA FLOW CYTOMETRIC ANALYSIS. Paula Imray and Chev Kidson. Queensland Institute of Medical Research, Brisbane, Australia, 4006.

Progression of normal and radiation sensitive lymphoblastoid cells from the point in the cell cycle at which they were irradiated through to a colcemid-induced metaphase block was followed by DNA flow cytometry. After 100 rad γ irradiation normal cells and Huntington's disease (HD) cells showed a proportion of cells blocked in G1. In contrast ataxia telangiectasia (AT) cells showed no disturbance in progression from G1 to S, or from S to G2 in the first cycle after irradiation. The cumulative mitotic index of irradiated cultures was reduced implying cells were delayed in G2. Normal and AT fibroblasts gave similar results to the lymphoblastoid cell lines. Thus AT cells did not recognise or respond to signals from γ-damaged DNA which in normal and HD cells caused a block in G1. The only point of arrest in cell cycle progression in irradiated AT cells was at G2.

Ultraviolet light (5 Jm^{-2}) did not affect the rate of movement of cells from G1 into S in normal, familial melanoma or xeroderma pigmentosum (XP) excision-deficient cells. The proportion of cells in S phase was increased and rate of entry of cells to the G2+M compartment reduced after UV in these cell types. Cells from an XP variant responded differently in that a large proportion of cells were blocked in G1 after UV irradiation. This suggests XP variant cells have a prereplicational repair defect.

1053 HYPERSENSITIVITY TO X RAYS IN LYMPHOCYTE LINES FROM PATIENTS WITH MUSCULAR DYSTROPHY, RETINITIS PIGMENTOSA, OR NEURODEGENERATION, F. Otsuka, R.E. Tarone, A.N. Moshell, R. Polinsky, J.D. Wirtschafter, R.A. Brumback, L.E. Nee, M.B. Ganges, S. Cayeux, and J.H. Robbins, NIH, Bethesda, MD, U. Minnesota, Minneapolis, MN, MDA Clinic and VA Med. Ctr., Fargo, ND

Photoreceptors, skeletal muscle, and neurons are highly differentiated, postmitotic excitable tissues which undergo premature death in retinitis pigmentosa, muscular dystrophy, and neurodegenerations, respectively. We have studied the post-X-ray survival of Epstein-Barr virus-transformed lymphocyte lines derived from patients with Usher syndrome (recessively inherited retinitis pigmentosa and congenital deafness), Duchenne muscular dystrophy, or the sporadic neurodegenerations of Parkinson and Alzheimer diseases. The cells, grown in RPMI medium 1640 supplemented with 20% fetal bovine serum, were irradiated with 100 rads from a 235-kilovolt X-ray machine. A line's post-X-ray viability ratio was calculated by dividing the number of viable (trypan blue-excluding) cells in an irradiated culture on the third postirradiation day by the number of viable cells in an unirradiated culture of the same line on that day. Two-sided P-values are reported. The 8 Usher syndrome, 7 Duchenne dystrophy, 6 Parkinson, and 3 Alzheimer disease kindreds had post-X-ray viability ratios (+SE) of 0.49 (+0.011), 0.49 (+0.009), 0.46 (+0.011), and 0.46 (+0.006), respectively, which were significantly less than the ratio of 0.54 (+0.011) of the 22 normal lines (P=0.011, 0.015, 0.002, and 0.016, respectively). Post-UV (254 nm) survival of these patients' lines was normal. The hypersensitivity to X rays might provide the basis for presymptomatic and prenatal tests for these diseases and for elucidating their pathogenesis. The death in vivo of excitable tissue in these diseases may result from unrepaired DNA damaged by hydrolysis and by endogenous radiomimetic chemicals.

1054 STATISTICAL ANALYSIS OF THE HYPERSENSITIVITY TO N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (MNNG) IN MUSCULAR DYSTROPHY (MD) OR NEURODEGENERATION, R.E. Tarone, D.A. Scudiero, R. A. Brumback, R. Polinsky, L.E. Nee, B.E. Clatterbuck, and J.H. Robbins, NIH, Bethesda, MD, NCI-Frederick Cancer Res. Facility, Frederick, MD, MDA Clinic and VA Med. Ctr., Fargo, ND

Huntington disease fibroblasts were hypersensitive to the lethal effects of MNNG in MNNG Series (S)-I-III (Scudiero, et al., PNAS 78:6451, 1981). We now report on S-IV-VI. As before, log-phase fibroblasts were incubated for 1 hr at 37° with MNNG (6-24 μM). The dose (D_{01}) of MNNG reducing each cell line's survival (measured as colony-forming ability at 3 weeks) to 1% was determined using methods which are applicable in the presence of inter-experiment variability and unequal experimental precision. Survival of a group of patient lines was compared to that of a group of normal lines using methods which adjust for genetic heterogeneity within the various groups. Two-sided P-values are reported. In S-IV the 5 normal lines had an average (av.) D_{01} of 16.8 (range: 14.3-19.9); in S-V and S-VI 8 new normal lines had avs. of 14.2 (range: 12.4-16.4) and 14.8 (range: 11.4-17.3), respectively. The 5 MD lines in S-IV (3 myotonic, 1 Becker, 1 Duchenne) had an av. D_{01} of 12.8 (P=0.02). The 4 MD lines of S-V (2 Duchenne, 1 Becker, 1 limb-girdle) had an av. D_{01} of 11.4 (P=0.01). Of the neurodegenerations, the 3 of S-IV (1 idiopathic orthostatic hypotension, 2 multiple system atrophy) had an av. D_{01} of 12.8 (P=0.03), the 3 S-V Parkinson disease lines had an av. D_{01} value of 12.2 (P=0.06), and the 4 S-VI Alzheimer disease lines (3 sporadic, 1 familial) had an av. D_{01} of 12.6 (P=0.04). The D_{01} value of each Parkinson and Alzheimer line was below that of at least 19 of the 21 normal lines we have studied. The hypersensitivity to MNNG in these diseases might result from defective repair of MNNG-induced DNA damage.

1055 NERVOUS SYSTEM DISEASE ASSOCIATED WITH DOMINANT CELLULAR RADIOSENSITIVITY. Chev Kidson, Philip Chen, Paula Imray and Effie Gipps. Queensland Institute of Medical Research, Brisbane, Australia. 4006.

A very marked association has been found between a number of relatively common degenerative diseases of the nervous system and cellular sensitivity to ionizing radiation, although this association was not absolute. Assays measuring clonogenicity and induced chromosome aberrations following γ-irradiation in air, gave the following data. (1) Huntington's disease (HD): of 13 patients and 17 first degree relatives, 70% and 36% respectively were sensitive. (2) Sporadic Alzheimer's disease (AD): of 16 patients, 60% were sensitive; one pedigree with familial AD exhibited a dominant sensitivity. (3) Familial non-specific dementia: in a large pedigree all 5 affected individuals were sensitive, 42% of 26 first-degree relatives were sensitive. (4) Amyotrophic Lateral Sclerosis-Parkinsonsian Dementia (ALS-PD) of Guam: of 21 patients 71% were sensitive, 31% of 32 first degree relatives were sensitive, 40 controls and 15 spouses were normal.

The true extent of mutations expressed as cellular radiosensitivity may be uncertain because of the limited resolution of available assays: only gene probes can identify them fully. We must assume that there are a range of mutations at different loci. In no case at this stage can extrinisic agents be causally excluded in the genesis of the pathology,i.e. radiosensitivity mutants may give predisposition. However, all the diseases studied involve selective loss of different, specific groups of neurons. Thus the mutations concerned may well be in genes involved in critical facets of nervous system development.

1056 ABNORMALLY LOW UV-INDUCED UNSCHEDULED DNA SYNTHESIS IN CELLS FROM A PATIENT WITH HYDROA VACCINIFORME, Alan D. Andrews, Charles L. G. Halasz, and Maureen B. Poh-Fitzpatrick, Columbia University, New York, NY 10032.

Hydroa vacciniforme (HV) is a rare disorder of unknown etiology which is clinically manifested by abnormal cutaneous sensitivity to sun exposure. Such exposure results in erythema, blistering and scarring, the scars usually being small and pitted, like those produced by infection with the vaccinia virus, hence the name of the disorder. Unlike the DNA repair-defective disorder xeroderma pigmentosum (XP), no significant pigmentary changes, xerosis, atrophy, or skin tumors have been reported in association with HV. Nonetheless, our studies have shown that cells from a 26 year old patient with HV are deficient in the rate of excision repair of ultraviolet (UV) light-induced DNA damage, as measured in an assay of unscheduled DNA synthesis (UDS). Log-phase cultured skin fibroblasts were irradiated with 30 J/M^2 of 254nm UV light from a GE G15T3 germicidal lamp. The cells were then incubated with ^{3}H-thymidine for three hours, fixed, and overlayed with NTB3 autoradiographic emulsion. After seven days at 4°C the autoradiograms were developed and stained, and grains over the non-S-phase nuclei were counted. The UDS rate was calculated by averaging the counts from 50 random nuclei. In three separate experiments, utilizing four different normal cell strains for comparison, the HV cell strain was found to have 51 to 59% of the normal rate of UDS. As the patient with HV had no skin tumors or other clinical signs of XP, these results suggest that a reduced rate of UV-induced UDS can be associated with clinical phenotypes markedly different from that of XP, and that a reduced rate of UV-induced UDS alone does not necessarily indicate that an individual is predisposed to UV-induced neoplasia.

1057 CELLS FROM PATIENTS WITH HEREDITARY MALIGNANT MELANOMA OF THE SKIN ARE ABNORMALLY SENSITIVE TO THE CYTOTOXIC AND MUTAGENIC EFFECT OF 4-NITRO-QUINOLINE-1-OXIDE, J. Justin McCormick, Jeffery N. Howell, Rebecca C. Corner and Veronica M. Maher, Michigan State University, East Lansing, MI 48824 and Mark H. Greene, Environmental Epidemiology Branch, National Cancer Institute, Bethesda, MD 20205

There is a hereditary form of malignant melanoma of the skin in which members of the affected families dominantly inherit a predisposition to develop dysplastic nevi at very high frequency and among those who develop such moles, the chance of developing malignant melanoma is very great. Smith et al. reported that skin fibroblasts from such melanoma patients are abnormally sensitive to the cytotoxic effect of UV (254 nm) and to 4-nitroquinoline-1-oxide (4NQO).(Proc. Am.As.Cancer Res.,1981) Because of the possible role of mutations in the etiology of cancer, we investigated their sensitivity to the mutagenic effect of these agents. We compared the frequency of TG resistant cells induced by 4NQO in fibroblasts from normal persons and from two non-related patients with this syndrome, designated 3012T and 3072T. Both of the patient-derived cell strains proved significantly more sensitive than normal to the cytotoxic and mutagenic effect of 4NQO. The slopes of their mutation frequency curves as a function of concentration were 5-fold steeper than normal; that of their corresponding survival curves were 3- and 5-fold steeper, respectively. For comparison, we measured the 4NQO sensitivity of XP12BE cells (complementation group A, with almost no ability to excise UV induced DNA damage). The slope of the 4NQO survival curve of XP12BE cells was also 5-fold steeper than normal. Whether the observed sensitivity of 3012T and 3072T cells reflects a deficiency in DNA excision repair isunder investigation,as is their response to the mutagenic action of UV radiation. Supported by NCI-CP-FS-11029-63 and CA 21253.

Inducible Responses/Mismatch Correction/Fidelity of Replication

1058 SOS INDUCTION OF LONG PATCH REPAIR AND RESISTANCE TO ALKYLATION DAMAGE, Mark Henteleff and Priscilla Cooper, Lawrence Berkeley Lab., Univ. of California, Berkeley, Ca. 94720

At least two distinct categories of inducible responses to DNA damage have been indetified in E. coli: SOS processes that occur in response to a wide variety of DNA damaging agents and are controlled by the rec-lex regulatory circuit, and adaptive responses that occur after damage by alkylating agents and are controlled by the ada gene. Both sets of responses result in increased resistance to killing, but the SOS responses include induced mutagenesis, whereas the adaptive response decreases mutagenesis. Long patch repair of UV damage is a uvr^+-dependent inducible rec-lex process (Cooper, MGG 185:189, 1982). Pre-induction of the long patch pathway via a tif mutation (recA441) correlates with both a large, uvr^+-dependent increase in UV resistance (Cooper, Chrom. Damage & Repair, p. 139, 1982; Castelazzi et al, J. Bact. 143:703, 1980) and a similarly uvr^+-dependent increase in ability to sustain DNA replication after irradiation (Cooper, ms. in prep.) We have characterized repair synthesis following treatment of E. coli cells with MNNG and find that, as in UV repair, there is a long patch component that requires new protein synthesis and is the result of an induced process. However, the initial step of this repair involves 3-meA DNA glycosylases I and II, the products of the tag and alk genes, rather than the uvr^+ complex. We have found that tif induction of SOS responses prior to treatment with MNNG produces a survival enhancement comparable to or greater than that resulting from adaptation and that this increased resistance is independent of uvr^+, as is the excision repair. The tif induction did not result in induction of either the inducible 3-meA glycosylase II or O^6-meG transferase. Thus, induced long patch repair occurs after MNNG treatment as after UV, and in both cases correlates with increased cell survival.

1059 DNA Damage Induces an E.coli Gene Required for Site-Specific Recombination, Harvey I. Miller, Genentech, South San Francisco, CA 94080.

The himA and himD genes of E.coli code for a protein, IHF, which, in concert with the phage lambda Int protein, catalyze lambda site-specific recombination. IHF is also involved in diverse cellular functions such as transposon precise excision and flagellar antigen regulation.

The himA gene exhibits a novel form of regulation. It is negatively regulated by both its own gene product and by the LexA protein. It is therefore one of the DNA damage inducible(din) genes. However, the himD gene is not UV inducible.

Studies on a cloned himA gene suggest how this dual regulation of the himA gene is accomplished.

1060 LETHAL EFFECTS OF ISOPSORALEN ON A umuC MUTANT OF ESCHERICHIA COLI, Stephan Miller, and Eric Eisenstadt, Dept. of Microbiology, Harvard School of Public Health, Boston, MA 02115.

Mutagenesis in Escherichia coli caused by ultra-violet (UV) radiation and a number of other SOS-dependent mutagens is thought to occur as a result of errors made during the repair of DNA after damage caused by these mutagens. This implies that formation of a mutation requires active processing of the original DNA lesions. umuC mutants isolated by Kato and Shinoura (1977) are nonmutable by both UV and 4-nitroquinoline-1-oxide. In addition, these umuC mutants were slightly more sensitive to the lethal effects of UV radiation.

We report here that the survival advantage conferred by the $UmuC^+$ phenotype in $uvrB^-$ cells is mutagen dependent. We measured the survival of E. coli following treatment with UV light and isopsoralen plus near UV light (IP). The survival of the $UmuC^+$ cells treated with 10μg/ml of IP and 3000ergs/mm^2 near UV was 13%, whereas the survival of the $UmuC^-$ cells was only 0.2%. In the case of UV, a dose of 27ergs/mm^2 gave a survival of 10% for $umuC^+$ and 3% for $UmuC^-$. The best IP dependent killing is obtained after a 1 hr. starvation period. Cell survival is increased if outgrowth after treatment is on minimal rather rich medium. The umuC dependent survival advantage, however, is greater on rich medium.

The great sensitivity of umuC mutants to IP has led us to begin a study of it's mutational specificity. IP killing of E. coli GM1 uvrB to an S/S_o of 5% gives a rifampicin mutation rate of 4×4^{-4}. About 6% of the lacI mutations are suppressible. These suppressible mutations are currently being analyzed to determine the specific base changes involved.

1061 MUTAGENESIS OF BACTERIOPHAGE T7, L.A. Dodson*, R.S. Foote†, S. Mitra†, and W.E. Masker†, *Univ. Tenn. Oak Ridge Grad. School Biomed. Sci. and †Biology Division, Oak Ridge Natl. Lab., Oak Ridge, TN 37830

We are examining chemical and radiation induced mutagenesis in bacteriophage T7 both in vivo and in vitro. Infective T7 phage particles can be synthesized in vitro by replicating T7 DNA and then encapsulating the product DNA into procapsids. Normally this system shows good fidelity, and phage are produced in vitro with about the same level of spontaneous mutagenesis typical of in vivo T7 phage bursts. However, certain conditions such as incorporation of abnormal precursors during in vitro DNA synthesis or DNA damage introduced by agents such as EMS or MMS cause mutations in phage produced by in vitro packaging. For example, we have observed in vitro mutagenesis by including O^6-methyldeoxyguanosine triphosphate in the DNA replication reaction and in this way directly demonstrated the premutagenic role of O^6-methylguanine. This system provides a sensitive biochemical tool for examining other mutagenic mechanisms in T7. UV irradiated or MMS treated T7 show enhanced survival upon infection of Escherichia coli hosts in which the SOS response has been induced. Enhanced survival is not observed for EMS treated T7. We have undertaken a study of the reactivation phenomenon in vivo and in vitro using E. coli mutants with altered DNA repair capabilities and have found that it persists in a umuC mutant. Mutagenesis of T7 irradiated with UV or treated with MMS or EMS does not require a functional umuC gene. Studies with other E. coli mutants as well as experiments with our in vitro system are being performed in the hope of identifying the mechanism(s) of reactivation and mutagenesis of T7. (†Operated by U.C.C. under contract W-7405-eng-26 with the U.S. D.O.E.)

1062 NEGATIVE COMPLEMENTATION OF THE E. coli $recA^+$ GENE AND ITS USES, Steven G. Sedgwick and Geoffrey T. Yarranton, Genetics Division, National Institute for Medical Research Mill Hill, London NW7 1AA, Great Britain.

Negative complementation of E. coli recombination occurred after derepression of plasmid sequences encoding the recA control region and between 22 and 79% of the contiguous structural gene. Plasmids having less than 22% of the structural gene had no effect, nor did plasmids expressing the final 27% of the gene from the lac promotor. We propose that negative complementation arises from oligomerization of full-size and truncated recA protein monomers through interactions in the amino terminal domains of the molecules. The presence of more than one physical domain in recA protein is suggested by the biphasic loss of circular dichrosim during denaturation. Although negative complementation impaired recombination there was no equivalent reduction in in vivo processes relying on recA protease action. Using this selective inhibition of recombinational activity we found that inducible post-replication repair of E. coli, and induced reactivation of λ, could be inhibited without reducing the frequencies of induced mutagenesis. It is concluded that the inducible repair systems contributing most to the survival of E. coli, and λ are largely recombinational and error-free and that mutagenesis arises from a separate process contributing little to the overall survival of a DNA damaged population.

1063 CONTROL OF THE SOS RESPONSE IN E. COLI BY THE LEVEL OF RECA PROTEASE.
John W. Little, Department of Biochemistry, University of Arizona, Tucson, Arizona 85721

Genetic and biochemical evidence has shown that the SOS response is regulated by the interaction of two proteins: the LexA repressor, which normally represses a set of unlinked genes (the SOS regulon); and the RecA protease, which is activated after DNA-damaging treatments, leading to cleavage of the LexA repressor and derepression of the SOS regulon. In order to characterize the regulatory circuit further, I have examined the stability of LexA protein in vivo, using antibody techniques. These experiments show that the stability of repressor varies markedly during the various phases of the regulatory cycle, and indicate that the level of RecA protease activity controls the state of the system and the transitions between its two states. The system can also exist in a "suoinduced" state at low levels of DNA damage. Data will also be presented which indicate that the central portion of the LexA repressor does not play a critical role in repressor function, but rather serves as a site of action for the RecA protease, thereby making the protein responsive to the regulatory system.

1064 SEPARATE THYMINE PRECURSOR FEEDS TO LEADING AND LAGGING DNA STRANDS: A MODEL FOR RECA INDUCTION.
Robert J. Melamede and Susan S. Wallace
New York Medical College, Valhalla, N.Y. 10595.

Thymidine is the preferred exogenously supplied thymine-containing DNA precursor in T4-infected plasmolysed cells. Thymine (1 μM) incorporation is not inhibited by a 200 fold excess of thymine, dTMP or dTTP. After short pulses the ^{3}HTdR is found exclusively in Okazaki pieces. In order to account for these data as well as for the complex patterns of TdR incorporation observed after infection by T4 recombination defective mutants we propose that there are separate thymine containing DNA precursor feeds for leading and lagging strand DNA synthesis, that is, thymidine is preferentially incorporated into lagging strands. To test this hypothesis, TdR and dTMP incorporation were compared in the plasmolysed cell system. In double label experiments ^{3}HTdR first appears in Okazaki pieces which then chase into higher molecular weight species while ^{32}PTMP is found in very small pieces, the bulk of which do not chase into larger species. Similar results were observed in uninfected plasmolysed E. coli. Thus, the two DNA precursors are differentially incorporated into DNA. The physiological function of these very small pieces remains unknown but similar small species of DNA have been reported by others using different methodologies. The model to be presented predicts that these small pieces are leading strand specific and in addition the model provides a detailed mechanism for recA induction. Supported by NCI CA33657 U.S. DHHS.

1065 REGULATION OF COLICIN E1 GENE EXPRESSION BY LEXA PROTEIN AND CYCLIC AMP
Atsushi Nakazawa, Yousuke Ebina, Mamoru Yamada and Yoshiyuki Takahara
Yamaguchi University School of Medicine, Ube, Japan 755

Colicin E1 induction is regarded as one of the *recA-lexA* dependent SOS responses of *E. coli*. We have determined the nucleotide sequence of the colicin E1 gene and its neighboring regions and assigned the initiation and termination sites of the transcription *in vivo* and *in vitro*. The promoter region of the colicin E1 gene has DNA homology to the operator regions that are commonly repressed by LexA protein such as *recA* and *lexA* operators. Using the *recA spr* double mutant, we demonstrated that LexA protein directly represses the gene expression. Cloning experiments of the structural gene for colicin E1 and of its promoter region indicated that no other part of ColE1 plasmid codes for the repressor of the colicin E1 gene expression.

The transcription *in vivo* of the colicin E1 gene is dependent on cyclic AMP in the *cya* mutant cells. However, upon deletion of the *Pvu*II fragments which are located in the mobility region of ColE1 plasmid, the induced synthesis occurred even without cyclic AMP. This region is thought to be related to the relaxation event of the plasmid (Dougan *et al. MGG, 158, 325 -327 (1978)*).

Thus the colicin E1 expression is negatively controlled by LexA protein and positively by cyclic AMP.

1066 INDUCTION OF lexA3 LYSOGENS OF BACTERIOPHAGE LAMBDA AFTER TREATMENT WITH N-NITROSO COMPOUNDS, R. Elespuru, S.Daley and S. Gandhi, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

Mutations in recA and lexA genes of E.coli are generally considered to eliminate the expression of SOS functions. We have observed, however, that induction of bacteriophage lambda not only occurs, but is enhanced in lexA3 strains compared to wild-type, after treatment with MNNG, MNU and related compounds. Chemicals were tested in a set of lambda lysogens originally designed for screening of carcinogens (Elespuru and Yarmolinsky, Environmental Mutagenesis 1: 65-78, 1979). Induction is monitored by the production of β-galactosidase 3 to 5 hr after treatment of a strain lysogenic for a lambda-lacZ fusion phage. Induced levels of enzyme were 2 to 5-fold greater in the lexA3 isogenote (provided by M.Yarmolinsky) after treatment with nitroso compounds (uninduced control levels were the same in lexA and lex$^+$ strains). Induction peaks did not shift to lower doses in the lexA strain. Induction of the lex mutant was dependent on the presence of ampicillin (10 μg/ml), originally added to reduce the uninduced control level. The lexA strain is uv-sensitive, as expected, and only slightly inducible by uv, 4NQO, bleomycin and cisplatin, in the presence or absence of ampicillin. Induction of λcI$_{857}$ lysogens (lexA or lex$^+$) by temperature shift indicated that the rate of enzyme synthesis is similar in the two strains in the presence of MNNG. We conclude that the function of genes repressed by lexA is not necessary for derepression of prophage after alkylation damage of DNA.

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1067 SOS INDUCTION BY MINI-P1KM PLASMIDS, Mike A. Capage and June R. Scott, Emory University, Atlanta, GA 30322

The replication of certain mini-P1Km plasmids constructed in vitro induces the expression of the SOS pathway in E. coli K12. Induction of SOS functions by these plasmids is indicated by three independent criteria: (i) el4, a cryptic genetic element which previously has been shown to excise from the E. coli chromosome when the SOS pathway is induced, excises in the presence of these mini-P1Km plasmids, (ii) the excision of el4 is not induced by the replication of these plasmids when the cells are RecA$^-$, and (iii) the level of spontaneous induction of λ$^+$ prophage is increased by a factor of 100 in lysogens carrying these plasmids. The induction of the SOS pathway is contingent upon the replication of these mini-plasmids from their P1 origin and is apparently caused by an aberrant initiation of DNA replication. When the region of the P1 genome delimited by EcoRI-6 is present in the cell, either as part of the mini-P1Km plasmid (i.e., in cis) or integrated into the E. coli chromosome (i.e., in trans), SOS induction does not occur. Thus, it appears that P1EcoRI-6 carries a gene coding for a product that inhibits SOS induction. This product may be necessary for normal initiation of DNA replication from the P1 origin or may be acting in a more general way to inhibit SOS induction.

1068 UV INDUCIBLE UV PROTECTION AND MUTATION GENES ON THE I GROUP PLASMID TP110. Peter Strike and Janice A. Glazebrook,Department of Genetics, University of Liverpool, Liverpool L69 3BX,England.

TP110 is typical of the IncI1 group plasmids in protecting E.coli cells from the lethal effects of UV light (1). Associated with this protection is an increase in both the spontaneous and induced levels of mutation. Introduction of this plasmid into umuC mutant hosts, which are normally non-mutable by UV (2), restores mutability to high levels. Introduction of the plasmid into recA or lexA mutant cells shows the protection and mutation effects to require the products of these two genes. Similar effects have previously been reported for the quite unrelated N group plasmid pKM101 (3). Using the Mu d(lac amp) fusion probe , we have isolated fusions of the lacZ gene to the promoter for the I group mutation and protection functions (imp). Expression of β-galactosidase in these fusion strains is inducible by DNA damaging agents, in amanner which is typical of other SOS genes. Experiments with spr and spr recA strains indicate that the lexA genes product is probably a direct repressor of the imp promoter. The imp genes have been cloned from TP110 into the low copy number vector pHSG415. Several Tn1000 inserts which inactivate the imp functions have been isolated, permitting precise location of the operon. The properties of strains carrying the cloned imp genes will be described, and a comparison will be made with genes cloned from other plasmids which have similar effects.

References. (1) Howarth,S.(1965) J.Gen.Micro.40,43-55 (2) Kato,T and Shinoura,Y (1977) Mol.Gen.Genet.156,121-131. (3)Walker,G.C. and Dobson,P.P.(1979)Mol.Gen.Genet.172,17-24

1069 LACK OF SINGLE-STRAND DNA-BINDING PROTEIN AMPLIFICATION UNDER CONDITIONS OF SOS INDUCTION IN *E. COLI*. Giuseppe Villani, Claude Paoletti and Bernard Salles. Laboratoire de Pharmacologie et de Toxicologie Fondamentales 205 route de Narbonne 31400 TOULOUSE FRANCE.

A two site immunoradiometric assay (IRMA) for quantification of the recA protein has been recently described (Paoletti et al. Biochimie 64, 239-246 (1982). We have used a similar technique to monitor the possible amplification of the ssb protein in *E. coli* after induction of the SOS repair process by various DNA damaging agents. Under these conditions, while we have been able to detect a full amplification of recA protein, we failed to observed any amplification of the ssb protein.

1070 THE GENETIC CONTROL OF DNA-DAMAGE-INDUCED RESTRICTION ALLEVIATION IN ESCHERICHIA COLI K12. Wilfried Wackernagel, Universität Oldenburg, 2900 Oldenburg and Brigitte Thoms, Ruhr-Universität, 4630 Bochum, W.-Germany.

The induction of a partial alleviation of the K12-specific DNA restriction was examined after treatment of cells with UV or nalidixic acid (Nal). Both induce the restriction alleviation (RA) function, which was monitored after expression at 30 °C by observing increased survival of unmodified phage λ. Induction was examined in strains with various mutant alleles of recA and lexA and other genes, which are presumably involved in the control of SOS functions. Essentially no induction by UV occurs in lexA3, recA1, 56, 430, 142 and recF143, but normal induction was observed in lexA spr (Def) and lexA tsl (Ts). Nal parallels the effect of UV, except that in recF143 Nal is an efficient inducer. Active recBC enzyme is generally required for efficient induction by UV and Nal. The expression of the RA function is temperature-sensitive in normal laboratory K12 strains, which hampers studies with ts allels of genes regulating SOS functions. Our results are interpreted to mean that the RA function is not under a direct control of the lexA protein (which makes RA different from most SOS functions) and that increased amounts of recA protein (activated by the recBC function) are required for induction.

1071 RECENT RESULTS OBTAINED WITH THE SOS CHROMOTEST, Maurice HOFNUNG and Philippe QUILLARDET, Unité de Programmation Moléculaire et Toxicologie Génétique, INSTITUT PASTEUR, Paris, France.

We have made use of a bacterial strain carrying a fusion of gene lacZ to gene sfiA to devise a new assay for genotoxic agents : the SOS Chromotest (P. Quillardet, O. Huisman, R.d'Ari, and M. Hofnung, Proc. Natl. Acad. Sci. USA, 79, 5971-5975, 1982). The response is rapid (a few hours) and quantitative. This response allows to classify compounds according to their SOS inducing potency, (SOSIP) defined as their ability to induce the expression of the sfiA-lacZ fusion. For most of the compounds tested the SOSIP is correlated with the mutagenic potency determined in the Salmonella/microsome assay. The sensitivity (lowest amount detected for each compound) is also comparable to that of the Salmonella/microsome assay.

Various aspects of the response such as relation to the initial number of lesions and dependance on the uvrABC system will be presented.

1072 PHENYLALANINE MUTAGENESIS IN Escherichia coli IS REGULATED BY THE uvrA, uvrB, lexA, and umuC GENES, Neil J. Sargentini and Kendric C. Smith, Stanford University School of Medicine, Stanford, CA 94305

Much of "spontaneous" mutagenesis in E. coli is controlled by the uvrA, uvrB, recA, lexA, and umuC genes, and it has been suggested that mutagenic lesions may be produced in DNA as a by-product of normal metabolism (Sargentini and Smith, 1981, Carcinogenesis 2:863-872). In order to identify normal cellular metabolites that might play a role in such mutagenesis, we have tested 20 common amino acids for mutagenicity (at 2 mM) using a lacZ53(Am)→Lac^+ reversion assay in E. coli K-12 uvrB5. After correcting for the effects of the amino acids on growth yield, only phenylalanine was significantly mutagenic. Repurified phenylalanine or phenylalanine from several sources were similarly mutagenic. Using the uvrB5 strain or a uvrA6 strain from another strain background, phenylalanine mutagenesis was also detected by assays using resistance to rifampicin, nalidixic acid, or bacteriophage T6, but not by assays involving trpE65(Oc)→Trp^+ or his-4(Oc)→His^+ reversion. A dose response for phenylalanine mutagenesis was demonstrable over the range of 0.5-2 mM in the uvrB strain but not in $uvrB^+$, uvrB umuC or uvrB lexA strains. This result indicates that putative phenylalanine-induced lesions are mutagenic via the error prone DNA repair process and that their mutagenic potential is normally reduced by a DNA excision repair process. Consistent with the concept that phenylalanine produces noncoding DNA damage, we were able to detect a small accumulation of DNA daughter-strand gaps in uvrB recA cells previously grown in the presence of phenylalanine. (Research supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute.)

1073 THE GENETICS AND SPECIFICITY OF CONSTITUTIVE AND INDUCIBLE REPAIR SYSTEMS OF Bacillus subtilis. Ronald E. Yasbin, P. I. Fields, B. M. Friedman and R. Miehl-Lester, University of Rochester, Rochester, NY 14642

An isogenic set of prophage cured DNA repair proficient and deficient strains of B. subtilis were constructed and analyzed for their sensitivities to selected mutagens. The results demosntrated that the lethal damage caused by UV and 4NQO was repaired via the bacterial excision and/or recombination repair systems. In contrast, the lethal damages caused by EMS and MMS were removed from the DNA by the recombination repair system of the bacteria, and not by the excision repair system. However, the mutagenic damage caused by EMS was processed by the excision repair system.

The bacteria required both a functional recombination repair system and a functional excision repair system in order to remove the DNA damage caused by the bifunctional alkaylating agent MITC.

Significantly, the W-reactivation system of B. subtilis can repair only pyrimidine dimers in bacteriophage DNA. This inducible repair system can be activated by treatment of the bacteria with UV, alkylating agents, cross-linking agents and gamma irradiation. However, bacteriophge treated with agents other than those that cause pyrimidine dimers to be produced were not repaired by this unique form of W-reactivation. In contrast, the W-reactivation system of Escherichia coli can repair a variety of damages placed in the bacteriophage DNA.

1074 INDUCIBLE COMPONENTS OF ERROR-PRONE REPAIR IN YEAST, Friederike Eckardt and Wolfram Siede, GSF, Radiation Biology, D-8042 Neuherberg, FRG. Mathematical analysis of biphasic mutation frequency curves as well as the calculation of the survival probability of induced mutants from mutant yield data ("apparent survival") indicate that UV irradiation induces (a) certain component(s) of error-prone repair. For experimental proof we used a thermo-conditional repair mutant, rad5-7 (=rev2) which shows enhanced mutagen sensitivity and depressed mutation frequencies of certain ochre alleles at 36°C as compared to 23°C. The biological activity of the RAD5 gene product can be followed by incubating mutagen treated cells in growth medium at the permissive temperature (RAD5 gene product active, hence increase in survival and mutation frequencies) before applying the restrictive temperature (RAD5 product inactive). Treatment with 5-100 µg/ml cycloheximide used to block protein synthesis at the permissive temperature, gives evidence that either the RAD5 product or precursor(s) in its pathway is newly synthesized after UV irradiation: survival in stationary cells is fully dependent on protein synthesis induced after UV, whereas a constitutive level in growing cells allows a slow increase in survival under conditions of protein synthesis blockage. The role of the inducible compound(s) in mutation induction is locus specific in stationary cells: RAD5 dependent reversion at the his5-2 allele is dependent on protein synthesis whereas reversion of the ade2-1 allele is not. The time courses for the increase in survival and mutation induction are different.

1075 STUDIES ON THE REGULATION OF EXPRESSION OF EXCISION REPAIR GENES OF Saccharomyces cerevisiae, Louie Naumovski and Errol C. Friedberg, Department of Pathology, Stanford University, Stanford, CA 94305.

The RAD3 gene of S. cerevisiae is required for the incision of DNA during the excision repair of pyrimidine dimers and of other forms of base damage. We have isolated a fragment of yeast DNA on a recombinant DNA plasmid (pNF3000) that complements the UV sensitivity of rad3 mutants in vivo. Genetic evidence indicates that this fragment contains the RAD3 structural gene. We are interested in studying the regulation of this and other cloned RAD genes, with particular emphasis on their possible inducibility by DNA damage. To this end we have constructed a hybrid gene by inserting a BamHI → BglII fragment of the E. coli β galactosidase gene that lacks its normal promoter and a portion of its amino terminal end ('Z), into a BamHI site known to be present within the cloned RAD3 gene. When present in a multicopy vector, this fusion expresses β galactosidase both in E. coli and in yeast. The level of β galactosidase in yeast increases as a function of cell growth, but is not enhanced by exposure of cells to UV radiation. In order to determine whether regulation of the hybrid gene is affected by its presence in a multicopy plasmid, the fusion will be integrated into the yeast genome as a single copy, and regulation will be examined by measuring β galactoside activity after UV-irradiation. These results will be compared to the quantitation of RAD3 mRNA in wild-type yeast cells similarly irradiated.

1076 ENHANCED REACTIVATION OF SV40 DNA REPLICATION IN CARCINOGEN TREATED MAMMALIAN CELLS, Janet A. Maga, and Kathleen Dixon, University of California, Los Angeles, CA 90024.

Pretreatment of mammalian cells with low UV fluences or certain chemical carcinogens such as N-acetoxy-2-acetylaminofluorene (AAAF) prior to infection with UV-irradiated virus induces a recovery pathway that results in enhanced survival or reactivation of UV-damaged virus. We have begun an investigation into the molecular bases of enhanced reactivation by examining SV40 DNA replication during the first round of infection. CV-1 cells were treated with AAAF 24 hours prior to infection with SV40 virus that had received UV fluences of 0-2600 J/m^2. The rate of SV40 DNA replication was then measured by a 1 hour ^{3}H-thymidine pulse. Enhanced survival of SV40 virus appears to result from an increased capacity of AAAF-treated cells to replicate SV40 DNA. AAAF treatment causes a two-fold decrease in the slope of the inactivation curve of SV40 DNA synthesis. This change in slope is not due to multiplicity reactivation or to differences in specific activity of viral DNA in AAAF-treated cells. Examination of the kinetics of SV40 DNA replication indicates that AAAF does not appear to affect the onset of detectable DNA synthesis. The increased capacity of AAAF-treated cells to replicate UV-damaged virus may result from an increased tolerance of UV lesions by the host replication machinery or induction of increased repair capacity. Experiments are now in progress to distinguish these two alternatives.

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1077 SPECIFICITY AND MODULATION OF ENHANCED REACTIVATION OF PARTIALLY INACTIVATED HERPES SIMPLEX VIRUS, Bubley, G., Crumpacker, C., and Schnipper, L.E., Beth Israel Hospital and Harvard Medical School, Boston, MA

Enhanced reactivation (ER) of UV-irradiated Herpes Simplex Virus (HSV) is induced in Vero cells by pretreatment with cytosine arabinoside, and the reactivation factor (RF) increases from 4 to 8, when cells are exposed to 0.125 and 0.50 ug/ml, respectively, prior to infection. In comparison, pretreatment with cis-platinum at concentrations sufficient to inhibit DNA synthesis was inefficient at induction of enhanced reactivation.

To assess the ability of caffeine to modulate Ara-C induced ER, cells were either exposed to both compounds simultaneously, or caffeine was added during the induction period. When caffeine was added with Ara-C, the ER of UV-HSV-1 is greater than with Ara-C alone. Pretreatment of cells with Ara-C, followed by the addition of caffeine (2, 6.2, 12.5 mM), resulted in reduction of the RF from 2.4 to 0.6, 0.85, and 0.6, respectively, and suggests that caffeine interferes with induction of ER by Ara-C.

Ara-C is incorporated into HSV-1 DNA in internucleotide linkage, following growth of virus in this analogue. To determine whether HSV, partially inactivated by growth in 5 x 10^{-7} and 5 x 10^{-6}M Ara-C can serve as a substrate for ER, cells were pretreated with Ara-C (.125 or .25 ug/ml) or UV-irradiation (12J/M^2), and infected after a 24 hour induction period. No enhanced reactivation (RF < 1.4) was observed with virus grown in Ara-C, whereas these same conditions of cell treatment yielded a RF of 5 for UV-HSV-1. These observations suggest that HSV-1 inactivated by passage in Ara-C is a poor substrate for the repair activity induced by either UV-irradiation or cytosine arabinoside.

1078 INDUCTION OF SOS-TYPE PROCESSES BY UV TREATMENT OF CULTURED HEPATOCYTES. James D. Yager, Jr., Joanne Zurlo, John E. Mignano and Arthur L. Penn, Department of Environmental Medicine, New York University Medical Center, New York, NY 10016

Carcinogen treatment of bacterial and mammalian cells causes an inhibition of DNA synthesis, which in bacteria, leads to the induction of SOS functions. One manifestation of SOS functions is the increased survival and mutagenesis of UV-irradiated phage upon infection of host bacteria pre-treated with UV or chemical carcinogens. Similarly, carcinogen treatment of cultured mammalian cells prior to infection with UV-irradiated virus results in enhanced virus survival and mutagenesis suggesting the induction of SOS-type processes. We have obtained data demonstrating similar responses occur in hepatocytes in primary culture following treatment with UV. Rat hepatocytes are inoculated into culture under conditions which stimulate DNA synthesis. Increased DNA synthesis is detectable 28 hr later at which point the cells are irradiated with UV. At various times thereafter, the cells are infected with control or UV-irradiated Herpes virus at low multiplicity (<0.1 pfu/cell). Virus survival is measured by direct plaque assay. UV-irradiated virus exhibits the expected 2-component survival curve in control and UV-pretreated hepatocytes. UV-enhanced viral reactivation in hepatocytes exhibits a dose/time dependent response. Additional studies employing SDS-PAGE demonstrate the appearance or increase in several nuclear proteins at the time of enhanced viral reactivation. pH step gradient alkaline elution of hepatocytes pulse labeled with ^{3}HdThd demonstrated that UV irradiation causes an accumulation of blocked replicons with recovery occurring by 24 hr. Taken together, these results suggest that carcinogen treatment of rat hepatocytes causes the induction of SOS-type functions that may have a role in the initiation of hepatocarcinogenesis.

1079 REPLICATION OF UV-IRRADIATED PARVOVIRAL SINGLE-STRANDED DNA IN MOUSE CELLS,Jean-Michel Vos and Jean Rommelaere, Dept. Molecular Biology,Université libre de Bruxelles,Belgium.

An immunological procedure was developed to measure the content of pyrimidine dimers induced by UV-light in the genome of the mammalian parvovirus Minute-Virus-of-Mice (MVM), using antibodies against these lesions. The sensitivity of this assay is enough to allow us to follow the fate of UV-damage present in viral DNA throughout its replication. In a previous work using the T_4 UV-specific endonuclease we showed that a minor fraction of pyrimidine dimers do not block the conversion of viral single-stranded DNA into duplex replicative forms (RF) when UV-irradiated MVM is grown in mouse fibroblasts (Vos, J-M and Rommelaere, J (1982) Biochimie, 64, 839-844). It was further observed that the fraction of tolerated dimers was little enhanced by UV-preirradiation of the host cells, although the level of RF formation was increased under these conditions (Rommelaere, J and D.C. Ward (1982) Nucleic Acid, Res. 10, (8), 2577-2596). A comparison of the enzymatic and immunological assays of dimer content will be presented and its implications will be discussed with respect to the tolerance of lesions during DNA replication and the mechanism of UV-enhanced viral reactivation in mammalian cells.

1080 INHIBITION OF ORNITHINE DECARBOXYLASE INDUCTION BY PSORALEN PLUS NEAR ULTRAVIOLET LIGHT IN HUMAN CELLS: THE ROLE OF MONOADDUCTS VERSUS DNA CROSSLINKS, E. Ben-Hur, A. Prager and M. Green, Nuclear Research Center—Negev, Beer-Sheva 84190, Israel.

Treatment of plateau-phase human breast carcinoma cells with psoralen-plus-UVA light (PUVA) inhibited the transcriptionally-controlled induction of ornithine decarboxylase (ODC). The influence response curve had a shoulder ($D_q = 56\ Jm^{-2}$) followed by an exponential decline ($D_o = 690\ Jm^{-2}$). The cells could not recover the capacity for ODC induction following PUVA doses that inhibited ODC induction by more than 50%. This is in contrast with the situation following UV-C exposure, following which recovery of DOC induction could be observed (E.Ben-Hur, A. Prager and F. Buonaguro [1982] Photochem. Photobiol. 35, 671-674). Consistently with the lack of ODC recovery there was no removal of PUVA damage in DNA following similar doses. However, removal of bound psoralen and DNA crosslinks was observed after lower doses during a 24 hour period. Using the two dose approach (E. Ben-Hur and M. M. Elkind [1973] Mutat. Res. 18, 315-324) it is shown that crosslinks are more efficient than psoralen monoadducts in inhibiting both ODC induction and RNA synthesis. It is concluded that DNA strant separation is an essential step during transcription in human cells.

1081 Characterization of a mitochondrial DNA polymerase induced by DNA damage, and production of monoclonal antibodies directed against the polymerase.

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Damage of the DNA of the protozoan Tetrahymena thermophila by ionizing radiation or by treatment with certain chemicals causes induction of a DNA polymerase with an increase in specific activity of up to 50 fold. The polymerase accumulate in the mitochondria but is encoded for by the nuclear genome.
In order to elucidate the role of the polymerase in the DNA repair processes the enzyme has been purified (more than 50 per cent pure), and stable murine hybridoma monoclones which produce antibodies directed against the polymerase have been established. These antibodies enable us to study the process of induction of the DNA polymerase and the function of the enzyme in the repair processes. Furthermore, we will be able to study the structure of the gene coding for the polymerase as well as the transport of the gene product from the cytoplasm to the mitochondrium.

1082 METHYL-DIRECTED CORRECTION OF DNA BASE PAIR MISMATCHES IN EXTRACTS OF ESCHERICHIA COLI, A-Lien Lu and Paul Modrich, Duke University, Durham, NC 27710

We have constructed DNA base pair mismatches within the EcoRI site of f1 strain R229 and have found such molecules to be subject to mismatch repair in vivo and in crude E. coli extracts. Although repair activity detected in vitro is low (maximal repair equivalent to about 5% of input DNA), the majority of the activity observed is dependent on the state of dam methylation of the DNA strands. Moreover, such molecules are subject to a form of in vitro repair synthesis which is dependent on the presence of a mismatch and state of methylation of DNA strands. Preliminary experiments indicate fractions derived from strains deficient in mutH, mutL, or mutS function to be deficient in the mismatch-dependent repair synthesis. However, activity is restored in mixed extracts.

1083 THE ROLE OF POST-REPLICATION MISMATCH REPAIR IN MODULATING ALKYLATION-INDUCED MUTAGENESIS, Steven M. Lyons, Paul F. Schendel, and Joseph F. Speyer, University of Connecticut, Storrs, CT 06268

A temperature-sensitive allele of the pur B gene in E. coli (mud) causes a reduction in spontaneous mutation frequencies. The antimutator phenotype of mud is conditional: growth at low temperature (24° C) or in the presence of adenine or adenosine restores normal mutation frequencies. Mud also diminishes the mutagenicity and cytotoxicity of alkylating agents such as MNNG. Unexpectedly, MNNG-treated cells grown with and without adenine have the same level of O^6-methyl guanine in their DNA, despite a several log difference in induced mutation frequency. This suggests that mud must be affecting a process subsequent to misreplication of O^6-methyl guanine, resulting in a lower mutation yield. We have devised model to explain the effect of mud on spontaneous and induced mutation, that involves a reduction in the rate of post-replication methylation of the DNA, thus allowing more time for mismatch repair to correct mismatched bases. Lower intracellular concentrations of ATP and of S-adenosyl methionine have been observed in mud cells. Also, experiments aimed at extending mismatch repair time in wild-type cells by using the methylation inhibitor, ethionine, have yielded results consistent with this hypothesis. It thus seems likely that the mismatch repair system has a significant modulating effect on the frequency of alkylation-induced mutation.

1084 INSERTION AND DELETION MUTATIONS IN THE dam GENE OF ESCHERICHIA COLI K-12. M.G. Marinus, Dept. of Pharmacology, Univ. of Mass. Med. School, Worcester, MA 01605.

The dam gene of E.coli encodes a DNA adenine methylase which methylates -GATC- sequences in newly synthesized DNA. DNA methylation has been proposed to play a role in DNA replicaton, repair and recombination. In order to determine if adenine methylation is essential for viability of the cell, three independent Mu cts dII301 (Amp, lac) insertions into the dam gene have been isolated. From these strains, derivatives have been obtained which are able to grow at high temperature, have lost Mu immunity but are still ampicillin (amp) resistant and Dam^-. These derivatives probably arose by deletion of the c end of Mu and some flanking dam DNA from the chromosome. Experimental evidence for insertion and deletions has come from genetic and physical data. The amp marker in the insertion/deletion strains is linked to aroB and mal at frequencies expected for a mutation in or near dam. The amp marker and the Dam^- phenotype co-seggregate showing that both arose from the same event. The insertion/deletion mutations are recessive and do not complement the dam-3 mutation. Preliminary data from hybridization studies (Southern blots) of the cloned dam gene to DNA from wild type and the insertion/deletion dam strains, indicate chromosomal rearrangements in the mutants compared to wild type. There is no detectable DNA adenine methylase or methylated adenines in the mutant strains. The existence of the mutants show that DNA adenine methylation is dispensible for viability of E.coli.

1085 VERY SHORT PATCH REPAIR OF SPECIFIC AMBER MUTATIONS. M. Lieb, Univ. of Southern California School of Medicine, Los Angeles CA 90033.

Mutation am6 in the cI gene of bacteriophage lambda recombines with other cI mutations more frequently than predicted by the physical distances involved. In 4-factor crosses of am6 with mutations located less than 50 base pairs to either side, cI+ recombinants that require three crossovers (triple recombinants) are as frequent as recombinants that require only one crossover. However, when am6 is crossed with a large insertion in cI, which may be expected to interfere with the formation of heteroduplex DNA by branch migration, the frequency of triple recombinants is very low. In addition, cI+ recombinants in crosses between am6 and adjacent mutations have a high probability of retaining the flanking markers of the am6 parent. These findings suggest that am6 is particularly susceptible to mismatch repair in heteroduplexes that include gene cI. A large fraction of such heteroduplexes are presumed to be the result of branch migration initiated by crossovers at some distance from am6. The absence of co-repair when am6 is crossed with adjacent cI mutations indicates that most repair tracts extend no farther than about 20bp to either side of the mismatch. Its location in a methylated sequence (5'CCAGG) may make am6 particularly susceptible to mismatch repair.

1086 CLONING OF mutH AND IDENTIFICATION OF THE GENE PRODUCT, Robert H. Grafstrom and Ronald H. Hoess, Basic Research Program - LBI, Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701

A specialized λ-transducing phage carrying the mutH gene and several deletion derivatives of this phage were characterized by restriction enzyme analysis. This analysis localized the mutH gene to a small region of bacterial DNA on the transducing phage and facilitated the subsequent cloning of this gene into the multicopy plasmid pBR322. The mutH gene is contained entirely on a 1.5-Kb HinIII fragment as judged by the ability of plasmids carrying this fragment to complement mutH$^-$ alleles on the bacterial chromosome. Using recombinant plasmids containing the 1.5-Kb HinIII fragment, we identified a 25,000-dalton protein as the product of the mutH gene in an in vitro transcription-translation system as well as in maxi-cells. Various deletion derivatives of the mutH-containing plasmids that exhibit a MutH$^-$ phenotype also have lost the 25,000-dalton protein. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-23909 with Litton Bionetics, Inc.

1087 MISMATCH NUCLEOTIDE PEPAIR AND GENE CONVERSION IN ESCHERICHIA COLI Richard A. Fishel and Richard Kolodner, Laboratory of Molecular Genetics, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.

The processes of genetic recombination, replication, and DNA irradiation frequently generate mismatch nucleotides. The most extensive occurance of heteroduplex DNA and subsequent mismatch repair is detected as gene conversion events during the process of general genetic recombination(Holliday.Genet.Res.5,282(1964);Warner,Fishel,and Wheeler.CSHQB.43,936(1978); Fishel,James,and Kolodner.Nature.294,184(1981). We have recently constructed a series of pBR322 derived DNA substrates that contain two 8-nucleotide intra-genic mismatch sites on opposite DNA strands separated by either 315, 627, or 1243 b.p. intervening DNA. Repair of either heteroduplex site without extension of the excision tract beyond the distance to the complimentary site will yield a functional tetracyclin-resistence (Tet) gene product. In the absence of repair DNA replication will separate the complimentary strands of the substrate and produce Tet-sensitive progeny. Initial experiments have shown that the generation of Tet-resistent transforments is moderatly frequent(2-5%) and is not affected by recA, mutS, uvrD(uvrE and recL) mutations and the state of DNA methylation and slightly dependent on recF and mutL gene products. These results differ from previosly reported mismatch experiments using inter-genic DNA substrates constructed by reannealing ϕX174, T7, or λ DNAs containing mutant sites in separate genes and where excision tracts of $>10^3$ nucleotides are not uncommon. The mechanism of intra-genic mismatch repair may more closly resemble the gene-conversion activities observed in general genetic recombination.

1088 GENETIC ANALYSIS OF THE SOS MUTATOR EFFECT IN MISMATCH CORRECTION DEFICIENT MUTANTS. Perrine Caillet-Fauquet, Geneviève Maenhaut-Michel and Miroslav Radman, Department of Molecular Biology, Université Libre de Bruxelles, B-1640 Rhode-St-Genèse, Belgium.

Mutagenesis induced by ultraviolet (UV) irradiation and by many chemicals depends upon transitory induction of a cellular mutator effect, which permits fixation of mutations opposite DNA lesions (targeted mutagenesis). The same SOS mutator effect generates mutations in the course of replication of undamaged DNA (untargeted mutagenesis). Inducible SOS mutator effect (untargeted mutagenesis) was measured in undamaged bacteriophage λ genome replicating in UV irradiated E.coli host. Two mutational targets were analysed : a forward mutation system in the λ repressor gene, permitting the analysis of pure and mixed mutational clones and the Ram221 → R$^+$ reversion system.

SOS mutator effect was found to be higher in mismatch correction deficient mutants (mutH,L,S) and lower in a adenine methylation deficient (dam$^-$) mutant, than in the wild type E.coli.

We present evidence that the observed decreased mutation frequency in dam$^-$ mutants is due to a killing of mismatch bearing chromosomes by the mismatch correction system presumably by the attack on both DNA strands.

We conclude that the majority of the SOS induced "untargeted" mutations (which occur as mixed mutational clones) are removed by the mismatch correction system. Our results suggest that the SOS mutator effect results from a decreased accuracy of DNA replication. On the basis of the pattern of effects of specific mut mutations on the two mutational targets, it can be argued that the mutational specificity of the SOS mutator effect is similar to that of spontaneous mutagenesis.

1089 MOLECULAR CHARACTERIZATION OF GENE(E) INVOLVED IN MISMATCH REPAIR IN *STREPTOCOCCUS PNEUMONIAE*, J.P. Claverys and M. Gherardi, CRBGC - CNRS 31062 Toulouse Cedex, France.

In *S. pneumoniae*, transforming efficiencies strongly depend upon the markers carried by donor DNA. These variations have been interpreted as repair of some mismatched base pairs in donor-recipient DNA heteroduplexes. The specificity of correction of mismatches is being analyzed. A few mutants defective in mismatch repair have been isolated and termed *hex*$^-$. However, neither the number of genes involved in mismatch repair nor their products have yet been identified. As an attempt to characterize such genes we made use of the "insertional mutagenesis", that is the inactivation of genes by insertion of heterologous DNA segments. A hybrid plasmid derivative of pBR325 (*E. coli*), carrying an erythromycin resistance determinant and which does not replicate autonomously into *S. pneumoniae*, was taken as heterologous DNA. In a shotgun type experiment, pneumococcal DNA fragments were joined *in vitro* to this DNA and used to transform a wild type pneumococcal recipient. Transformants resistant to erythromycin were selected ; they arise *via* integration into the chromosome of *in vitro* generated chimeric plasmids, by homology-dependent mechanism. We applied to a population of ery^r clones an enrichment procedure for *hex*$^-$ mutants. This approach proved to be efficient for the isolation of *hex*$^-$ mutants. Various *hex*$^-$ mutants can be compared by Southern blot hybridization using heterologous vector DNA as a probe. Moreover, regions adjacent to the site of insertion can readily be cloned. This constitutes the first step toward the cloning of genes involved in mismatch repair in *S. pneumoniae*.

1090 NUCLEOTIDE POOLS DURING BASE ANALOG MUTAGENESIS, Randi L. Hopkins and Myron F. Goodman, University of Southern California, Los Angeles, California, 90089-1481

DNA AND RNA precursors are measured during 2-aminopurine (AP) induced mutagenesis of E. coli infected with T4 bacteriophage allelic for gene 43 (DNA polymerase gene). Preliminary data suggest that the pool of dAPTP is 5-6% of dATP in "mutator" and "wild type," while only 0.4-1% in "antimutator" phage. [tsL56(43), 43$^+$, and tsL141(43), respectively, all in an ocUV199(r_{11}B) background are the genotypes of the allelic phage.] AP can cause up to a 10-fold expansion in the pool of ATP. Other deoxyribo- and ribonucleoside triphosphate pools expand, but to a much smaller extent. Expansion is more pronounced in antimutator than in the other phages.

Using the framework of previous genetic and biochemical studies with these organisms in vivo and their purified gene products in vitro in conjunction with AP compounds, these data are used to address fidelity in DNA replication. A multiwavelength UV monitored HPLC technique which is very powerful for detection and identification of very rare nucleotides, and many dNTP and rNTP analogs is described.

1091 ROLE OF DNA POLYMERASES IN THE BASE-PAIRING PATTERN OF O^6-METHYLGUANINE DURING IN VITRO DNA REPLICATION. Elizabeth T. Snow*, Robert S. Foote and Sankar Mitra. Univ.Tenn. Oak Ridge Grad. Sch. of Biomed. Sci. and Biol. Div., Oak Ridge Natl. Lab.†, Oak Ridge, TN 37830

O^6-methylguanine (m^6G), because of its ability to "mispair" with thymine during replication, is an important premutagenic lesion produced in DNA by simple methylating carcinogens. The kinetics of incorporation of nucleotide precursors opposite m^6G were analyzed during replication of synthetic deoxynucleotide polymers, containing m^6dG as the only modified base, using T5 and T4 phage DNA polymerases and E. coli DNA polymerase I. The incorporation of m^6dGMP during replication of both natural and synthetic DNA templates was also analysed using the same DNA polymerases. In all cases thymidine is the preferred base-partner for m^6dG; however, the relative incorporation of dTMP and dCMP opposite m^6dG is a function of DNA polymerase activity as well as dNTP pool concentrations and DNA sequence. Both dT:m^6dG and dC:m^6dG are recognized as "abnormal" base-pairs by the DNA polymerases as evidenced by high turnover of dTMP and dCMP during incorporation opposite m^6dG, high apparent Km's for incorporation opposite m^6dG, and decreased rate of replication of m^6dG-containing templates. The relative utilization and turnover of m^6dGTP during DNA replication is dependent on both the DNA polymerase and the DNA primer-template. O^6-methylguanine is incorporated into DNA as an analogue of adenine which acts as a potent competitive inhibitor of m^6dGMP incorporation. The data fit a model in which m^6dGTP acts as both an alternate substrate and an inhibitor during replication of poly(dA-dT) by E. coli DNA polymerase I. *Univ. of Tenn. Research Assistant. †Operated by UCC under contract W-7405-eng-26 with the U.S. Dept. of Energy.

1092 BASE INSERTION OPPOSITE NON-CODING LESIONS IN DNA, Daphna Sagher, Department of Microbiology, The University of Chicago, Chicago, IL 60637

AP (apyrimidinic) DNA was prepared from M13 phage grown on an ung⁻ dut⁻ E. coli mutant and treated in vitro with glycosylase to remove uracil. This modified DNA, primed with a restriction fragment, served as a template for several DNA polymerases and the nucleotide inserted opposite the AP site on the parent strand was examined. Our studies showed that synthesis by T4 pol stopped almost always before the putative AP site whereas pol I, pol α and AMV reverse transcriptase stopped either before or at the site. T4 pol was then used to synthesize to the position before the AP sites with all 4 nucleotide triphosphates, and the products purified and incubated with a single nucleotide at a time and the polymerase tested. The degree of insertion of the particular nucleotide opposite the site was determined by scanning the autoradiograms of the sequencing gels. In the sites thus far analyzed, the degree of insertion of the 4 nucleotides in the presence of Mg^{++} was dA >> dG > T > dC, dA close to 100%. Mn^{++} relaxed the specificity, so that greater incorporation of dG and of the pyrimidines was observed. The special preference for dA is demonstrated also by pol α and AMV reverse transcriptase. These findings suggest a possible role for the polymerase in selecting a base when non-coding lesions on the parent strand are involved.

1093 REPLICATION OF UV IRRADIATED DNA IN YEAST CRUDE EXTRACTS, Peter D. Moore*+ and Michael A. Resnick*, *National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709, +Department of Microbiology, University of Chicago.

Many of the mutagenic and lethal effects of UV induced pyrimidine dimers occur during DNA replication. In prokaryotes, dimers block DNA synthesis; however some of these may be by-passed following induction of error prone repair as part of the SOS response. Pyrimidine dimers also inhibit DNA synthesis in all eukaryotes that have been examined; the nature of the inhibition and the subsequent replication by-pass is poorly understood. For yeast it has been argued [C. Lawrence, Mol Gen. Genet 182: (1981) 511] that dimers are instructive and may allow some extent of replication: in any case it is supposed that some DNA synthetic activity, either constitutive or induced, is capable of replicating past dimers. We are studying in vitro replication of UV irradiated DNA in crude extracts from yeast; the products are analyzed using a modification of the sequencing techniques previously used with purified enzymes [P.D. Moore, et al., Proc Natn Acad Sci 78: (1981) 110]. Using these procedures we can observe termination of synthesis at dimers and are comparing synthesis in extracts from mitotic and meiotic cells or from cells pre-treated with UV light.

1094 SPECIFICITY OF NUCLEOTIDE INCORPORATION OPPOSITE LESIONS THAT TERMINATE DNA SYNTHESIS, Samuel D. Rabkin, Department of Microbiology, The University of Chicago, Chicago, IL 60637, and Peter D. Moore, Natl. Inst. Environ. Hlth. Sci., Research Triangle Park, NC 27709

Knowledge of the ways in which a polymerase deals with a bulky lesion after DNA synthesis has come to a halt is critical to our understanding of the mechanism of targeted mutagenesis in "SOS" induced cells. We have developed an in vitro model system with E. coli DNA polymerase I (Klenow fragment) to examine the question of the coding or non-coding capabilities of bases reacted with acetoxy acetylaminofluorene or irradiated with UV light, such that purines or pyrimidines have been damaged. By substituting Mn^{2+} as the divalent cation and consequently relaxing the base specificity of the polymerase we have tried to mimic one possible mode of error-prone DNA synthesis. The experiments reveal a preference for purines, particularly A. The specificity of nucleotide incorporation opposite the lesion in this "relaxed" system will be compared to that observed under "normal" conditions. The correlation of these results with in vivo mutational data suggests a model for the process of mutation at lesions that block DNA synthesis.

1095 PREVENTION OF MUTAGENESIS BY DNA REPAIR ENZYMES. Jacques LAVAL and Serge BOITEUX, LA 147 CNRS, U140 INSERM, Institut Gustave Roussy, 94805 Villejuif Cedex, France.

Cytosine deamination yields uracil which is a premutagenic DNA modification as it codes for dAMP when replicated by DNA polymerase leading to GC-AT transitions. It has been assumed that the biological role of uracil-DNA glycosylase is to prevent this adverse effect. Mutants deficient in uracil-DNA glycosylase are GC-AT mutators (Duncan and Miller Nature, 287, 569, 1980).

Our in vitro system uses the replication by E.coli polymerase I or III of poly(dC) containing dUMP residues. It leads to the incorporation in an acid precipitable form of dGMP and dAMP.

We show that treatment of the polynucleotide by uracil-DNA glycosylase prior to its replication in the presence of Mg^{++} abolishes the incorporation of dAMP. The AP sites thus genarated are replicated in the presence of Mn^{++} leading to the preferential incorporation of dAMP. This dAMP incorporation is in turn prevented if the polynucleotide containing AP sites is pretreated by Micrococcus luteus AP-endonuclease.

Analysis of the product of the reaction showsthat the dAMP incorporated bands with dGMP in alkaline Cs SO_4 density gradients.

This work was supported by Grant CRL and PRC awarded by the Institut National de la Santé et de la Recherche Médicale.

1096 ELONGATION OF MISMATCHED PRIMERS BY DNA POLYMERASE α SUBSPECIES FROM CALF THYMUS, Gerhard Krauss, Bernd Reckmann and Frank Grosse, Med. Hochschule Hannover, W.-Germany

We have described earlier the purification from calf thymus of two subspecies of DNA polymerase α, sedimenting at 9 S and 5.7 S. The 5.7 S enzyme has been shown to be more error prone. We now have compared the ability of the subspecies to elongate mismatched primers, using the in vitro replication of poly dA (dT_8dG). In general the elongation of a mismatch occurs at a low frequency. The polymerase subspecies sedimenting at 9 S elongates the mismatch at a 4-5 fold lower rate than the 5.7 S subspecies. The presence of Mn^{2+} has little influence on the frequency of elongation of the mismatch. However, a primer of the structure (dT_8dGdT) is much better elongated in the presence of Mn^{2+} than in the presence of Mg^{2+}. Utilizing primers of the structure (dT_8dGdT_n) we have determined the minimal number of correct base pairs (n=5) that have to follow a mismatch in order to restore the normal elongation rate.

1097 Analysis of Structure and Function of Chick DNA polymerase α by Monoclonal Antibodies

Akio Matsukage, Masamitsu Yamaguchi,
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We have established mouse hybridoma clones which produce monoclonal antibodies against chick DNA polymerase α. Antibodies produced by 4 clones were detected by a specific immunobinding assay for DNA polymerase α activity. All four antibodies are of the IgG class. Using these antibodies, we have established an immunoblotting method, an antibody-column chromatography separation and an immunofluorescence scan for the detection and purification of DNA polymerase α. With combination of the immunoblotting and antibody-column, the association of the high molecular weight polypeptide (130-150K) and the low molecular weight one (50-60K) was confirmed. Furthermore, the antibody column retained concomitantly DNA primase activity and DNA polymerase α. This provides direct evidence for the association of the DNA primase to DNA polymerase α. Furthermore, the variation of the enzyme distribution during cell cycle differentiation has been monitored by immunofluorence.

1098 AN ANTIMUTATOR PHENOTYPE IN HERPES SIMPLEX VIRUS RESULTS FROM ALTERED DNA POLYMERASES AND AFFECTS REPLICATION FIDELITY, J.D. Hall[1], D.M. Coen[2], P.A. Schaffer[2], P.A. Furman[3] and C. Knopf[4]; U. of Arizona[1], Tucson, AZ 85721; Harvard Med. School[2], Boston, MA 02115; Burroughs Wellcome Co.[3], Research Triangle Park, NC 27709; German Cancer Research Center[4], Heidelberg, West Germany.

To study the role of DNA polymerase in spontaneous mutagenesis of herpes simplex virus type 1, we measured the production of mutants by viral derivatives carrying altered polymerases resistant to phosphonoacetic acid (PAA). Several derivatives produced 9 to 123 fold fewer thymidine kinase deficient (TK^-) mutants than their wild type parents. To locate the antimutator mutation in one strain (PAA^r-5), we constructed PAA-resistant, recombinant viruses using wild type viral DNA and DNA restriction fragments coding for resistance (marker transfer). The resultant recombinants were also antimutators, indicating a close linkage between the drug resistant locus and the sequences controlling mutant production. N-methyl-N'-nitro-N-nitrosoguanidine, a mutagen thought to produce base substitutions due to mispairing of O^6-methylguanine residues, stimulated the production of thymidine kinase mutants for wild type (KOS) but not PAA^R-5 at low drug concentrations. Double mutants containing the PAA-resistance locus from PAA^r-5 and a temperature sensitive allele (F17 or F18) produced fewer temperature resistant revertants than the temperature sensitive parental strains. These results suggest that the reduced yield of mutants in PAA^r-5 results from enhanced replication fidelity by the altered DNA polymerase. Fidelity assays with purified polymerases from KOS (wild type) and PAA^r-5 were conducted to confirm this hypothesis.

1099 STUDIES OF DNA POLYMERASE ALPHA VERSUS REPLICATIVE AND REPAIR CAPACITY OF HUMAN CELLS, Sharon W. Krauss and Stuart Linn, Univ. of California, Berkeley, CA 94720.

Changes in DNA polymerases α, β, and γ occur as human fibroblasts of defined life-span are increasingly passaged: accuracy of DNA polymerization in vitro decreases, levels of α but not β-polymerases decline and chromatographic properties of α-polymerase species are altered. Qualitatively similar changes also are observed with DNA polymerases from post-confluent early passage fibroblasts (Krauss and Linn, Biochemistry 21, 1002, 1982). In both cases, IMR-90 fibroblasts appear to remain competent to perform UV-stimulated repair synthesis. We now find that stationary phase HeLa cells and butyrate-treated diploid human fibroblasts contain α-polymerases with altered fractionation properties on DEAE-cellulose similar to those of senescent diploid human fibroblasts. Moreover, the polymerases from the stationary phase HeLa cells also have decreased fidelity in vitro. The butyrate-arrested fibroblasts contain normal levels of α-polymerase activity yet are not engaged in DNA replication and have unchanged UV-induced repair synthesis. This contrasts to senescent or postconfluent fibroblasts which have decreased levels of α-polymerase activity yet maintain normal levels of UV-induced repair synthesis. Therefore, although α-polymerases may be involved in DNA repair synthesis as well as DNA replicative synthesis, apparently α-polymerase levels do not necessarily correlate with repair capacity or with replicative potential. Additionally, some changes in DNA polymerase properties reported in "aging" cultured cells might be a consequence of entry into a non-replicative state. (Supported by Grant AG00819 from NIH.)

1100 STUDY OF THE ROLE OF GATC SEQUENCES IN MISMATCH REPAIR, Geneviève Maenhaut-Michel Department of Molecular Biology, Université Libre de Bruxelles, B-1640 Rhode-St-Genèse, Belgium.

It was demonstrated that adenine methylation of 5' GATC sequences (6-meA) by the E.coli Dam methylase, inhibits mismatch repair on the methylated strand (1). This observation generated the hypothesis that the (non-methylated) GATC sequences may be required for mismatch repair, e.g. by playing the role of a signal sequence to instruct mismatch repair enzymes.
We have addressed this question by constructing heteroduplex DNA of phage ØX174, which is devoid of GATC sequences, containing a short (about 60 nucleotides) intergenic insert (2) of pBR322 plasmid DNA, containing two GATC sequences in one case and none in another, and the same am/+ mismatch. The analysis of the phage progeny from infective centres of transfected E.coli indicates that : i) GATC sequence plays a role in mismatch repair but is not absolutely required for it ; ii) mutL and mutS mutations and the recA mutation affect the efficiency of the recovery of the two mismatched genetic markers.

(1) Radman, M., Wagner, R.E., Glickman, B.W. and Meselson, M.(1980) Progress in Environmental Mutagenesis. M. Alacevic ed., Elsevier/ North-Holland, Biomedical Press, pp. 121-130.
(2) Miller, U. and Wells, R. (1980) J. Mol. Biol. 141, 1-24 et 25-41.

1101 THE PRESENCE OF REPLICATION ENZYME COMPLEX AND ATTACHMENT SITES ON THE NUCLEAR MATRIX OF CELLS INFECTED WITH SIMIAN VIRUS 40, Clint Jones and Robert T. Su, Univ. of Kansas, Lawrence, Kansas 66045

To investigate the roles of the nuclear matrix in mammalian chromosome replication, the matrices prepared from SV40 infected cells at various times after infection was hybridize to SV40 DNA fragments generated by restriction endonuclease Hind III. Specific SV40 DNA sequences within Hind III fragments C and A were consistently found in the matrices during the early infection cycle. When DNA synthesis was performed in vitro using the nuclear matrices from nuclei without the treatment of DNase I, limited DNA synthesized was mainly localized within Hind III fragments C and B, as determined by the Southern blotting technique. The result implied that early replicative intermediates were associated with the matrix. Previously, a tenacious complex containing α-DNA polymerase and T-antigen was obtained from the matrix from SV40 infected cells(Jones and Su, 1982. NAR, 18:5517-5532). SV40 DNA in this complex when hybridized to Hind III restriction fragments, was predominantly in the fragment C where the replication origin of SV40 DNA is included. The results suggested that subnuclear structure, the nuclear matrix, might involved in the early stage of viral chromosome replication.

Mutagenesis/Post-Replicational Responses/Neoplastic Transformation

1102 SPECIFIC MUTAGENESIS IN E. COLI B/r ung BY THYMINE-CYTOSINE DIMERS, Douglas Fix and Richard Bockrath, Indiana University School of Medicine, Indpls, IN 46223

Mutations in E. coli by ultraviolet light (UV) are thought to result from error-prone repair at or near sites containing photoproducts. Recently, we demonstrated that class 2 suppressor mutations produced by UV in uracil-DNA-glycosylase deficient strains (ung) became refractory to photoreactivation (PR) if held at an elevated temperature after irradiation (1). This suggested the production of mutation by thymine-cytosine dimers (T=C) at precise genetic loci. Subsequent deamination of the cytosine residues and photoenzymatic monomerization then would reverse the resultant T=U dimers and, in an ung strain, produce a G:C → A:T transition. Our model proposed that the slope of the mutation frequency response after PR developing with thermal treatment was proportional to the deamination rate of cytosine residues in dimers (k) and the UV dependent probability of a precisely placed T=C dimer (Q_o). Here, we have tested this model by altering either the temperature or the initial UV fluence. Holding irradiated ung cells at 37° to 48°C gave larger slopes with increasing temperature. Plotting the log of the slope versus the reciprocal of absolute temperature (Arrhenius plot) allowed estimation of activation energies near 28 kcal/mol in an excision repair defective strain. Extrapolation towards 60°C approached a value published for the deamination rate of cytosine residues in dimers at this temperature (2). Likewise, holding excision repair proficient cells at a constant temperature following several different UV doses (5-30 Jm^{-2} at 254 nm) gave larger slopes after increasing initial fluences. This relationship was linear and revealed a value of k consistent with that found previously. Supported by N.I.H. GM21788.

(1) Mol. gen. Genet. (1981) 182, 7-11; (2) J. Mol. Biol. (1966) 17, 237-254.

1103 NONTARGETED VS. TARGETED MUTAGENESIS IN BACTERIOPHAGE LAMBDA, Richard D. Wood and Franklin Hutchinson, Yale University, New Haven, CT 06511

Ultraviolet light-induced mutations in bacteriophage lambda can be separated into those that arise when irradiated phage are introduced into lightly irradiated host cells ("targeted mutagenesis") and those that arise when unirradiated phage are introduced into heavily irradiated host cells ("nontargeted mutagenesis"). These two classes of mutations appear to be the result of different processes which lead to distinct endpoints. Some characteristics of these processes were examined by studying UV-induced clear plaque mutants in a lambda phage/E. coli system.

A number of forward mutations in the cI gene were sequenced to determine mutational specificity. Nontargeted mutants (uvr^+ host given 125 J/m^2, no UV to phage DNA) were principally frameshift mutations in runs of identical bases. In contrast, mutants induced when phage DNA is UV-irradiated consist mainly of transitions, although a variety of other alterations are produced.

Targeted UV mutagenesis is dependent on a functional umuC gene, while nontargeted mutagenesis can occur in $umuC^-$ host cells. Although targeted mutants arise with a mutant burst size of about 30, nontargeted mutants have a mutant burst size of 1 to 3. These genetic, physiological, and spectral differences for the two cases require that different models be constructed for targeted and nontargeted mutagenesis.

1104 THE INVOLVEMENT OF recF IN SPONTANEOUS AND UV-INDUCED MUTATION IN ESCHERICHIA COLI, Bryn A. Bridges and Maurice Southworth, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, England

The inducible error-prone system of E.coli can, when fully induced, result in mutagenesis in infecting phage lambda being almost independent of the presence of photoproducts in the lambda DNA. Under these conditions UV mutagenesis of lambda is recF-independent except for a minority of unmixed mutant clones which are seen only in $recF^+$ strains and which may arise as mutations at photoproduct sites. In bacteria themselves, UV mutagenesis is overwhelmingly dependent on the presence of photoproducts in the DNA but is apparently recF independent. We shall present results of further experiments on the role of recF in UV and spontaneous mutagenesis.

1105 BASE SUBSTITUTION MUTATIONS INDUCED BY METABOLICALLY ACTIVATED AFLATOXIN B_1, Patricia L. Foster, Eric Eisenstadt and Jeffrey H. Miller, Harvard School of Public Health, Boston, MA 02115 and Universite' de Geneve, Geneva, Switzerland

We have determined the base substitutions generated by metabolically activated aflatoxin B_1 (AFB1) in the lacI gene of a $uvrB^-$ strain of Escherichia coli. By monitoring over 70 different nonsense mutation sites, we show that activated AFB1 specifically induces G:C to T:A transversions. One possible pathway leading to this base change involves depurination at guanine residues. We consider this mechanism of mutagenesis in light of our other findings that the carcinogens benzo(a)pyrene diol epoxide (Eisenstadt, E., Warren, A.J., Porter, J., Atkins, D. and Miller, J.H. (1982) Proc. Natl. Acad. Sci USA 79, 1945-1949) and N-acetoxyacetylaminofluorene (Miller, J.H. and Eisenstadt, E., in preparation) also specifically induce G:C to T:A transversions.

1106 ROLE OF DIMERS AND (6-4) LESIONS IN UV MUTAGENESIS, Douglas E. Brash and William A. Haseltine, Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115

UV-induced nonsense mutation hotspots in the E. coli lacI gene occur at hotspots for formation of both pyrimidine dimers and pyrimidine-pyrimidine (6-4) photoproducts (Brash & Haseltine, Nature 298:189, 1982). To determine which lesion(s) are mutagenic, we first showed that pyrimidine dimers, but not (6-4) lesions, can be photoreactivated in vitro by E. coli photoreactivating enzyme plus fluorescent light. We next examined photoreactivability of UV-induced $lacI^-$ mutations (in uvr^+ and uvr^- cells) and found a 1-1.5 log photoreactivation, with little change in distribution of mutation hotspots in uvr^-. To test whether this result could be accounted for by photoreactivation of the SOS induction signal alone, we photoreactivated induction of beta-galactosidase in dinD1::Mud(Ap^R, lac) uvr^+ and uvr^- strains under conditions identical to those used for generating and photoreactivating mutations. SOS induction was substantially photoreactivated. We next constructed spr and tif derivatives of the lacI mutation strain P90C and the trp mutation strain WP2, and are examining photoreactivability of mutagenesis under conditions of constitutive SOS.

1107 THE PROPORTION OF UNTARGETED MUTATIONS IN THE *lacI* GENE OF *E.coli*, J.R.Christensen, R.B.Christensen and C.W.Lawrence, University of Rochester, Rochester, NY 14642

The condition of reduced polymerase accuracy(mutator condition) which can often be detected when undamaged phage replicate in UV-irradiated *E.coli* is thought to have only a negligible influence on the replication of the host-cell genome (1), and SOS functions other than excision appear to be unimportant for the repair of chromosomal DNA (2,3). If so, nearly all chromosomal mutations should be targeted at sites of damage, and almost none untargeted, the result of the mutator condition. We have tested the validity of this conclusion by estimating the proportion of untargeted mutations at the *lacI* locus in excision deficient (*uvrA*) strains. Contrary to expectation, we find that about 10% of mutations from $lacI^s$ to $lacI^-$ are untargeted. The frequency of untargeted mutations was estimated by transfering an unirradiated *F'pro lac* into an irradiated (3.5, 7.0 Jm^{-2}) recipient in which the *pro lac* region is deleted. Irradiation of the *F'* prior to conjugation gave the estimate of total mutation frequency. Induced mutation rates in conjugating cells were 2-3 fold higher than in cells containing a resident *F'*, and 1.5 times higher when irradiated *F'* was transfered to unirradiated recipients. Conjugation may therefore lead to partial induction of SOS functions, though damage in ss-DNA may be more mutagenic than damage in ds-DNA. Supported by DOE and NIH grants GM 21858 & GM26147.

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1108 UV MUTAGENESIS OF M13LAC HYBRID PHAGE DNA: NUCLEOTIDE SPECIFICITY IN A FORWARD MUTATION SYSTEM. J. Eugene LeClerc, Robert C. Hayes, Randi L. Kubrick and Nancy L. Istock, Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14642

We have used Messing's M13lac hybrid phage as a forward mutation system to study the nucleotide specificity of UV mutagenesis. M13lac hybrid phage contains the M13 single-stranded DNA genome with an insert of the regulatory region and part (α peptide) of the lacZ gene for lactose metabolism in E. coli. Infection of E. coli lacZ∆m15 cells with M13lac hybrid phage allows production of a functional β-galactosidase by intracistronic complementation. Infected cells are therefore lac$^+$, detected as blue plaques on indicator plates. Inactivation of α-complementing activity by mutagenesis of the lacZ'(α) gene of the hybrid phage genome gives rise to light blue or colorless plaques of infected cells, easily distinguishable from the blue plaques of M13lac$^+$-infected cells. Nucleotide changes in the nonessential lac DNA are determined by direct nucleotide sequencing of the DNA from mutant clones.

We analyzed 115 mutant clones derived from UV-irradiated phage grown in UV-irradiated cells and identified 78 single base substitutions, 19 large deletions, 18 single base deletions, 6 tandem double base substitutions and 3 single base additions. 74% of the single base substitutions occurred at adjacent pyrimidine sites, of which TT sites predominated. Several mutant clones contained multiple, non-tandem nucleotide changes.

1109 THE SPECIFICITY OF MUTAGENESIS AT APURINIC SITES AS DETERMINED IN A FORWARD MUTATIONAL SYSTEM, Kunkel, Thomas A., NIEHS, Research Triangle Park, NC 27709

Depurination, the loss of a purine base from DNA, has been estimated to be a frequent cellular event. Since such lesions are clearly non-coding, they may, if unrepaired provide a significant mutagenic challenge to an organism. The mutagenic consequences of unrepaired apurinic sites are being examined in a forward mutational system. Transfection of depurinated single strand M13mp2 DNA into competent cells results in a large (10 to 50-fold) increase in the frequency of mutant (white or light blue) plaques compared to a non-depurinated DNA control. Mutagenicity is proportional to the number of apurinic sites in the DNA and is largely dependent on a functional error-prone repair system in the competent cells. The specificity of depurination dependent mutagenesis, determined by DNA sequence analysis of mutants, will be discussed.

1110 MOLECULAR MECHANISMS OF MUTATION BY SITE-SPECIFIC CARCINOGEN-INDUCED COVALENT ADDUCTS OF DNA, Robert W. Chambers, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

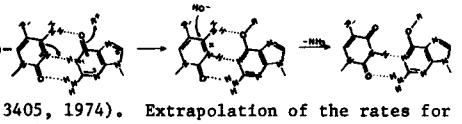
We have developed a site-specific mutagenesis system that employs bacteriophage ΦX174 and E. coli to study the mutagenic effect of site-specific covalent adducts representing the products that are formed when a particular carcinogen reacts with DNA. These adducts are introduced one at a time into a single preselected site in gene G (an essential gene). The mutagenic effect of the adduct is determined in vivo in a variety of DNA repair backgrounds by transfection of spheroplasts carrying mutations in different DNA repair genes. This approach is now operational and is being used to answer the following questions: (1) Which of the various adducts that form when a given carcinogen reacts with DNA actually produce mutations? (2) What kind of mutation does each different premutational lesion produce? (3) What role do various DNA repair systems play in producing these mutations? Three examples of DNA damage in gene G of ΦX174 are currently under investigation: several different adducts produced from carcinogenic alkyl nitroso compounds, a deoxyribose ("AP site") which is formed by "repair" of some of these alkyl adducts, and a "thymine dimer". The principles on which our approach rests are general and we are planning to extend our experiments to animal cells in culture.

1111 ANALYSIS OF MUTATIONS INDUCED BY THE ULTIMATE CARCINOGEN N-ACETOXY-N-2-ACETYLAMINOFLUORENE : A MODEL FOR FRAMESHIFT MUTATIONAL HOT SPOTS, Robert P.P. Fuchs, Nicole Schwartz and Michel P. Daune, Groupe de Biophysique, IBMC, CNRS, 15 rue Descartes, 67084 Strasbourg cêdex, France.

N-Acetoxy-N-2-acetylaminofluorene (N-Aco-AAF), a model ultimate metabolite of the rat liver carcinogen N-2-acetylaminofluorene is known to be a strong mutagen both in bacterial and eukaryotic systems. N-Aco-AAF binds covalently to guanine residues in DNA (mainly at position C-8). Being interested in the way the initial premutagenic lesion (i.e. the guanine-AAF adduct) is converted into a stable mutation we developped an "in vitro" site directed mutagenesis assay. We describe here the analysis at the sequence level of forward mutations induced in the tetracycline resistance gene of pBR322 by directing the chemical reaction of the carcinogen to a small restriction fragment (BamH1-SalI) inside the antibiotic resistance gene. Mutants are selected for ampicillin (Ap) resistance and tetracycline (Tc) sensitivity. The plasmid DNA of such mutants was analysed for sequence changes in the fragment where the AAF binding had been directed. It is shown that the mutations are mainly frameshifts involving the addition or the deletion of one or two GC base pairs. Moreover, the mutations are clustered at a small number of sites which would appear to be mutational hot spots. A mechanism explaining the presence of mutational hot spots as a consequence of the local nucleotide sequence is proposed.

1112 CROSS-STRAND CATALYZED DEAMINATION - A NOVEL MECHANISM FOR MUTAGENESIS, Lawrence C. Sowers, Barbara R. Shaw and W. David Sedwick, Duke University Medical School and the Dept. of Chemistry, Durham, NC 27710

This paper describes a novel catalytic mechanism for the deamination of cytosine residues within a double helical DNA structure. Mutagen binding to the N-3, O-6 and C-8 positions of guanine significantly increases the acidity of the N-1 proton from its usual pK of 9.8. Subsequent ionization of the N-1 proton can result in protonation of the N-3 position of the base-paired cytosine via proton transfer. This reaction is catalyzed by a variety of alkylating agents, metal complexes and metallic ions such as silver. Our work with N-3 methyl derivatives of deoxycytidine (protonated under physiological conditions) yields rate constants for hydroxide attack on protonated cytosine derivatives identical to those of Lindahl (Biochem. 13:3405, 1974). Extrapolation of the rates for this reaction predicts that at 37°C and physiological pH, deamination should occur with a T 1/2 of approximately 100 years for dCMP, and 10,000 years for a cytosine residue in native DNA. In direct support of our suggested model for cross-strand catalyzed deamination of cytosine addition of silver to the alternating co-polymer dG-dC increased the rate of deamination of cytosine paired to a modified guanine 280-fold relative to dCMP and 28,000-fold relative to cytosine base-paired with an unmodified guanine in DNA at 37°C and pH 7.4. The possible impact of this mechanism on mutagenesis of alkylating and other purine-modifying agents at cytosine and 5-methylcytosine base pairs in DNA will be illustrated.



1113 EXTRACELLULARLY UV-IRRADIATED DNA CONFERS A MUTATOR PHENOTYPE TO TRANSFECTED MAMMALIAN CELLS, C. Dinsart, J.J. Cornelis and J. Rommelaere, ULB, Dpt. of Molecular Biology, Rhode Saint Genèse, Belgium , B1640.

The frequency of mutants among the progeny of intact parvovirus H-1 is increased when human or rat cells are exposed to low doses of UV radiation or chemicals prior to virus infection. Enhanced parvovirus mutagenesis is also observed if cells are pretreated with UV-killed Simian virus 40 (SV40), suggesting that UV-lesions per se serve as a signal triggering a mutator phenotype (1). In order to identify the signalling lesions, we tested whether the introduction of UV-damaged exogeneous DNA into the cells enhanced their ability to mutagenize parvovirus H-1. Double-stranded circular supercoiled DNA of SV40, double-stranded salmon sperm DNA and single-stranded circular ØX174 DNA all triggered an induction of H-1 backmutations in transfected cells. The magnitude of the induction increased with the number of UV-lesions present in transfecting DNA up to a saturation level. Unirradiated DNA displayed little triggering capacity. The mutator effect was very similar in human and rat cells and did not depend on the transfection method used. Altogether, these data indicate that UV-damaged DNA and/or products of its intracellular processing modulate the expression of mutator functions in mammalian cells. Whether this modulation consists in a de novo induction remains to be elucidated.

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1114 THE MEI-9 AND MUS(2)201 EXCISION-DEFECTIVE MUTANTS OF DROSOPHILA MELANOGASTER ARE HYPERMUTABLE BY MONOFUNCTIONAL ALKYLATING AGENTS, P. Dennis Smith, Ruth L. Dusenbery, Jeffrey L. Jefferson, Emory University, Atlanta, GA 30322.

The mei-9 and mus(2)201 mutants of Drosophila melanogaster exhibit larval hypersensitivity to a variety of mutagenic agents and have been shown to be defective in the repair of DNA damage induced by UV, X-rays, and a number of alkylating agents. The effects of these mutants on mutation induction were determined for a series of monofunctional alkylating agents which vary systematically with respect to their relative electrophilicities and yield varied patterns of primary alkylation lesions in DNA. The sex-linked recessive lethal test, which simultaneously monitors mutagenesis at approximately 700 loci, was employed as a simple and objective measure of mutation induction. Two experimental designs were employed. In one case, repair-deficient females were mated to mutagen-treated repair-proficient males and mutations produced from lesions induced in mature sperm were assayed. Alternatively, repair-deficient mutagen-treated males were mated to repair-proficient females and mutation induction in all stages of spermatogenesis was determined. With both experimental designs, mutation induction in the repair-deficient genotypes was enhanced in comparison to repair-proficient controls. The relative increase in mutability for both excision-deficient strains was shown to parallel very closely the expected production of 3-alkyl adenine, suggesting that, in the excision-defective strains, this lesion may persist and act as a promutagenic DNA alteration. Moreover, studies of mutation induction during spermatogenic stages indicated that excision repair is active in gonial cells but is shut down during meiosis, remaining inactive in postmeiotic cells.

1115 MUTATION IN DNA TRANSFECTED INTO MAMMALIAN CELLS, Michele Calos, Jane Lebkowski, and Michael Botchan*, Stanford University, Stanford, CA 94305, *University of California, Berkeley, CA 94720

The lacI gene of E. coli was used to score mutation in mammalian cells by constructing recombinant DNA vectors containing lacI and sequences that provide for replication (ori_{pBR322}, ori_{SV40}) and selection (amp^R, TK) in both bacterial and mammalian cells. Plasmid DNA was introduced into COS-7 simian cells by DEAE dextran transfection. After replication in the mammalian cells, it was returned to E. coli for analysis of mutations. Mutations in lacI occured at frequencies of 1% and 10% for two different plasmid constructions, compared to a spontaneous mutation rate in E. coli of less than 10^{-5}. The mutations consist of base substitutions, deletions, duplications, and more complex rearrangements. We have characterized examples of each class at the DNA sequence level and argue that many of the mutations are due to error-prone recombination or replication/repair systems induced by the transfected lac DNA.

1116 DNA-MEDIATED GENE TRANSFER OF HUMAN DNA REPAIR GENE(S) INTO DNA REPAIR DEFICIENT CHINESE HAMSTER OVARY CELLS, Jaime S. Rubin, Alexandra L. Joyner, Alan Bernstein and Gordon F. Whitmore, Department of Medical Biophysics, Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9.

Chinese hamster ovary (CHO) cell lines that are deficient in the initial incision step of the DNA repair excision pathway have been used as recipients for DNA transfer. These cells are sensitive to a variety of agents including UV-light and Mitomycin-C (MM-C). Cotransfer of both genomic HeLa DNA and the selectable bacterial gene Ecogpt in an SV-40 pBR322 vector, and treatment in growth media containing both MM-C and MAX (mycophenolic acid, adenine and xanthine) result in a stable cotransformation frequency of approximately 10^{-7}. CHO cells from two different DNA repair complementation groups have been successfully transformed to the MM-C resistant phenotype. The presence of both human DNA sequences and the bacterial gene in the genome of the transformed cells has been demonstrated. Using highly repetitive human DNA sequences as probes, similar ("Alu") sequences can be specifically detected in the CHO cell genetic background by Southern gel analysis under stringent hybridization conditions. Secondary transformants have also been generated at the same frequency using cellular DNA from the primary transformants. Southern gel analysis is currently in progress to determine whether these secondary transformants also contain specific human sequences. With this approach, and by using CHO mutants from different complementation groups as recipients for DNA transfer, we hope to identify the human gene(s) involved in the repair of DNA.

1117 DETECTION OF DELETION MUTATIONS IN pSV2gpt TRANSFORMED CHO CELLS, Kenneth R. Tindall[1,2] and A. W. Hsie[2], Yale University[1], New Haven, CT 06511, and Biology Division[2], Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Deletion mutations were detected at the xanthine-guanine phosphoribosyl transferase (gpt) locus in hypoxanthine-guanine phosphoribosyl transferase (HGPRT)-deficient Chinese hamster ovary (CHO) cells transformed by the pSV2gpt plasmid vector. The pSV2gpt vector carries the E. coli gpt gene in an SV40-pBR322 recombinant plasmid and expresses the gpt gene product, XGPRT, in pSV2gpt transformed mammalian cells. The purine analog, 6-thioguanine (TG), is metabolized by XGPRT and was shown to be applicable for the isolation of mutants at the gpt locus in pSV2gpt transformed CHO cells. Southern blot hybridization analysis established the presence of a single copy of the gpt gene integrated in the CHO genomic DNA in one pSV2gpt transformant. Induction of TG^r mutants in this cell line by X-rays was quantitative. All isolated TG^r mutants were demonstrated to lack XGPRT activity. In addition, specific deletions of integrated pSV2gpt sequences approximating 1.0 kb in a spontaneous TG^r mutant and 3.1 kb in a TG^r mutant isolated following an X-ray dose of 400 rads were detected by Southern blot hybridization. The sites of these deletions provide a molecular basis for the observed TG^r phenotype and the lack of XGPRT activity associated with these cell lines. These data demonstrate the utility of this gpt transformed cell line for quantitative and molecular analyses of gene mutation in CHO cells.

1118 THE MECHANISM OF MUTATION INDUCTION IN DIPLOID HUMAN CELLS CAN DIFFER FROM THAT IN BACTERIA, Veronica M. Maher, Ann E. Aust, Norman R. Drinkwater, and J. Justin McCormick, Michigan State University, East Lansing, MI 48824

To investigate whether chemicals induce mutations in human cells by mechanisms similar to those in Salmonella typhimurium, we compared the frequency of mutations conferring diphtheria toxin (DT) resistance induced in the gene coding for elongation factor -2, (EF-2) with mutations conferring 6-thioguanine (TG) resistance induced in the gene coding for the HPRT enzyme. Since recovery of DT cells requires that the EF-2 retain protein synthesis activity, the mutation cannot involve major changes in DNA. In contrast, cells can acquire TG resistance by any mechanism which eliminates HIPRT activity e.g., base substitution, frameshift, deletion, loss of chromosomes, etc. Therefore, comparison of the relative frequencies of TG^r and DT^r mutuants induced by a series of chemicals allows classification of the compounds as to their abilities to induce base substitution or other types of mutations. We found that ethylnitrosourea (ENU), a well-characterized base substitution mutagen in bacteria, gave a ratio of DT^r to TG^r mutants of 2.0. ICR 191, a classical frameshifter in bacteria, gave a ratio of DT^r to TG^r mutants of 0.1. This ratio was also found with X-rays, as expected of an agent inducing gross chromosomal changes. But, when we tested the diol epoxide of benzo(a)pyrene (BPDE) and N-acetoxy-2-acetylaminofluorene(N-AcO-AAF), carcinogens which have been reported to cause frameshifts in bacteria, we obtained DT^r to TG^r ratios of 1.7 and 1.6 respectively. These results suggest that BPDE and N-AcO-AAF, like ENU, yield predominantly base substitutions. We are currently extending these studies to other structurally-related chemicals. Supported by grants CA21253, CA06523, ES07076, and by a grant from the Michigan Osteopathic College Foundation.

1119 A COMPARISON OF MUTATION INDUCTION IN V79 CHINESE HAMSTER CELLS AND IN E. COLI BY ETHYLATING AGENTS ON THE BASIS OF THE AMOUNT OF DIFFERENT ETHYLATION PRODUCTS IN DNA, A.A. van Zeeland and G.R. Mohn, Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Leiden, The Netherlands.

The induction of O^6-ethylguanine and 7-ethylguanine in DNA of V79 Chinese hamster cells as well as in E. coli has been measured following exposure to different concentrations of ^{3}H-labelled ethylmethanesulphonate (EMS) and ethylnitrosourea (ENU). In V79 cells, both the amount of 7-ethylguanine and O^6-ethylguanine in DNA increased linearly with the exposure concentration of the mutagen used. In parallel to the dosimetry measurements, the induction of gene mutations at the HGPRT-locus has been measured. The results do not show a relationship between mutation induction and the level of 7-ethylguanine in the DNA. However, at equal levels of O^6-ethylguanine, mutation induction by EMS and ENU was the same. In E. coli the amount of 7-ethylguanine in DNA increased linearly with the exposure concentration of both mutagens. In contrast, there was a exponential increase in the amount of O^6-ethylguanine. At high concentrations the ration of O^6-ethylguanine to 7-ethylguanine approached the values found in V79 cells. These measurements suggest that at low exposure concentrations O^6-ethylguanine is selectively removed from the DNA in E. coli, possibly because of the presence of a constitutive fraction of the adaptive response. Mutation induction in E. coli measured as nalidixic acid-resistance and as valine-resistance, increased also exponentially with the exposure concentration, suggesting that in E. coli O^6-ethylguanine is also responsible for mutation induction.

1120 ULTRAVIOLET-INDUCED HYPERMUTABILITY IN PATIENTS WITH HEREDITARY CUTANEOUS MELANOMA, Kenneth H. Kraemer, Mark H. Greene and Mohan I.R. Perera, National Cancer Institute Bethesda, MD 20205

The dysplastic nevus syndrome (DNS) is a pre-neoplastic form of hereditary cutaneous melanoma in which affected individuals have increased numbers of abnormal (dysplastic) nevi and a markedly increased risk of developing cutaneous melanoma. We have carried out a series of quantitative investigations to determine the frequency of ultraviolet (UV)-induced mutations in EB virus-transformed lymphoblastoid cell lines (LCL) derived from 2 patients with the DNS and melanoma (DNS3Be, DNS5Be) and in 2 normal LCL (HH4, GM606). Cell survival was assessed by the ability of cells treated with 254nm UV (2.25-9.0J/m^2) to initiate microcultures in 96 well tissue culture plates (Kraemer et al Mutat Res 72:285,1980). The microculture initiating efficiency of the untreated LCL used was 10-100%. UV survival of DNS3Be and DNS5Be was similar to that of the normal lines. The cell mutation assay was performed by innoculating $4x10^4$ cells per well in medium containing 6-thioguanine and estimating the frequency of mutation by counting the number of microculture-containing wells after a 2 week incubation (Furth et al Anal Biochem 110:1-8,1981). Mutation frequency in all the untreated LCL was similar. DNS3Be and DNS5Be showed a 2- to 4-fold greater ($p<0.05$) frequency of induced mutants per surviving cell than the normal LCL following 6.7 and 9.0J/m^2 UV. This UV-induced hypermutability of DNS cells suggests that DNS patients may have an increased susceptibility to UV-induced somatic mutations. This abnormality may be etiologically related to their high frequency of melanomas.

1121 MUTAGENESIS IN MAMMALIAN CELLS STUDIED WITH A SHUTTLE-VECTOR SYSTEM, William C. Summers, Uma B. Dasgupta and Saumyen Sarkar. Yale University School of Medicine, New Haven, CT 06510

The goal of this project is to understand the molecular basis for mutagenesis in mammalian cells. We have constructed a shuttle vector plasmid which can replicate in mammalian cells and then can be assayed for mutant phenotypes in E. coli. Sequence analysis of the mutant gene is used to characterize the mutations which occurred during replication in the mammalian cells.

The vector is a derivative of E. coli plasmid PBR322 which provides a replication origin for growth in bacteria and the ampicillin resistance gene for selection in E. coli. The replication origin and T-antigen gene of SV40 are included to drive replication of the plasmid in mammalian cells. As a target for mutagenesis, we have inserted, at a unique EcoRI site, the 200 nucleotide fragment from plasmid πVX which encodes the supF gene (tyrosine-suppressor tRNA) of E. coli. The plasmid confers the suppressor phenotype on E. coli. Mutations in the tRNA gene which lead to loss of suppressor phenotype can be positively selected by the galactose killing method (only su^- cells with gal K (amber) gal E mutations can survive in galactose-glycerol medium). The 200 nucleotide EcoRI fragment is directly excised and sequenced to locate the mutation in the tRNA gene.

1122 MECHANISM OF ACTIVATION OF THE T24 HUMAN BLADDER CARCINOMA ONCOGENE. Mariano Barbacid, Eugenio Santos, Simonetta Pulciani and E. Premkumar Reddy. National Cancer Institute, Bethesda, Maryland 20205

It has been firmly established that a significant fraction of human tumors contain dominant transforming genes (oncogenes). Several of these oncogenes appear to have originated by activation of a family of evolutionarily conserved proto-oncogenes, generically designated as c-ras. Members of this proto-oncogene family have been occasionally transduced from the genome of rodents into retroviruses originating the well-characterized Harvey, Kirsten and BALB murine sarcoma viruses. A human oncogene present in T24 and EJ bladder carcinoma cell lines has been molecularly cloned. When this oncogene was compared to its homologue gene present in normal human cells, only DNA sequence analysis revealed the presence of minor genetic differences. Hybrid molecular clones containing different domains of the T24 oncogene and its normal counterpart were constructed and tested for their ability to transform NIH/3T3 mouse cells. These experiments helped to localize the genetic changes that led to the activation of the T24 oncogene to within a 930bp DNA fragment that encompasses putative 5' regulatory sequences and the first exon. Comparative sequence analysis of this region in the T24 oncogene and its normal human counterpart revealed a single base pair difference. A guanosine residue of the normal gene was substituted by a thymidine residue in the T24 oncogene. This point mutation resulted in the replacement of glycine for valine as the twelfth aminoacid residue of p21, the gene product of the T24 oncogene. These results demonstrate that mutagenesis plays a direct role in the activation of cellular oncogenes.

1123 GENE AND GENOME AMPLIFICATION IN MAMMALIAN CELLS FOLLOWING EXPOSURE TO 3.4 MeV ALPHA PARTICELS, Christine Lücke-Huhle, Ludwig Hieber, Nick Kennedy and Peter Herrlich, Kernforschungszentrum Karlsruhe, Institut für Genetik, Postfach 3640, D-7500 Karlsruhe 1, Federal Republic of Germany

Various mammalian cell lines (Chinese hamster V79, rat XC 81 and SV40 transformed human fibroblasts) were exposed to alpha particles emitted from an Americium-241 source. As the dose increased, the division probability of cells in the first cell cycle after irradiation decreased. This was due to an arrest in G2.

For Chinese hamster V79 cells we observed genome amplification (up to 4 fold) after doses causing irreversible arrest in 95 % of the cells. The amplification process was initiated 15-20 hrs after irradiation. Flowcytofluorometry and premature chromosome condensation analysis revealed that endoreduplicating cells reached a maximum 39 hrs after irradiation. At this time about 50 % of all cells had a DNA content highet than 4N. Polyploid cells in various phases were detected within the first post-irradiation cell cycle.

Since the relatively high dose of 4.38 Gy caused genome amplification, we investigated whether lower doses of alpha irradiation gave rise to the amplification of specific genes (SV40, mmtv, tk, dhfr). In addition, SV40 transformed human cell lines from normal donors and from patients suffering from either Xeroderma pigmentosum or Ataxia telangiectasia offered the possibility to investigate the importance of this phenomenon for repair functions.

1124 STRUCTURAL ALTERATIONS IN DNA DURING CHEMICAL CARCINOGENESIS, Daniel L. Stout and Frederick F. Becker, M.D. Anderson Hospital & Tumor Institute, Houston, TX 77030.

We have previously reported that DNA from N-2-acetylaminofluorene-induced premalignant, rat liver nodules and diethylnitrosamine/phenobarbital-induced γ-glutamyltransferase-positive rat liver foci show damage which progressed with increasing risk for malignancy. This damage manifested itself by an increased rate of DNA elution as determined by alkaline elution, when compared to normal rat liver. Since the alkaline elution technique cannot distinguish between double- and single-strand breaks or apurinic sites, additional methods have been used to characterize the structural alteration in DNA from these cell populations. Using BD-cellulose chromatography to separate DNA containing single-stranded regions from bulk duplex DNA, we found that DNA from hyperplastic nodules contained up to 5 times more single-stranded regions than did DNA from normal liver. However, when Tb^{3+} fluorescence was used to quantitate total single-stranded content, DNA from these liver lesions was found to contain only about twice as much single-strand as DNA from normal liver. Further, Exonuclease III, a double-strand specific 3' exonuclease with apurinic endonuclease activity, digests DNA from nodules 10-50 times faster than normal liver DNA. In toto, these data suggest that the DNA damage detected in the rat liver lesions by alkaline elution consist principally of gaps. However, because apurinic sites could contribute to both increased Tb^{3+} fluorescence and Exonuclease III sensitivity, the presence of these alkali labile lesions cannot be ruled out.

This work was supported by NCI Grant CA 20659.

1125 PROTECTIVE ROLE OF THIOLS IN CARCINOGEN INDUCED DNA DAMAGE IN RAT LIVER, John Y.H. Chan, Daniel L. Stout and Frederick F. Becker, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

In attempting to block the DNA damaging effects of carcinogens with thiol agents, we investigated the effects of diethylmaleate (DEM), a thiol depletor, and α-mercaptopropionylglycine (αMPG), a liver protective thiol, on DNA damage caused by the direct acting carcinogens N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and methylnitrosourea (MNU). By depleting liver thiols to more than 70%, DEM caused a 4-8 fold increase in DNA damage in rats treated with MNNG (ip) as measured by alkaline elution procedure. DEM alone did not cause any measurable DNA damage. However, DEM had little synergistic effect with MNU, despite a similar level of thiol depletion. Administration of αMPG, a synthetic thiol reported to have no effect on the covalent binding of drugs to macromolecules, but which prevents their deleterious effects caused a 70% decrease in DNA damage induced by MNNG. These data indicate that biological thiols are essential for the protection of DNA damage caused by MNNG in rat liver and that synthetic thiols such as αMPG may be useful in blocking that damage. Although MNU and MNNG are both direct-acting methylating compounds, our results suggest that they may have a distinct mechanism(s) of action in rat liver. This work was supported by NCI Grant CA 20657.

1126 ALTERATION OF DNA METHYLATION BY THE CARCINOGEN BENZO[A]PYRENE, Martin Wojciechowski and Thomas Meehan, Michigan Molecular Institute, Midland, MI 48640

Evidence has accumulated implicating DNA cytosine methylation at 5'-CpG-3' sites as a regulatory signal for gene expression in vertebrate systems. Methylation patterns are clonally and epigenetically maintained by enzymes recognizing hemimethylated sites, produced during DNA replication and subsequently methylated. Benzo[a]pyrene (BP) is a potent precarcinogen widely distributed in the environment. The active form of the carcinogen, 7*r*,8*t*-dihydroxy-9*t*,10*t*-epoxy-7,8,9,10,-tetrahydroBP (BPDE), preferentially alkylates guanine residues. Since carcinogen adducts probably occur at CpG-containing sites, BPDE binding may disrupt methylation patterns. To investigate this possibility, we have isolated a DNA methyltransferase from *Haemophilus parainfluenza*, and are using this enzyme as a model methylase. The enzyme methylates the internal C of the sequence CCGG, and has properties in common with mammalian methylases. The binding of BPDE to several DNAs inhibits the *in vitro* methylation of CCGG sites by the enzyme, and the inhibition is proportional to carcinogen binding levels over a wide range of covalent modification. This inhibition is greater than would be expected from the number of BPDE-modified CCGG sites alone. Thus, binding of BPDE to sequences other than CCGG results in an inhibition of methylation at CCGG sites, presumably by dissociating the processive enzyme upstream from methylatable sites. These results are consistent with the hypothesis that carcinogens may alter methylation patterns and in turn gene expression, possibly of endogenous onc genes, or other as yet unrecognized gene(s), involved in the transformation process. (Supported by the E. U. Pardee Foundation and NCI 07199).

1127 POLY (ADP-RIBOSE) IN ONCOGENIC TRANSFORMATION. Carmia Borek and James E. Cleaver, Columbia University, New York, NY 10032 and University of San Francisco, California 94143

DNA damaging agents including radiation and some chemicals carcinogens inhibit cellular glycolysis, decrease cellular NAD content and stimulate poly (ADP-ribose) synthesis. Compounds such as 3 amino benzamide prevent the decrease in cellular NAD content, inhibit the synthesis of poly ADP-ribose and may increase the frequency of mutations and sister chromatid exchanges.

Using the C3HT10½ cell line and short term cultures of hamster embryo cells, we have investigated whether inhibition of poly ADP ribosylation modifies radiogenic transformation. We find that in these 2 cell systems radiogenic transformation following exposure to 300 rad is significantly inhibited by benzamide and 3 aminobenzamide. Thus implicating cellular consequences of delaying ligation in the process of radiogenic transformation, and suggesting that some molecular events associated with mutagenesis and sister chromatid exchanges are different from those associated with transformation. Experiments in progress are evaluating the effects of benzamide and 3 aminobenzamide on chemically induced oncogenic transformation.

1128 CELLULAR AGE RESPONSE FOR KILLING AND MUTATION INDUCTION AFTER 4-NITROQUINOLINE-1-OXIDE, Regine Goth-Goldstein, Lawrence Berkeley Laboratory, Berkeley, CA 94720

The cell-cycle response for killing and mutation induction by 4-nitroquinoline-1-oxide (4NQO) which is considered a UV mimetic agent was measured in synchronous Chinese hamster ovary cells (CHO wild-type) and a UV-hypersensitive mutant derived from this line. For the wild-type a characteristic age response is seen for killing by 4NQO with maximum sensitivity in G_1 and resistance increasing through the S-phase. The induction of resistance to 6-thioguanine, ouabain and diptheria toxin is also highest in G_1. This characteristic age response which is very different from that observed after UV could be caused either by cell cycle variation in the metabolic activation of 4NQO or by cell cycle variation in the activity of the DNA repair process which removes potentially lethal and mutagenic lesions.

The UV-sensitive mutant, which was found to be also hypersensitive to 4NQO shows a relatively flat response to 4 NQO throughout the cycle for both killing and mutation induction. As the two cell lines should metabolize 4NQO equally well, the results indicate that the age response in the wild-type is due to a reduced repair of 4NQO induced lesions in G_1. This repair is absent in the mutant.

1129 DECREASED IN VITRO LIFESPAN OF FIBROBLASTS DERIVED FROM SKIN EXPOSED TO PUVA IN VIVO, David Jacoboson-Kram, Richard W. Gange, Judith L. Roe, John A. Parrish and Jerry R. Williams, The George Washington University School of Medicine and Health Sciences, Washington, DC 20037.
A prospective study of oral methoxsalen photochemotherapy, PUVA (8-methoxypsoralen plus 320-400 nm UV), has identified a population of persons at increased risk for non-melanoma skin cancers. Relative risk for treated patients is 8 times that of controls, while previous history of ionizing radiation treatment raises the risk to 25. We have initiated studies to determine cellular mechanisms which underlie the extreme sensitivity of some individuals to PUVA-induced carcinoma. We are in the process of studying several parameters of DNA repair in fibroblasts of treated patients and controls. To date we have initiated 6 cell lines from skin biopsies of a 42 year old female who has received extensive PUVA and x-ray therapy, developing 13 skin tumors. Cells derived from a biopsy site which was unexposed to either treatment senesced after 45 population doublings, while cells from PUVA + x-ray exposed biopsies senesced at 17 to 19 doublings. These observations indicate that progeny of cells exposed to PUVA in vivo retain a memory of this exposure which is manifested as a decreased in vitro lifespan. It is of interest that this highly mutagenic treatment results in neoplasia as well as in premature senescence in vitro.

1130 THE RELATIONSHIP BETWEEN DNA DAMAGE AND RECOMBINATION: MODELING AND ANALYSES OF SUCROSE GRADIENT SEDIMENTATION PATTERNS. Thomas A. Darden and Michael A. Resnick, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709.
The mechanisms by which unexcised DNA lesions are tolerated by cells undergoing DNA replication is a question of continuing interest. In bacteria the importance of recombinational mechanisms is well established. The evidence for recombinational exchanges in lower eucaryotes and mammalian cells has not been as clear, perhaps because of limitations in the sensitivity of the assays. However, the enhancement of sister chromatid exchanges by various mutagens and carcinogens suggests an association between recombination and DNA damage. A convenient and sensitive method for the detection of rare exchanges has been to look for covalent associations between stretches of newly synthesized DNA and of parental DNA containing enzyme sensitive lesions (e.g. UV induced pyrimidine dimers) induced before replication [Ganesan, J. Mol. Biol. 87 (1974) 103]. If exchanges have occurred, traditional analyses of the resulting alkaline sucrose sedimentation profiles based on molecular weight calculations may be misleading. A mathematical model encompassing exchanges due to repair events or to genetic recombination has been developed, which leads to new methods of analyzing sucrose gradient results. Using these methods, the nature and extent of recombination in various cellular systems are currently being investigated.

1131 EXPRESSION OF CLONED BACTERIOPHAGE T4 UVSW AND UVSY RECOMBINATION REPAIR GENES, Michael F. Laspia, JoAnne K. DeVries and Susan S. Wallace, New York Medical College, Valhalla, N.Y. 10595.
The expression of T4 uvsY and uvsW from hybrid plasmids containing part of the late region of the T4 genome has been studied. Certain of these hybrid plasmids express the uvsY or uvsW gene products as indicated by their ability to enhance the survival of UV-irradiated uvsY and uvsW mutant phage, respectively. One plasmid, which contains both the uvsY and uvsW genes, was found to increase the survival of UV-irradiated, MMS-treated or EMS-treated recA hosts. Whether this protective effect is due to complimentation of recA function or to inhibition of recB,C degradation of the host chromosome is being examined. Identification of the uvsY and uvsW gene products is currently being examined following UV-irradiation of maxicell strains containing the hybrid plasmids. Presumptive assignation of a 16,000 kdal protein to uvsY is being confirmed.

These studies were supported by CA33657 awarded by the NCI U.S. DHHS.

1132 Carcinogen stimulated recombination in E. coli. William D. Taylor, Cynthia Luisi-DeLuca and Ronald D. Porter. Penn State Univ., University Park, PA 16802

We have discovered that acetoxyacetylamino fluorene and other carcinogenic mutagens stimulate a non-reciprocal recombination between a gene on a plasmid and an homologous gene on the E. coli genome. Carcinogen treatment of the plasmid in vitro followed by transformation into recipient cells carrying a deletion of the gene results in a very low frequency of mutation of the plasmid gene.

When the recipient cells carry the homologous gene, the level of mutations in the carcinogen treated plasmid appears to be much higher, but we have shown that these apparent mutations are actually due to carcinogen stimulated recombination between the two gene copies. The recombination does not generate mutations per se, but merely transfers the allele from the cellular genome to the plasmid. The level of this stimulation depends on the amount of carcinogen bound to the plasmid DNA. This stimulation is totally dependent on a functional recA gene in the recipient cell, but the effect does not depend on either lexA or uvrA. It is suggested that this strong stimulation of non-reciprocal recombination by carcinogens could play a major role in carcinogenesis.

1133 CHARACTERIZATION OF A NEW RADIATION-SENSITIVE MUTANT, Escherichia coli, K-12 radC, Israel Felzenszwalb[1], Neil J. Sargentini and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford CA 94305, [1]On leave from the: Instituto de Biologia, Universidade do Estado do Rio de Janeiro, RJ 20551, Brazil

After N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis of Escherchia coli K-12 (xthA14), an X-ray sensitive mutant was isolated. The sensitivity is due to a mutation, radC, which is located at 80.7 min on the E. coli linkage map. For X radiation, but not for UV radiation, radC sensitization was dependent upon the cells being grown in and plated on rich medium. For this reason, all characterization studies were performed using cells grown in and plated on rich medium. Compared to the wild-type strain, the radC mutant is sensitive to X-rays and to UV radiation. The radC mutant was normal for gamma radiation mutagenesis, but showed less UV radiation mutagenesis than the wild-type strain; it showed normal amounts of X-ray and UV radiation-induced DNA degradation, and it was~60% deficient in recombination ability. The radC gene product is suggested to play a role in Type III DNA repair after X irradiation and in postreplication repair after UV irradiation for the following reasons: the radC strain was normal for Host Cell Reactivation of gamma- and UV-irradiated bacteriophage λ ; the radC mutation did not sensitize a recA strain, but did sensitize a polA strain to X and UV radiation; the radC mutation sensitized a uvrA strain to UV radiation. (Research supported by Public Health Service research grant CA-06437 and research program grant CA-10372 from the National Cancer Institute. One of us (I.F.) is supported by funds from International Atomic Energy Agency, BRA/8101 and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, 200018-81BF.)

1134 RECOMBINATION AND REPAIR OF NONREPLICATING UV-IRRADIATED DNA, John B. Hays, Theodore A.G. Smith, Anton Alldrick, and Gary Hellman, University of Maryland Baltimore County, Catonsville, MD 21228

Repair and recombination in the absence of DNA replication are assayed by a two-step procedure: (i) infection of E. coli homoimmune lysogens by UV-irradiated λ duplication phages (repressed infection); (ii) extraction of phage DNA, measurement of recombination (transfection of recA recB spheroplasts, EDTA assay for loss of chromosomal duplication) and/or repair (infectivity in transfection of uvrB recA recB spheroplasts, frequency of sites sensitive to dimer-specific M. luteus UV endonuclease). Most, but not all UV-stimulated recombination is due to excision repair of pyrimidine dimers: recombination is 1/4 normal levels in uvrA,B,C mutants; acetophenone-sensitized 313 nm radiation (dimers predominant photoproduct) stimulates recombination; photoreactivation reduces recombination at least 50%. Recombination, negligible in recA repressed infections, is slightly lower in recB cells (2/3 normal) and reduced drastically in recF (1/5 normal). Recombination is 3/5 normal in the presence of chloramphenicol plus rifampin. Repair of nonreplicating DNA (restoration of infectivity), reduced about 50% in recA and recB infections, is 1/4 normal in recA recB; repair is normal in recF cells. Repair is 1/10 as efficient at 30° as at 40°, and is almost completely inhibited by rifampin plus chloramphenicol. Restoration of infectivity correlates with removal of dimers (UV-endonuclease-sensitive sites).

1135 THE ROLE OF E.Coli GYRASE IN CUTTING IN TRANS, Era Cassuto, Stephen West and Paul Howard-Flanders, Yale University, New Haven, CT 06511

Cutting in trans has been described as the cutting of covalent circular λ DNA which occurs in response to infection of a recombination proficient E.Coli λ lysogen with undamaged and crosslinked λ phage. More recently several authors have reported a reduction of recombination and repair frequency in cells lacking gyrase B-. We have observed in a gyrase B ts mutant, cutting in trans is completely inhibited at non permissive temperature and is greatly reduced in WT cells treated with coumermicyn, an antagonist of gyr B_T. The role of gyrase in cutting in trans is studied in experiments involving purified gyrase and recA protein, covalent circular DNA and homologous gapped DNA.

1136 THE ENZYMATIC REACTIONS OF RECOMBINATIONAL REPAIR. Paul Howard-Flanders, Steve West, Era Cassuto, Jill Countryman & James Rusche. Yale University, Box 6666 New Haven, CT06511. We now know some of the important intermediate and final structures in postreplication and recombinational repair. These include circular duplex DNA molecules containing postreplication gaps, homologous duplexes nicked in the cutting in trans reaction, circular duplexes joined by a single strand Holliday crossover in a figure 8, and recombinant circular duplexes. Postreplication gaps formed by the replication of DNA containing damaged bases, initiate sister exchanges in vivo with high efficiency. In studies with purified enzymes, recA protein binds strongly to single stranded regions in gapped duplex DNA and promotes homologous pairing with intact duplexes to produce joint molecules, which are stabilized if the structure permits heteroduplex formation. When suitably gapped and nicked duplex molecules are incubated together, recA protein transfers a 3' ended complementary strand into the single stranded gap and so initiates a strand exchange and branch migration. Branch migration involving three strands requires single strand binding protein (Cox & Lehman, 1981), whereas reactions involving four strands proceed in its absence, forming two heteroduplexes linked by a Holliday crossover. RecA mediated reactions enable the construction of biparental figure 8 molecules from two genetically marked plasmids. Figure 8 molecules containing a Holliday crossover are biologically active. They represent an intermediate structure in recombinational repair and, when transfected into recA bacteria, they are resolved into progeny plasmids of both recombinant and parental types. (see abstract: West et al.).

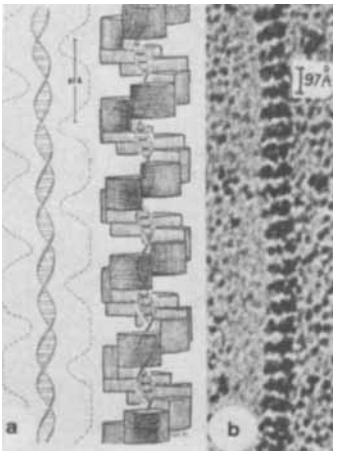
1137 A SYSTEM FOR ANALYZING THE BIOCHEMISTRY OF DNA RECOMBINATION, James R. Rusche and Paul Howard-Flanders, Yale University, New Haven, Ct., 06511.

Homologous recombination between DNA molecules can be envisioned to occur in three biochemically distinct phases: i. pairing of partially duplex DNA with an intact homologous duplex DNA ii. incision of the intact DNA duplex allowing reciprocal exchange of strands and iii. resolution of the strand exchange structure to produce two recombinant DNA molecules. While proteins from both procaryotic and eucaryotic cells have been shown to pair homologous DNA strands (phase 1), there is scant biochemical evidence to delineate the latter two phases of the strand exchange process.

We have developed a sensitive assay for recombinant formation between two E. coli plasmids that contain mutations in an antibiotic resistance gene. Plasmids containing site specific nicks or single strand gaps are subsequently incubated in vitro with fractionated cell extracts. Any molecules that recombine in vitro can be quantitated by transfection of the incubation mixture into a recombination deficient strain.

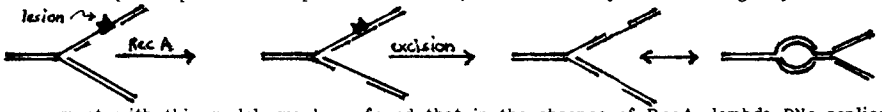
1138 STRUCTURE OF RECA-DNA COMPLEXES, A.Stasiak, E.DiCapua, Th.Koller, Institute for Cell Biology, ETH, Zürich

Purified recA protein in the presence of ATP and Mg promotes the complete recombination process including homologous recognition and strand transfer. Thus the study of the structure of recA-DNA complexes will give insight into the molecular mechanism of recombination and the structure of recombining DNA molecules. In the presence of ATPγS recA-DNA complexes are stable. The structure of the DNA in these complexes ressembles the structure of intercalated DNA. It is stretched by a factor 1.5 and is unwound from 10.5 base pairs per turn into 18.5 base per turn thus following the protein helix visible in EM (fig.b.), which contains 1 recA per 3bp. (Stasiak & DiCapua(1982) Nature 299,185). The DNA seems to be located in the central part of the complex as it is protected against DN-ases(see model fig.a.). Since it is likely that the DNA in these complexes has a configuration ready for homologous recognition or for strand transfer we intend to relate this structure to the mechanism of recombination.



1139 ISOLATION OF PUTATIVE POST-REPLICATIONAL DNA REPAIR INTERMEDIATES FROM BOTH E. COLI AND BACTERIOPHAGE LAMBDA, Manuel S. Valenzuela, Biophysics Laboratory, University of Wisconsin, Madison, WI 53706

Multiply branched DNA structures of the type shown in the diagram (where arms b&c are homologous) were found among DNA replicative intermediates of E. coli and phage lambda (1). The condition that leads to their formation appears to be thymidine deprivation (2). More recently we have observed that in a RecA$^-$ background, these molecules are absent, indicating that a recombinational step may be required for their formation. Based on this requirement and the fact that DNA lesions accumulate under thymidine starvation conditions, we would like to propose that multiply branched molecules are the result of a snap-back reaction of a post-replicational repair intermediate, as indicated by the following sequence:



In agreement with this model, we have found that in the absence of RecA, lambda DNa replicative intermediates show a high incidence of growing points with internal single stranded gaps.

(1) Valenzuela, M.S. & R.B. Inman (1981) Mol. Gen. Genet. 184: 450.
(2) Valenzuela, M.S. & R.B. Inman (1981) Nucleic Acids Res. 24: 6975.

1140 AMPLIFIED EXPRESSION OF THE E. coli recB AND recC GENES, AND PURIFICATION AND CHARACTERISATION OF THE GENE PRODUCTS, Peter T. Emmerson, Karen E. Atkinson, Linda Hutton and Ian D. Hickson, The University, Newcastle upon Tyne, UK.

Expression of the recB and recC genes has been amplified by individually subcloning them downstream of the phage lambda leftward promoter in the plasmid pPLc24 (Remaut et al., Gene 15, 81-83, 1981). The RecB and RecC proteins could be readily seen as prominant bands on SDS-PAGE analysis and amounted to about 1 or 2 percent of total cell proteins, an approximately 2,000-fold amplification. These proteins have been purified and their individual and combined properties are currently being investigated. To amplify the RecBC DNase in vivo and to facilitate purification of the native enzyme a plaque forming lambda phage has been constructed which carries recB and recC together with Sam7 and Eam. In addition to roles in DNA repair and genetic recombination, the recB and recC genes are involved in transposon excision. Mutations texA343 and texA344 isolated on the basis of increased precise transposon excision (Lundblad and Kleckner, in Molecular and Cellular Mechanisms of Mutagenesis, Lemontt and Generosa, Eds., Plenum Press, 1982) are complemented by plasmids carrying recC$^+$ and recB$^+$ respectively but not by those in which these genes are inactivated by insertion of Tn1000.

1141 SUPPRESSION OF THE recF GENE OF ESCHERICHIA COLI BY MUTATIONS LINKED TO recA, Michael R. Volkert and Margaret A. Hartke, Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 27709.

In recB recC sbcB mutant strains of E. coli, genetic recombination and several pathways of DNA repair require the recF gene. In wild-type genetic backgrounds recF has no detectable effect on recombination, but causes sensitivity to UV and several other DNA-damaging agents. srfA mutations are newly isolated suppressors of recF mapping in or very near recA. They restore both genetic recombination and DNA repair to recB recC sbcB recF mutant strains. srfA mutations partially suppress UV sensitivity of recF single mutants, and suppress more completely the UV sensitivity of a uvrA recF strain without effect on a uvrA single mutant. Moreover, since srfA mutations also have no effect on recB recC sbcB mutant strains, srfA mutations are specific suppressors of recF. srfA mutations raise the possibility that recA protein can be altered to carry out recombination and DNA repair in the absence of recBC or recF activities, or that a gene which is tightly linked to recA can be altered to substitute for recF.

1142 GENETIC AND MOLECULAR BASES FOR THE TWO INDEPENDENT PATHWAYS OF POSTREPLICATION REPAIR IN UV-IRRADIATED ESCHERICHIA COLI K-12, Tzu-chien V. Wang and Kendric C. Smith, Dept. of Radiology, Stanford University School of Medicine, Stanford, CA 94305

Based on genetic studies, two major independent pathways of postreplication repair have been reported (Wang and Smith, MGG 183, 37, 1981). While both pathways require functional recA and ssb genes, one pathway is dependent on the recF gene and the other is dependent on the recB, lexA and uvrD genes. Sedimentation analysis of DNA from UV-irradiated uvrB cells suggests that the recF-dependent pathway is a gap-filling repair (GFR) process that repairs DNA daughter-strand gaps directly, and the recB-dependent pathway is a sister-duplex recombination (SDR) process that involves double-stranded exchanges at sites of homology between two duplex DNA molecules to bypass unrepaired DNA daughter-strand gaps. A mutation at umuC sensitizes uvrB, uvrB recB and uvrB recF cells to UV irradiation, but not uvrB recB recF cells. A mutation at umuC inhibits a small portion of GFR, but does not appear to affect the SDR process. We suggest that the umuC gene product is involved in a minor recF-independent GFR process. (Research supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute.)

1143 A NEW MUTATION (mmrA1) IN ESCHERICHIA COLI K-12 THAT PREVENTS THE RICH-MEDIUM INHIBITION OF POSTREPLICATION REPAIR AFTER ULTRAVIOLET IRRADIATION, Rakesh C. Sharma, Neil J. Sargentini and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305

Ultraviolet-irradiated uvrA, uvrB and uvrC cells of E. coli K-12 showed higher survival if plated on minimal growth medium rather than on rich growth medium. We have called this phenomenon "minimal medium recovery" (MMR) (K.C. Smith, 1971, Photophysiology 6, 209-278). MMR is not observed in a uvrA strain carrying an mmrA1 mutation, and the uvrA mmrA strain showed the same survival on minimal and rich growth medium plates as the uvrA strain showed on minimal medium plates. The mmrA1 mutation was isolated as a hidden mutation from a uvrA6 polA1 strain, and was mapped at 84.3 min on the E. coli K-12 linkage map. When compared to the uvrA strain, the uvrA mmrA strain did not show the postirradiation inhibition by rich growth medium of the repair of DNA daughter-strand gaps. However, both strains were similar in their ability to repair DNA daughter-strand gaps if compared in minimal growth medium. These results are consistent with the idea that the mmr gene product is not involved directly in the repair of DNA lesions, but prevents rich growth medium from inhibiting a portion of postreplication repair. (Supported by USPHS research grant CA-02896 and research program project grant CA-10372 from NCI, DHHS).

1144 THE ROLE OF REPAIR GENES DURING MEIOSIS IN YEAST. Michael A. Resnick*, John Nitiss*+, and John C. Game•, *National Inst. of Environmental Health Sciences, Research Triangle Park, N.C. 27709, +Illinois Inst. of Technology, Chicago Ill. 60616. •Dept. of Medical Physics, Univ. of California, Berkeley, 94720.

The process of meiosis in eukaryotes includes a period of intense genetic activity. Since intermediates in meiotic recombination might be expected to occur during repair in mitotic cells, it is not surprising that some mitotically defined repair mutations affect normal meiosis. The excision repair pathway as defined by mutants of the RAD1 gene is not required for meiosis; however, it functions to remove UV-induced damage during meiosis.

The pathway identified with the repair of ionizing radiation damage in mitotic cells is essential to normal meiosis. Of particular interest is the RAD52 gene which is essential in mitotic gene conversion and the repair of double-strand breaks. The rad52-1 mutant begins to die with the onset of the meiotic round of DNA synthesis and no recombination is observed among the cells recovered, thus strongly implicating the RAD52 gene in normal meiotic recombination. At the molecular level single-strand interruptions (SSI's) are observed in meiotic rad52-1 cells and their frequency (of the order of 200 per cell) is comparable to that of recombination in wild-type cells. The regions of these rare SSI's are being isolated using sucrose gradients containing restriction enzyme layers. There are major differences in the DNA recovered from mitotic wild type and rad52-1 cells and from mitotic vs. meiotic cells. Comparable results have been obtained with a mutant of the RAD57 gene which is also involved in double-strand break repair.

1145 CLONING OF THE RAD52 GENE OF SACCHAROMYCES CEREVISIAE, David Schild,* Boyana Konforti,* Carl Perez,* Warren Gish,† and Robert Mortimer,* Dept. of Biophysics and Medical Physics †Dept. of Molecular Biology, University of California, Berkeley, CA 94720 USA

The RAD52 gene of Saccharomyces cerevisiae has previously been shown to be involved in both recombination and DNA repair. Here we report on the cloning of this gene. A plasmid containing a 5.9kb yeast insert in the YEp13 vector has been isolated and shown to complement the X-ray sensitive phenotype of the rad52-1 mutation. From this insert various fragments have been subcloned into the YRp7 vector. A spontaneous integration event of one of the subclones has been genetically mapped to the chromosomal location of RAD52, indicating that the structural gene has been cloned. A 1.97kb BamH1 fragment subcloned into YRp7 in one orientation has been shown to complement the rad52-1 mutation, while the same fragment in the opposite orientation fails to complement. Various other subclones indicate that a BglII site, in the BamH1 fragment, is in the RAD52 gene. This BglII site has been deleted by S1-nuclease digestion and the resulting deletion inactivates the RAD52 gene. This deletion is presumed to cause a frameshift and is being used to determine whether RAD52 codes for an essential function; if it is a non-essential gene, we plan to isolate a strain totally lacking any RAD52 activity to determine whether rad52-1 is a leaky allele. BAL31 deletions from one end of a 1.9kb Sal1-BamH1 fragment have been isolated; up to ~.9kb can be deleted without loss of RAD52 activity, demonstrating that the RAD52 gene is approximately 1kb or less in length. We are currently using Northern analysis to determine whether RAD52 is transcriptionally regulated, and if so, what conditions induce it.

1146 THE IDENTIFICATION OF A DEOXYRIBONUCLEASE CONTROLLED BY THE RAD52 GENE OF SACCHAROMYCES CEREVISIAE. Terry Chow and Michael A. Resnick, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709.

Genetic and biochemical studies on rad52 mutants implicate the RAD52 gene product as having a key role in mitotic and meiotic recombination, as well as in DNA double-strand break repair. As part of our investigations, we have examined deoxyribonuclease levels in extracts of wild-type and rad52 mutants and have observed no significant differences. However, major differences were observed when we employed anti-serum raised against a purified single strand DNA-binding endo-exonuclease from Neurospora crassa in assays developed in this lab. As much as forty percent of the alkaline deoxyribonuclease in wild-type extracts exhibited immunocrossreactivity, whereas none was found in extracts from rad52 strains. This DNase activity was also followed through meiosis; maximum activity was observed in wild-type cells, at a time corresponding to an early stage of premeiotic DNA-synthesis and commitment to recombination. This DNase activity was not found in meiotic rad52 strains.

This immunocrossreactive DNase has been partially purified (> 200 X's) with DEAE-Sepharose, hydroxylapatite, and SS-DNA-cellulose chromatography. The DNase is Mg^{2+} dependent and exhibits endo-exonucleolytic activity with SS-DNA and exonucleolytic activity with DS-DNA.

1147 CLONING OF A DNA REPAIR GENE IN SACCHAROMYCES CEREVISIAE. Louise Prakash and Renata Polakowska, University of Rochester School of Medicine, Rochester, NY 14642

The rad6 mutants of the yeast Saccharomyces cerevisiae appear to be similar in many ways to the recA mutants of Escherichia coli. The rad6 mutants are highly sensitive to UV, X-rays and alkylating agents and are defective in induced mutagenesis by all these agents. The rad6-1 mutation, a nonsense allele, enhances the UV sensitivity of the excision defective mutants and results in defective postreplication repair of UV induced DNA damage. The rad6 mutants are proficient in genetic recombination, and thus are like the lexB30 allele of recA, which is also recombination proficient. In order to gain insight in the structure, function and regulation of the RAD6 gene, we have isolated a fragment of DNA on a recombinant plasmid, YEp13, which restores resistance to UV, gamma rays, and MMS to both rad6-1 and rad6-3 mutants. The cloned DNA segment complementing rad6 was transferred to an integrating plasmid containing the yeast URA3 gene, and then used to determine the site of recombination of the cloned DNA. Genetic evidence indicates that the cloned segment integrates at the rad6 locus. Subcloning of this segment has yielded a 1.9kb fragment which still functions in complementation of rad6. When this fragment is used as a probe for hybridization to total yeast RNA, two transcripts are observed. Experiments are in progress to define the rad6 transcript and study its regulation.

1148 RECOMBINATION OF PARENT AND DAUGHTER STRAND DNA AFTER UV-IRRADIATION (UVR) IN MAMMALIAN CELLS, A.J. Fornace Jr., NIH, Bethesda, Maryland, 20205

During DNA synthesis after UVR in bacteria, parental DNA containing pyrimidine dimers has previously been shown to recombine with daughter strand DNA by a recA dependent mechanism. When growing mammalian cells were UV-irradiated and then incubated with labeled thymidine (tdr), pyrimidine dimers, measured by endonuclease sensitive sites (ESS) produced by a dimer specific endonuclease from M. luteus, were initially interpreted to be in the newly synthesized DNA. Further investigations revealed that most or all of these ESS were actually only adjacent to newly synthesized DNA and in daughter strand DNA present prior to UVR. In order to demonstrate clearly such a recombination event, cells were irradiated in the G_0 or G_1 phases of the cell cycle and then allowed to enter DNA synthesis. Pyrimidine dimers were measured by a recently developed very sensitive assay using alkaline elution to detect ESS. Human peripheral blood lymphocytes (PBL), normal human (NF) and Group A xeroderma pigmentosum fibroblasts, and 3T3 cells were irradiated and then stimulated to enter DNA synthesis by addition of phytohemagglutinin or release from contact contact inhibition. [^{14}C]-tdr was added to cells 20h after UVR and the cells were analysed 35h after UVR. The frequency of ESS in daughter strand DNA increased with UV dose in all cells and was 1-3% of the ESS estimated to be present in the parent DNA. After 4 Jm^{-2}, 0.2, 1.2, or 1.3 ESS/10^9d of DNA were detected in the newly synthesized DNA of NF, 3T3 cells, or PBL respectively, and 1.0 ESS/10^9d after 1 Jm^{-2} in XP. Analysis by fluorescence activated cell sorter analysis, incorporation studies, and autoradiography indicated that the cells were irradiated prior to S phase. Appreciable incorporation into parent strand DNA by repair synthesis was also excluded.

1149 RESTORATION OF NORMAL RESISTANCE TO KILLING AND POST REPLICATION RECOVERY (PRR) BY TREATMENT OF MUTANT CHINESE HAMSTER OVARY CELLS (CHO-UV-1) WITH DNA FROM WILD TYPE CHO-K1 CELLS, C. Waldren[1], Denise Snead[1] and T. Stamato[2], [1]Eleanor Roosevelt Institute, Denver, CO 80262 and [2]The Wistar Institute, Philadelphia, PA 19104.

CHO-UV-1, a mutant derived from CHO-K1, is hypersensitive to killing by a number of agents including ultraviolet light (UV) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). UV-1 has normal UV-induced excision repair but it is defective in PRR. We now show that treatment of UV-1 with a co-precipitate of calcium phosphate and CHO-K1 DNA, followed by selection in MNNG, produces clones with normal resistance to killing by the above agents and normal PRR. Resistant clones occurred, in two separate experiments, at a frequency of approximately 10(-5) DNA-treated cell. Neither spontaneous nor mutagen-induced reversion to normal resistance has been observed in more than 10(8) UV-1 cells tested nor has DNA from UV-1 produced resistance. D_{50}, the dose of MNNG which reduces the plating efficiency to 50%, ranged from 0.05 to 0.15 μgm/ml for the eight clones tested, compared to 0.15 μg/ml and 0.003 μg/ml for CHO-K1 and UV-1 respectively. Resistant clones have been in culture for more than six months without change in phenotype. Cytogenetic analysis has not revealed notable differences between the chromosomes of resistant clones and UV-1. We conclude that genetic material, possibly repair genes, from CHO-K1 has been stably integrated into the genome of UV-1, conferring normal resistance to killing and correcting defective PRR. Transfection has now been carried out with human DNA and CHO-K1 DNA combined with retrievable bacterial DNA which should allow purification and characterization of repair genes. ERICR contribution no. 415. Supported by ES02808, ES02274, ES02470 and MOD 15-10.

1150 THE EFFECT OF CAFFEINE ON INITIATION OF DNA REPLICATION IN B16CL4 MOUSE MELANOMA CELLS. Helene Z. Hill, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

Caffeine (CAF), while non-toxic itself, enhances the lethality of other agents. This study using undamaged cells was undertaken to better understand the mechanism of action of CAF. DNA pulsed-labelled with ^{3}HdThd in the presence of CAF is smaller and more homogeneous than control DNA. It has been suggested that this is due to stimulation of initiations at abnormally frequent sites. These experiments were designed to compare distances between initiation sites using B16CL4 mouse melanoma cells. DNA pulse-labelled in the presence of CAF compared to similarly sized DNA pulsed in its absence should chase into the high MW fraction more rapidly than the control, if the initiation sites are more frequent, or more slowly, if they are less frequent, provided that the effects of CAF are rapidly reversed once it is removed from the medium. Preliminary experiments indicated that DNA synthesis, which is about 50% of normal in the presence of 8mM CAF, returns to 90% of normal within 5 min after removal of CAF. Pulse times were designed so that sucrose density gradient profiles of newly synthesized DNA were similar re distribution of label in various MW classes. This required a shorter labelling period for cells with less or no CAF. The small MW DNA synthesized in the presence of 1mM CAF moved into the high MW fraction slower than control DNA, while that synthesized in 4mM CAF entered the high MW fraction at the same rate as the control DNA. These results suggest that initiations that occur in the presence of CAF are not more spatially frequent than normal. The uniform, symmetric nature of the small MW peak produced by pulse-labelling in the presence of CAF may be due to the synchronous initiation of replicon clusters that normally initiate in an asynchronous manner.

1151 *ARREST SITES FOR DNA REPLICATION IN VIVO IN UV-IRRADIATED SV40*, *James M. Clark and* Philip C. Hanawalt, Biological Sciences, Stanford University, Stanford, CA 94305
We are using SV40 to study the process by which mammalian cells replicate DNA containing UV-induced photoproducts. We are focusing on a particular sequence of SV40 near the origin of replication in order to locate sites at which replication is arrested in vivo. Intracellular replication of a temperature-sensitive mutant of SV40 (tsA58) in CV-1P monkey kidney cells is synchronized using temperature shifts plus aphidicolin treatment prior to irradiation with 254 nm UV light. We use sequential CsCl and CsCl/ethidium bromide gradients and BND-cellulose chromatography to purify viral replicative intermediates which are then cleaved with MspI and end-labeled with ^{32}P to provide a unique site from which to map arrest sites downstream. ^{32}P-labeled nascent DNA is separated into leading and lagging strands by hybridization to the appropriate mercurated single strand M13-SV40 hybrid DNA probes, purified away from non-hybridized DNA by sulfhydryl-cellulose chromatography, and then analyzed on denaturing polyacrylamide sequencing gels. Discrete, UV-specific, arrest sites are identified and their positions located with respect to the template strand by comparison of nascent DNA chain lengths with DNA sequencing ladders. Concurrent studies on Form I DNA isolated from UV-irradiated cells map the locations of pyrimidine dimers in the template strand with respect to the MspI site. Correlations between the locations of UV-specific arrest sites and pyrimidine dimer sites in the DNA template will be presented.

1152 SV40 REPLICATIVE INTERMEDIATE DNA FROM UV-IRRADIATED INFECTED CELLS, James H. White and Kathleen Dixon, UCLA, Los Angeles, CA 90024, NCI-FCRF, Frederick, MD 21701.
To investigate the mechanism by which pyrimidine dimers are accommodated during SV40 DNA replication, replicative intermediate (RI) DNA was analyzed following UV irradiation of infected cells. RI DNA was isolated by passage of total SV40 DNA over BND cellulose columns. A UV fluence inducing 1 pyrimidine dimer/genome equivalent of SV40 DNA (d/g) did not significantly reduce the incorporation of ^{3}H-dT into RI DNA in continuous labels up to 45 min post-UV, while 2 d/g reduced it only slightly. At the same time, incorporation into completed molecules was reduced but by less than the amount expected for completion of only undamaged templates, indicating that some dimer-containing templates are completely replicated (Stacks and Dixon, this volume). Analysis of lengths of daughter strands in late RI molecules after cleavage with the restriction enzyme Bgl I (which cleaves SV40 DNA only at the replication origin) suggests that replication does not proceed past the normal terminus after UV.

The most prominent species of molecule in RI DNA, labeled from 30 min to 1 h after 1 d/g, migrates as a relaxed circle on agarose gels and in sucrose gradients. Due to their behavior on BND cellulose, these molecules are presumed to contain gaps. These gapped molecules contain discontinuities in their daughter strands, and their abundance and specific activity also suggest that they are replicative products rather than excision repair intermediates. Electron microscopy of total RI DNA suggests that these relaxed circles are the only new structure induced in appreciable amounts after UV.

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1153 REPLICATION FORK MOVEMENT ON UV-DAMAGED SV40 DNA TEMPLATES, Susan W. Barnett, Elliot Landaw, and Kathleen Dixon, University of California, Los Angeles, CA 90024.
The rate of DNA replication fork movement on UV-damaged SV40 templates was measured by following a protocol fashioned after the experiments of Danna and Nathans (1). SV40-infected cells were UV-irradiated and then pulse-labeled with ^{3}H-thymidine. Form I completed molecules were isolated, digested with restriction enzymes and the relative intensity of labeling along the SV40 genome was determined. Our previous work indicates that UV-irradiation inhibits total SV40 DNA synthesis in a fluence-dependent fashion, and that this inhibition is most pronounced in the production of form I molecules. Mathematical modeling was employed to test models for describing how replication fork movement is affected by pyrimidine dimers in template strands. We were primarily concerned with distinguishing between two mechanisms of inhibition: 1) Specific blockage of replication forks by the presence of dimers in a subset of DNA templates, and 2) an overall slowdown in fork movement affecting all replicating molecules in an irradiated population. Data from both the relative intensity of labeling along the SV40 genome and the incorporation of label into completed molecules were analyzed to distinguish between the two models. The results of this analysis indicate that there is not a uniform slowdown of replication on all molecules; instead, there appears to be specific blockage on some template molecules, presumably those containing dimers.

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(1) K.J. Danna and D. Nathans, Proc. Natl. Acad. Sci. U.S.A. 69, 3097 (1972).

1154 PYRIMIDINE DIMERS PERSIST IN FULLY REPLICATED SV40 DNA, Pamela C. Stacks and Kathleen Dixon, Dept. Biological Chemistry, University of California, Los Angeles, CA 90024.

When SV40-infected TC7 cells are UV-irradiated with fluences introducing 1-3 pyrimidine dimers per SV40 genome, subsequent SV40 DNA replication is inhibited. This reduction appears to be due in part to blockage of replication by pyrimidine dimers. However, a careful analysis of progeny molecules replicated after UV irradiation reveals that a large proportion of these molecules contain pyrimidine dimers. These newly replicated molecules were separated from the bulk of unreplicated viral DNA by labeling with BUdR after UV irradiation and then recovering hybrid density molecules from CsCl density gradients. Pyrimidine dimers were present in 38% and 63% of completed form I molecules made 30-180 min after a UV fluence yielding 1 and 2 dimers per SV40 genome respectively. If a UV-induced recombinational mechanism is responsible for the introduction of pyrimidine dimers into hybrid density progeny molecules, this exchange must involve less than 180 base pairs of fully light DNA (based on limits of resolution in our alkaline CsCl gradients).

Replication of SV40 DNA continues to decline as a function of time after UV irradiation of infected cells. This appears to be due in part to a marked reduction of entry of new molecules into the replication pool. When cells are labeled with ^{3}HdT before UV irradiation, and BUdR is added 30 min thereafter, a much larger proportion of the ^{3}H-label remains as fully light density than in unirradiated controls.

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1155 **UV-INHIBITION OF SIMIAN VIRUS 40 REPLICATION,** **Howard J. Edenberg, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223.**

Ultraviolet light (UV) can cause mutations, cancer, or cell death. These cellular responses can best be analyzed after first determining the mechanism by which UV inhibits DNA synthesis. I am examining the events that occur when replication forks encounter UV-lesions in the template DNA of simian virus 40 (SV40), which replicates in the nucleus of monkey cells using the cellular enzymatic machinery. UV inhibits elongation of nascent DNA strands; the residual synthesis decreases rapidly with time after irradiation, and at all times the extent of incorporation of ^{3}H-dT into DNA decreases with increasing UV fluence. The completion of replication is inhibited even more severely than is elongation; post-UV incorporation is predominantly into replicative intermediates. The small fraction of Form I molecules completed after UV contain post-UV label predominantly in the terminal portions, as expected if they represent molecules largely completed before UV. DNA strands made after UV are approximately the size of parental DNA which has been cleaved at pyrimidine dimers by a UV-endonuclease, indicating that they do not extend past dimers. The hypothesis that replication forks are halted upon encountering pyrimidine dimers in the template strand is consistent with these data.