MECHANISMS AND CONSEQUENCES OF DNA DAMAGE PROCESSING

Organizers: Errol Friedberg and Philip Hanawalt

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Mechanisms and Consequences of DNA Damage Processing

Introductory Lecture

E 001 RETROSPECTIVE PERSPECTIVES ON DNA REPAIR

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Many important advances since the first UCLA Symposium on this topic 14 years ago are providing a solid mechanistic basis for understanding the biological consequences of damaged cellular DNA. Among the highlights at Squaw Valley in 1974 was the first presentation of the "SOS hypothesis" (1) and an early report of a procedure for the *in vitro* construction of recombinant plasmid DNA molecules (2). Since that time the approaches of recombinant DNA have become an essential technology in this field. Many genes involved in DNA repair have been cloned and their protein products are being characterized. Functional complexes that carry out repair *in vitro* have been assembled. Specially constructed shuttle vectors are being used to probe the mechanisms of repair and mutagenesis at the DNA sequence level. We now fully appreciate that the processing of damaged DNA is as essential a transaction as are replication and transcription. The subject of DNA repair has come of age in that it is now treated in a comprehensive textbook (3).

One of the areas of current interest to my colleagues and me has been the role of chromatin structure and gene expression in the accessibility of DNA damage to repair enzymes in mammalian cells. This topic will be briefly reviewed with emphasis upon the preferential repair of expressed genes (4-6) and our recent discovery of selective repair in the transcribed strand of an active gene (7). These results combined with those that demonstrate translesion synthesis or lesion persistence (8) lead us to suggest that repair of most bulky DNA lesions is needed to permit transcription of essential genes but that it is not necessary for the completion of genomic replication. Furthermore, it is likely that some differentiated cell systems may repair only those genes remaining active and essential for cell function. The evident and hypothesized relationships of DNA repair to transcription and chromosomal configuration will be discussed.

1. Radman, M. in *Molecular Mechanisms for the Repair of DNA* (eds. P. C.Hanawalt and R. B. Setlow) pp. 355-367 Plenum Press, N. Y. (1975)
2. Cohen, S. N. and Chang, A. C. Y. *Ibid.* pp. 355-344
3. Friedberg, E. C. *DNA Repair*, W. H. Freeman and Co., N. Y. (1985)
4. Bohr, V. A., Smith, C. A., Okumoto, D. S. and Hanawalt, P. C. *Cell 40*: 359-369 (1985)
5. Mellon, I., Bohr, V. A., Smith, C. A. and Hanawalt, P. C. *Proc. Natl. Acad. Sci. USA 83*: 8878-8882 (1986)
6. Madhani, H. D., Bohr, V. A. and Hanawalt, P. C. *Cell 45*: 417-422 (1986)
7. Mellon, I., Spivak, G., and Hanawalt, P. C. *Cell 51*: (in press)
8. Vos, J. M. and Hanawalt, P. C. *Cell* 50: 789-799 (1987)

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DNA Repair Mechanisms in E. coli

E 002 THE DYNAMICS OF THE E. coli *uvr* NUCLEOTIDE EXCISION REPAIR SYSTEM.

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Having reagent quality Uvr proteins in sufficient quantities at our disposal has permitted in-depth studies into the four-step nucleotide excision reactions (incision, excision, resynthesis and ligation) shown below. Evidence will be presented describing the highly plastic nature of the Uvr proteins as well as the DNA substrate-effector relationship between DNA and the UvrAB protein complex.

A protease was isolated which converts UvrB to UvrB which is a cryptic ATPase and can participate only in step 2 of the pre-incision reaction but none of the ensuing steps. This degradative conversion appears to provide a mechanism for regulating the effective concentration of UvrB minimizing non-specific incision of undamaged DNA. The responsible protease appears to be similar to the E. coli Ada protease which is also responsible for the turnover of the Ada protein.

I.PRE-INCISION REACTIONS

step 1 - UvrA DIMERIZATION

$$4ATP + 2UvrA \rightarrow (UvrA\text{-}ATP_2)_2$$

step 2 - NUCLEOPROTEIN FORMATION

$$(UvrA\text{-}ATP_2)_2 + DNA \rightarrow (UvrA\text{ - }ATP_2)_2\text{ - }DNA$$

$$(UvrA\text{-}ATP_2)_2\text{ - }DNA + UvrB \rightarrow (UvrA\text{-}ATP_2)_2\text{ - }UvrB\text{-}DNA$$

step 3 - TOPOLOGICAL UNWINDING

$$(UvrA\text{-}ATP_2)_2\text{ - }UvrB\text{ - }DNA + ATP \rightarrow (UvrA\text{-}ATP_2)_2\text{ - }UvrB\text{ - }{}_{tu}{}^{*}DNA$$

[tu=topologically unwound]

step 4 - TRANSLOCATION

$$(UvrA\text{ - }ATP_2)_2\text{ - }UvrB\text{ - }t_uDNA + ATP \rightarrow (UvrA\text{-}ATP_2)_2\text{ - }UvrB\text{-}{}_{tu}DNA^{+}$$

[damaged site primed for incision by UvrC]

II.INCISION REACTION

step 5 - DUAL ENDONUCLEOLYTIC EVENTS

$$UvrA_2B\cdot tuDNA^{+} + UvrC \rightarrow UvrA_2BC\cdot tuDNA^{+} \rightarrow UvrA_2BC\cdot inDNA^{+}$$

[inDNA = incised damaged DNA]

III. EXCISION REACTIONS.

step 6 - UvrD:DNA POLYMERASE I INTERACTIONS

$$UvrA_2BC\cdot inDNA^{+} + UvrD + polI + 4\ dNTPs \rightarrow 2UvrA + UvrB + UvrC$$

+ resynthesized, nicked DNA

IV. LIGATION REACTION

step 7 - POLYNUCLEOTIDE LIGASE REACTION

E 003 MOLECULAR ANALYSIS OF PLASMID DNA REPAIR WITHIN UV-IRRADIATED E. COLI: IMPLICATIONS OF DISTRIBUTIVE AND PROCESSIVE REPAIR PATHWAYS, Elliott A. Gruskin and R. Stephen Lloyd, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

The initial step in DNA repair is the specific binding of repair enzymes to damaged sites. An important aspect of this process is the method by which these enzymes locate a damaged site within large domains of DNA. Repair enzymes might either "scan" DNA for damage, or encounter damage by diffusional mechanisms. In order to discriminate between these two mechanisms in E. coli, an in vivo DNA repair assay was developed to study: 1) the kinetics of plasmid DNA repair; 2) the effect of repair on plasmid topology and; 3) the dimer distribution in plasmid DNA as a function of time during repair. The use of specific E. coli mutants allowed independent analysis of the UvrABC-initiated repair of dimer sites and the photolyase-catalyzed dimer cleavage. E. coli (uvrA$^-$ recA$^-$) harboring pBR322 were analyzed for photolyase-catalyzed repair. E. coli (uvrA$^-$ recA$^+$) harboring pBR322 were analyzed for UvrABC-initiated repair. In addition, to serve as a model system, uvrA$^-$ recA$^-$ E. coli harboring the pGX2608-16 which expresses the denV gene was analyzed for dimer repair initiated by the pyrimidine dimer-specific T4 endonuclease V (the denV gene product) and completed by endogenous E. coli proteins.

Our results demonstrate that repair initiated by the endogenous UvrABC complex and by T4 endonuclease V occurs by a processive DNA scanning mechanism. In contrast, photoreversal of pyrimidine dimers catalyzed by endogenous photolyase occurs by a distributive mechanism. Excision repair initiated by T4 endonuclease V proceeds through a nicked circular plasmid intermediate which becomes supercoiled during a post-repair process. Excision repair initiated by UvrABC occurs without any apparent change in plasmid topology. These results suggest that excision repair initiated by UvrABC consists of two coupled phases: the dual incision events followed by repair patch synthesis. The net result is excision repair without a transient loss of DNA superhelix density. Supported by NIH ES 04091 and NIH ES 00267. EG is a predoctoral fellow funded through CIIT.

E 004 ENZYMOLOGY OF *E. coli* METHYL-DIRECTED DNA MISMATCH CORRECTION, K. G. Au, M. Grilley, R. S. Lahue, S.-S. Su, B. Yashar, K. Welsh and P. Modrich, Dept. of Biochemistry, Duke University Medical Center, Durham, NC 27710.

Methyl-directed mismatch correction has been reconstituted in a purified system. The reaction requires MutH, MutL, and MutS proteins, DNA helicase II, single strand DNA binding protein, and DNA polymerase III holoenzyme. Repair of covalently closed, circular heteroduplexes by this set of proteins is directed by the state of d(GATC) methylation and displays a mismatch specificity similar to that observed in crude extracts. However, this reaction is inhibited substantially by the presence of DNA ligase. We have identified and partially purified a high molecular weight, thermolabile factor which circumvents this inhibition to permit high efficiency mismatch correction by the defined system in the presence of DNA ligase.

Analysis of individual *mutH* and *mutS* gene products has indicated that these proteins function in strand discrimination amd mismatch recognition, respectively. Near homogeneous MutH protein posesses a weak endonuclease activity which cleaves an unmethylated strand 5' to the dG of a d(GATC) site. MutS protein has been found to recognize all eight possible base-base mismatches, with the hierarchy of affinities of MutS for the different mispairs correlating to some extent with their efficiencies of correction. Preliminary electron microscopic studies, performed in collaboration with R. Thresher and J. Griffith of the University of North Carolina, have demonstrated formation of protein-stabilized DNA loops in the presence of MutS protein. This reaction requires ATP and the presence of a mismatch within the DNA substrate.

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E 005 REGULATION OF THE ADAPTIVE RESPONSE TO ALKYLATION DAMAGE BY THE ESCHERICHIA COLI ADA PROTEIN, Barbara Sedgwick, ICRF, Clare Hall Laboratories, South Mimms, Herts, England, EN6 3LD, U.K.

E.coli is induced for the adaptive response by exposure to simple alkylating agents and acquires increased resistance to their toxic and mutagenic effects. The multifunctional ada gene product acts both as a DNA repair enzyme and as a regulator of expression of the inducible genes of this response. It repairs several O-alkylated lesions in DNA by transferring the methyl groups on to two of its own cysteine residues. Cys-321 accepts methyl groups from O^6-methylguanine, whereas cys-69 was recently identified as the acceptor for methyl groups from the Sp stereoisomers of methylphosphotriesters. This post-translational modification of the Ada protein by methylation of cys-69 converts it onto an efficient transcriptional activator. It can then readily bind to the promoters of the inducible ada and alkA genes in the region of a specific DNA sequence, the 'Ada box'. The Ada protein is readily cleaved in a central region by a sequence specific endogenous proteolytic activity. Several less specific reagent proteases also cleave preferentially in this region indicating that the Ada protein is comprised of two domains held together by a susceptible hinge region. The C-terminal domain was purified previously and shown to retain the activity which repairs the mutagenic lesion O^6-methylguanine. The N-terminal domain apparently retains the DNA binding site which recognises the 'Ada box' sequence in the promoters of the inducible genes. Methylation of cys-69 reduces the sensitivity of this domain to trypsin suggesting a conformational change of the protein which would allow DNA binding and thus transcriptional activation. Several independently isolated constitutive mutants of the adaptive response are not mutated in the promoter region of the ada gene, but have a single amino acid change in the N-terminal domain of the protein.

E 006 DIVERGED DNA REPAIR SYSTEMS IN PROKARYOTES AND THEIR RELATION TO THE EUKARYOTIC REPAIR SYSTEMS, Mutsuo Sekiguchi, Dept. of Biochemistry, Kyushu University Faculty of Medicine, Fukuoka 812, Japan

The DNA repair systems presumably differentiated along with independent evolution of the organisms, probably resulting in a variety of excision repair systems. T4 endonuclease V, a bacteriophage T4-coded repair enzyme, may be a primitive form of the enzymes participating in the incision step of the excision repair of UV-damaged DNA; it is controlled by only one gene. In Escherichia coli, the incision step is more complex and controlled by at least three genes. Recent discovery that Micrococcus luteus cells possess both types of repair enzymes poses an interesting question as to the evolutionary differentiation of the systems.

Many bacteria possess both constitutive and inducible systems for repair of mutagenic and cell-killing effects of a large group of chemical agents. In E. coli two types of 3-methyladenine-DNA glycosylases, one being constitutive and the other inducible, are present. The induction process is controlled by Ada protein, which itself is a DNA repair enzyme, O^6-methylguanine-DNA methyltransferase, and is induced by alkylating agents. Besides the inducible enzyme, a constitutive methyltransferase, which is simpler in both structure and function, appears to exist. In most mammalian cells only the latter type of the enzyme is found. In spite of these differences, impaired repair capacities of some mammalian cell lines can be restored by introduction of cloned bacterial genes or enzymes into cell.

E 007 THE AP ENDONUCLEASES OF *E. COLI*, Bernard Weiss, Department of Molecular Biology and Genetics), The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We are studying pathways of DNA repair by isolating and characterizing bacterial mutants that lack specific enzymes. The major AP endonuclease activities of *E. coli* are associated with the following enzymes and their respective genes: exo III, *xth* (~85%); endo IV, *nfo* (~5-10%); and endo III (thymine glycol- or urea-DNA glycosylase), *nth* (5-10%). Endo III cleaves 3' to the base-free sugar, probably via β-elimination, leaving an end that cannot prime DNA synthesis. Its DNase activity appears to be of no use and may be deleterious. Apart from being weak mutators, *nth* mutants have no other apparent defect. They are not unusually sensitive to γ-rays or H_2O_2, both of which produce substrates for the glycosylase activity. Exo III and endo IV cleave 5' to an AP site, generating good primers for repair synthesis, and permitting the removal of the base-free sugar via the 5'→ 3' exonuclease activity of PolI. Primarily, endo IV provides a backup for exo III, which is more active. Thus, an *nfo* mutation enhances the sensitivity of *xth* mutants to γ-rays, alkylating agents, and oxidants. Endo IV is the prototype for simple 5' AP endonucleases found so far in all organisms. In *E. coli* it appeared to be a minor activity, until it was discovered that the enzyme could be induced to levels as high as that of exo III. The inducing agents are all compounds that generate superoxide radicals in vivo, such as paraquat and plumbagin. Induction is independent of the *oxyR* and SOS regulons. Preliminary evidence suggests that the *nfo* gene may be under negative control; cells bearing multicopy *nfo* plasmids have a lower level of endo IV than expected from the plasmid copy number and are strongly induced by a normally weak inducer such as H_2O_2. The mechanism of control is now being explored through the use of operon fusions.

Altered DNA Conformations: Implications and Applications

E 008 THE ROLE OF DNA STRUCTURE IN FRAMESHIFT MUTAGENESIS
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The alteration of the DNA secondary structure induced by the covalent binding of the chemical carcinogen N-Acetoxy-N-2acetylaminofluorene to the C8 position of guanine residues depends upon the local sequence. *In vitro* studies have shown that when binding occurs within a "random" DNA sequence the helix is locally distorted as described by the Insertion-Denaturation model. However, if binding occurs within alternating GC or GT sequences, -AAF adducts trigger the B to Z transition.

Using a forward mutation assay, we have shown that -AAF adducts induce more than 90% of frameshift mutations located at specific sequences (mutation hot spots). Two classes of mutation hot spots were identified :

i) -1 frameshift mutations occuring at runs of guanine residues; these mutations can be explained by the so-called Streisinger model of strand slippage during replication.

ii) -2 frameshift mutations occuring within small stretches of alternating GC sequences. Since -AAF adducts are known to favor the B->Z transition within alternating GC sequences, we wanted to investigate the role of Z-DNA structure in this mutation pathway .

Plasmids containing stretches of either $(GC)_n$, $(GT)_n$ or $(AT)_n$ sequences (with n = 12 or 13) located in front of the lacZ gene were constructed such that frameshift mutations can easily be scored phenotypically. A high level of spontaneous frameshift mutation frequency was found with the GC containing plasmids as compared to the frequency observed with both the GT and AT containing plasmids. The molecular nature of the mutational events was determined for the different constructions in wild type and *mutL* strains. From these experiments it can be concluded that i) in the AT and GT containing plasmids frameshift mutations spontaneously arise through a strand slippage mechanism and ii) that most of the mutations arising in the GC containing plasmid are independant from such a slippage mechanism and may be related to the formation of a Z-DNA structure within the GC stretch. This view is supported by the analysis of the topoisomer distribution present in the plasmids used in the mutation studies.

E 009 VISUALIZATION OF THE BENDING OF DNA BY NATURAL SEQUENCES, DRUGS, DNA DAMAGING AGENTS, AND SITE SPECIFIC DNA-BINDING PROTEINS. Jack D. Griffith, Caroline Laundon, and Hsilin Cheng, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514.
The axis of the DNA helix can be bent from the straight by proteins that bind DNA, by DNA damaging agents, drugs, and by naturally occurring sequences. Gel electrophoresis provides a powerful method for examining the bending of DNA. This technique, however, appears to require that the bends be planar for the DNA fragment to show a significant retardation of mobility and the location of the bend relative to the ends of the DNA determines the degree of retardation. Using electron microscopy it has been possible to directly visualize the bending of a 200 bp highly bent DNA segment isolated from the kinetoplast DNA of Crithidia fasciculata (1). Using this segment embedded in a larger non-bent DNA (890 bp), and EM examination, we found that the fraction of most highly bent molecules could be increased by up to 40 fold when the DNA was incubated with certain cationic metals (2). The order of effectiveness of the metals was that zinc and cobalt are more effective than manganese and calcium which in turn are more effective than magnesium. When this highly bent helix segment was cloned into 2 sites in pBR325 and the resulting DNA reconstituted with a limiting amount of histone proteins, the bent helix segments showed a 3 to 10 fold higher affinity for nucleosome formation. In addition, the bent helix segments were found to reside at the ends of the interwound supertwisted DNA rods as seen by EM. Using this approach a highly bent helix segment was identified at the locus in SV40 DNA that corresponds to the terminus of replication and ends of the early and late transcripts. This segment binds histones tightly and locates at the ends of supertwisted DNA rods, but shows a relatively small retardation on gel electrophoresis, presumably due to the non-planar nature of the bend. The bending of DNA by binding drugs will be discussed.

1. Griffith, J.D., Bleyman, M., Rauch, c., Kitchin, P.A., and Englund, P.T. (1986) Cell, 46, 717-724,
2. Laundon, C., and Griffith, J., (1987() Biochemistry, 26, 3759-3762.

E 010 SITE SPECIFIC PSORALEN LESIONS AS SUBSTRATES FOR DNA REPAIR ENZYMES, John E. Hearst, Department of Chemistry, University of California, Berkeley, Chemical Biodynamics Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720.
Synthetic oligonucleotides which are site specifically modified with psoralen monoadduct have been used as hybridization probes. The ability to rapidly photocrosslink these oligonucleotides to their target sequences upon hybridization provides a unique tool for the understanding of the dynamics and equilibria associated with the probing of single strand DNA targets of complementary sequence. These same modified oligonucleotides plus RecA provide a unique method for understanding the dynamics of the interactions in the ternary complex associated with DNA recombination. The utility of such reactions for clinical diagnostics will be briefly discussed.

E 011 Z-DNA AND HOMOLOGOUS GENETIC RECOMBINATION, Alexander Rich, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

By using a Z-DNA affinity column, Z-DNA binding proteins have been isolated from human tumor cells (acute lymphoblastic leukemia). One fraction from this column contains a strand transferase activity. The transferase activity is also dependent upon ATP (Fishel, R., Detmer, K. and Rich, A., PNAS, in press). This recombination enzyme has properties similar to Rec 1 isolated by W. Holloman from ustilago. Both of these enzymes are Z-DNA binding proteins and their properties suggest that they carry out recombination through the formation of a paranemic joint. A paranemic joint consists of equal numbers of B-DNA and left-handed Z-DNA. This joining makes it possible for two DNA molecules to recognize homologous sequences without an initial strand cleavage.

Genes and Gene Products Involved in DNA Repair in Eukaryotic Cells: I. The Yeast Saccharomyces cerevisiae

E 012 THE EXCISION REPAIR *RAD* GENES AND Rad PROTEINS OF S. cerevisiae: RECENT PROGRESS, Helmut Burtscher, Andrea J. Cooper, Linda B. Couto, Itzik Harosh, Clare Lambert, Louie Naumovski, Wolfram Siede, William A. Weiss, Larry H. Thompson* and Errol C. Friedberg, Department of Pathology, Stanford University, Stanford, CA 94305, and Lawrence Livermore National Laboratory*, Livermore, CA 94550.

In an effort to understand the molecular biology and biochemistry of nucleotide excision repair in eukaryotes, we have been studying the yeast *Saccharomyces cerevisiae* as a model system. In this organism at least 5 unlinked genes (*RAD1*, *RAD2*, *RAD3*, *RAD4* and *RAD10*) are required for the incision of DNA containing bulky base adducts. We have isolated these genes by molecular cloning and are using them to explore the biology of nucleotide excision repair and to overexpress Rad proteins in yeast and in *E.coli*. Recent progress in this work is focused on the following specific areas:

Overexpression of Rad proteins in yeast. The Rad1, Rad2, Rad3 and Rad10 proteins have been overexpressed in *E.coli* (and injected into rabbits to obtain a source of affinity-purified polyclonal antisera) and in yeast cells. Several of the overexpressed proteins are mainly insoluble in aqueous solvents; however some fraction of all of them are present in the soluble fraction of crude extracts as polypeptides of apparent Mr ~150kDa, ~150kDa, ~90kDa and ~24kDa, respectively. The Rad3 and Rad10 proteins have been partially purified. The former has an ATPase activity which is also detectable in affinity-purified preparations of a Rad3-ß galactosidase fusion protein.

The *RAD10* gene partially complements an excision repair-defective mammalian cell mutant. The *RAD10* gene was tailored into a mammalian expression vector and transfected into the UV sensitive, excision repair-defective CHO cell line UV20 (genetic complementation group 2), after ligation to a plasmid containing the mouse *DHFR* gene. *RAD10* sequences were amplified after exposure of stable transformants to methotrexate. *RAD10* mRNA and Rad10 protein could be detected in these cells. Cells with amplified copies of *RAD10* as well as stable integrants without amplified copies showed partial complementation of UV sensitivity and sensitivity to mitomycin C. The *RAD10* gene had no effect on the UV sensitivity of excision-defective CHO cell lines from other genetic complementation groups.

DNA damage-inducibility of the *RAD2* gene. Induction of *RAD2* by DNA-damaging agents is positively regulated. Deletion mapping of the *RAD2* upstream region has identified a putative upstream activator sequence(UAS) as well as several AT-rich sequences which are required for normal induction. Induction of the *RAD2* gene can be demonstrated in stationary phase cultures, suggesting that the phenomenon is not the result of cell cycle regulation.

The *RAD4* gene. The *RAD4* gene (which is toxic to *E.coli*) can be propagated following insertional mutagenesis with random DNA fragments. Removal of the inserts restores normal *RAD4* activity. The nucleotide sequence of the cloned gene has been determined. The *RAD4* coding region contains 2262 bp (754 codons) and could encode a polypeptide of calculated Mr = 87.1kDa. Computer-assisted searches at both the DNA and amino acid sequence levels revealed no extensive regions of homology between *RAD4* and other genes or proteins.

E 013 UBIQUITIN, RAD6 AND DNA REPAIR, Stefan Jentsch, John P. McGrath and Alexander Varshavsky, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA, (617) 253-6788.

Ubiquitin is one of the most highly conserved proteins known and is present in all eucaryotic cells. It exists in cells either free or covalently joined to a variety of cytoplasmic, nuclear and integral membrane proteins. Conjugation of ubiquitin to intracellular proteins is essential for their selective degradation. Alternatively, reversible joining of ubiquitin to proteins could also modulate protein function.

We undertook the purification of the enzymes involved in ubiquitin conjugation and cloned several of the corresponding genes from S. cerevisiae. UBA1 codes for an ~120-kD, ATP-dependent ubiquitin-activating enzyme. UBC1 codes for a heat-shock inducible ~30-kD ubiquitin-conjugating enzyme. ubc1 mutants exhibit slow growth and defects in cell cycle progression. CDC34, a gene required for the transition to S phase, encodes another ubiquitin-conjugation enzyme. RAD6/(UBC2) is required for DNA repair, induced mutagenesis, and sporulation. Recently we showed (Nature, 329, 131-134, 1987) that RAD6/(UBC2) encodes an enzyme which catalyses the covalent attachment of ubiquitin to histones H2A and H2B in vitro. Ubiquitination of chromosomal proteins by the RAD6/(UBC2) enzyme may induce structural changes of chromatin, thereby allowing access by enzymes of repair and mutagenesis pathways to the sites of DNA lesions.

E 014 STRUCTURE AND FUNCTION OF THE RAD50 GENE OF SACCAROMYCES CEREVISIAE, W. Raymond, E. Alani, L. Cao, R. Padmore and N. Kleckner, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Previous work in other laboratories has shown that the RAD50 gene is required during vegetative growth for repair of X-ray and MMS-induced DNA damage and is required during meiosis for an early step in genetic recombination, for synaptonemal complex formation and for chromosome segregation. The following new information about RAD50 will be discussed:

(1) The RAD50 gene encodes a 150kD protein with two outstanding features: a region similar to purine nucleotide binding domains and two 30 kD regions capable of forming alpha-helical coiled coils; one such region shows weak but significant homology to the S2 domain of rabbit myosin.

(2) Both the RAD50 transcript and its protein product are present in very small amounts in both mitotic and meiotic cells. The level of protein is the same in mitotic and meiotic cells under all conditions; under some conditions, the steady state transcript level increases during meiosis.

(3) Strains carrying a complete RAD50 deletion have a significant mitotic growth defect. Several lines of evidence suggest that mitotic DNA synthesis is normal in rad50 strains, and that the growth defect arises from a delay, and occasional failure, of nuclear division.

(4) Thin section microscopy of rad50 strains undergoing meiosis has revealed that rad50 strains not only lack intact synaptonemal complexes, as reported by B. Byers, but also lack isolated lateral elements and contain aberrant SC-related structures called polycomplexes that are observed in other strains defective in early steps of meiosis. FACS analysis suggests that meiotic DNA replication is essentially normal in rad50 mutants.

Since cdc mutants defective in mitotic DNA metabolism are also defective in subsequent nuclear division, RAD50 may be important during vegetative growth for proper metabolism of newly replicated DNA. The absence of lateral elements and the presence of polycomplex bodies, when added to previous genetic and physical evidence that rad50 mutations blocks recombination at a very early step, suggests that RAD50 function is required for completion of events that are prerequisites for initiation of the major processes of chromosome metabolism that are specific to meiosis I. The relationships among the vegetative growth, DNA repair and meiotic functions of RAD50 are not known. These functions could be carried out by a single activity, or by two or more different activities of the RAD50 protein.

E 015 THE *RAD6* GENE AND PROTEIN OF *SACCAHROMYCES CEREVISIAE*, Louise Prakash, Department of Biophysics, University of Rochester School of Medicine, Rochester, NY, 14642.

The *RAD6* gene of *Saccharomyces cerevisiae* is required for postreplication repair of UV damaged DNA, induced mutagenesis by UV, γ-rays, and alkylating agents, and for sporulation. *rad6* mutants are highly sensitive to UV, γ-rays, alkylating agents and various other chemicals. The nucleotide sequence of the *RAD6* gene indicates that it encodes a protein of 172 amino acids of Mr 19,704. The most striking feature of the RAD6 protein is its highly acidic carboxyl terminus: of the 23 terminal amino acids residues, 20 are acidic, including 13 consecutive aspartic acid residues (1). The RAD6 protein was purified from a yeast strain carying a RAD6 overproducing plasmid and its structure and function examined. The RAD6 protein is a monomer with two structurally distinct domains: the amino terminal 149 residues comprise a globular domain, whereas the last 23 acidic residues form a linear, extended tail. However, deletion of the entire polyacidic tail does not affect the UV repair or mutagenesis functions of *RAD6*, but severely reduces the sporulation efficiency.

The RAD6 protein has recently been shown to possess ubiquitin-conjugating activity. It is one of several ubiquitin-conjugating enzymes and ubiquitinates histones such as H2A and H2B (2). The ubiquitin conjugating enzymes are highly conserved in eukaryotic organisms. Using affinity-purified RAD6 antibody, we have observed a single cross-reacting protein from *Schizo-saccharomyces pombe, Drosophila melanogaster,* and humans.

The polyacidic tail of RAD6 protein could facilitate interaction with histones. We are currently investigating the role of the polyacidic sequence in histone ubiquitination.

(1) Reynolds, P., S. Weber, and L. Prakash, 1985, Proc. Natl. Acad. Sci. USA 82: 168-172.
(2) Jentsch, S., J. P. McGrath, and A. Varshavsky, 1987, Nature 329:131-134.

E 016 THE *RAD3* GENE AND PROTEIN OF *SACCHAROMYCES CEREVISIAE*, Patrick Sung and Satya Prakash, Department of Biology, University of Rochester, Rochester, NY 14627.

The *RAD3* gene is one of ten genes of *Saccharomyces cerevisiae* involved in excision repair of DNA damaged by UV light and by various other agents. In contrast to other DNA repair genes of yeast, *RAD3* is also required for cell viability. The *RAD3* gene encodes a protein of 778 amino acids with an Mr of 89,779, and the encoded protein contains a consensus amino acid sequence present in various enzymes that bind and hydrolyze ATP.

The RAD3 protein has been overproduced and purified to near homogeneity from yeast cells (1). For overproducing the RAD3 protein, the *RAD3* gene was fused to the highly expressed constitutive yeast alcohol dehydrogenase I (*ADC1*) promoter. RAD3 protein was purified from yeast cells carrying the RAD3 overproducing plasmid by a combination of chromatographic steps: DEAE-sephacel, DNA agarose, Bio-Gel HTP, Bio-Rex 70 and finally, a DEAE sephacel column step. The RAD3 protein catalyzes the hydrolysis of ATP to ADP and P_i in the presence of single-stranded DNA. No ATPase activity occurs in the absence of DNA or with double-stranded DNA, and UV irradiation of DNA does not stimulate the ATPase activity. The ATPase activity coelutes with the RAD3 protein in various column steps and is inhibited by anti-RAD3 antibodies (1).

To correlate ATPase activity with the *in vivo* biological functions of the RAD3 protein, we have mutated the lysine^{48} residue in the consensus sequence found in ATP binding and hydrolyzing enzymes. The mutant rad3 proteins have been overproduced and purified from yeast cells and their ATP binding and hydrolysis activities are being investigated. These biochemical studies coupled with genetic studies of these mutants should clarify the role of RAD3 ATPase activity in excision repair and cell viability.

(1) Sung, P., L. Prakash, S. WSeber, and L. Prakash. 1987. The *RAD3* gene of *Saccharomyces cerevisiae* encodes a DNA-dependent ATPase. Proc. Natl. Acad. Sci. USA 84:6045-6049.

E 017 Analysis of Yeast Recombination-Repair Genes.

David Schild, Gary Cole and Robert Mortimer. Div. of Biology and Medicine, Lawrence Berkeley Lab., and Depts. of Biophysics and Medical Physics and of Genetics, Univ. of Calif., Berkeley, Ca. 94720.

The *RAD52, 54* and *57* genes of the yeast *Saccharomyces cerevisiae* are involved in both DNA repair of x-ray induced double strand breaks and mitotic and meiotic recombination. These genes have been cloned and the cloned genes are being used to better characterize these genes at both the molecular and genetic levels. One-step gene disruptions (and disruption-deletions) of these genes have been isolated in order to study null alleles of these genes and to compare isogenic Rad^+ and Rad^- strains. In addition, transcriptional regulation of the *RAD52* and *RAD54* genes has been studied in both mitotic and meiotic cells.

Yeast strains carrying the disruption-deletion of *rad57* are viable at both 23° and 36°, but are considerably more x-ray sensitive at 23° than at 36°. A similar cold-sensitive x-ray sensitive phenotype was also recently reported for *rad55* disruption-deletions[1]. Since all existing alleles and even deletions in these genes result in a c.s. phenotype, the effect is not due to instability of the proteins at 23°. One model which could explain these results is that both *RAD55* and *RAD57* might function, either together or separately, to stabilize a recombinational-repair multi-enzyme complex which is less stable at 23° than at 36°.

Strains with disruptions in either *rad52* or *rad54* are also viable, but show equal x-ray sensitivity at all temperatures tested. Diploids homozygous for the *rad54* disruption-deletion show a considerably greater defect in meiosis than had previously been reported for *rad54* point mutations. The meiotic defect observed varied significantly depending on the strain background. Preliminary results indicate that *rad54*-disruption homozygous diploids constructed from unrelated haploid strains have much lower spore viability (1 to 20%) than diploids constructed from isogenic or closely related haploids (>50%). These results may indicate a role for *RAD54* in meiotic mismatch repair during meiosis.

Previous results demonstrate that *RAD54*, but not *RAD52*, is induced in mitotic cells by DNA damage[2]. Preliminary results indicate that both of these genes are induced during meiosis at about the time of meiotic recombination. Experiments to confirm these results are currently in progress, using diploids homozygous for mating-type (therefore unable to enter meiosis) as negative controls. We are also further defining the regions 5' to *RAD54* to determine which sequence(s) are involved in mitotic and meiotic induction, and to determine whether induction is positively or negatively regulated.

[1] Lovett,S.T. and R.K. Mortimer, 1987. Genetics 116: 547-553.

[2] Cole, G.M., D. Schild and R.K. Mortimer, 1987. Mol. Cell. Bio. 7: 1078-1084, and H. Emery, D. Schild and R.K. Mortimer, unpublished observations.

Human Hereditary Diseases with Abnormal Responses to DNA Damage

E 018 DEFECTIVE REPAIR AND MUTAGENESIS IN XERODERMA PIGMENTOSUM ASSESSED WITH PLASMID VECTORS,

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We employed plasmid vectors to measure DNA repair and mutagenesis in xeroderma pigmentosum (XP) cells. Plasmids were used which could replicate in bacteria and utilize human host cell enzymes for expression or replication. Plasmids were damaged in vitro (permitting precise quantification of the extent of damage and possible further in vitro modification) and transfected into XP or repair proficient human cells. Expression, repair, replication and mutation preceded for 2-3 days and then an end-point was assayed. Repair was measured by determining the extent of expression of novel enzyme activity coded for by the damaged plasmid. Similarly, transformation of indicator bacteria by the harvested plasmid was used to assess plasmid survival and mutation frequency and DNA sequencing was performed on mutated plasmids recovered to determine exactly which bases were mutated.

Using non-replicating plasmids (pRSVcat, pSV2cat) coding for the bacterial cat (chloramphenicol acetyl transferase) gene, XP group A and D cells showed less activity than repair proficient cells. One pyrimidine dimer inhibited expression in the XP cells. Use of photolyase specifically to remove cyclobutane dimers revealed that non-dimer photoproducts were also blocks to transcription in XP group A cells.

Using a replicating shuttle vector plasmid (pZ189) we found a restricted spectrum of ultraviolet induced mutations with the XP group A and D cells. Plasmid survival was reduced and fewer plasmids had multiple base substitution mutations than with the normal line. The major UV photoproduct, the TT dimer was only weakly mutagenic with XP or normal cells. The majority of mutant plasmids had G:C to A:T base substitution transition mutations. Treatment of the UV damaged plasmid with photolyase prior to transfection of the XP group A and D cells showed increased plasmid survival and decreased mutation frequency indicating that cyclobutane dimers contributed to a major portion of lethality and mutagenicity. Since the major class of mutations involved G:C base pairs, cytosine rather than thymine containing dimers were implicated. The remaining non-dimer photoproducts were also mutagenic and were major contributors to transversion mutations.

E 019 MOLECULAR DEFICIENCIES IN HUMAN CANCER-PRONE SYNDROMES.

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A structural defect of DNA ligase I appears to be a consistent feature of Bloom's syndrome (BS). Eight different cell lines representative of this inherited disease contain a DNA ligase I with altered biochemical properties, whereas a number of control enzymes (including DNA ligase II and uracil-DNA glycosylase) are present at normal levels. No defects in either DNA ligases I or II have been detected in cell lines derived from patients with Fanconi's anemia, Cockayne's syndrome, xeroderma pigmentosum (including variant), ataxia telangiectasia, Friedreich's ataxia, or Werner's syndrome. Most available BS lines are from Ashkenazi individuals, and such cells exhibit an anomalously heat-labile ligase I present at a reduced level of activity. Other BS cell lines of Anglo-Saxon origin have a ligase I of normal heat resistance which is present as a dimer rather than monomer at low or physiological ionic strength. The dimer form is dissociated into active monomers by exposure to 1M NaCl. These data indicate that BS is due to a missense mutation at one of at least two alternative sites in the structural gene for DNA ligase I, resulting in a malfunctioning variant of this essential enzyme.
Xeroderma pigmentosum-derived cell lines are known to be deficient in their ability to remove pyrimidine dimers from DNA. A soluble cell-free system has been prepared from normal human cell lines which performs repair replication of UV-irradiated DNA. Cell extracts are made by procedures similar to those employed by others to carry out transcription in vitro. DNA repair replication requires Mg^{2+}, ATP, and deoxynucleoside triphosphates. Pyrimidine hydrates and similar minor UV lesions susceptible to DNA glycosylases have been removed from the DNA substrate prior to use. The activity is strongly reduced in extracts of cells derived from xeroderma pigmentosum complementation groups A and C.

E 020 *FANCONI'S ANEMIA : GENETIC AND MOLECULAR STUDIES, E. Moustacchi, C. Diatloff-Zito, D. Papadopoulo and D. Averbeck, Institut Curie - Biologie, Paris, France.*

Fanconi's anemia (FA), an inherited autosomal recessive disorder, belongs to a class of diseases characterized by chromosomal instability, predisposition to cancer and hypersensitivity to genotoxic agents possibly linked to deficiencies in processing DNA lesions. Cells from individuals with FA have an increased sensitivity to DNA interstrand crosslinking agents and a cell-cycle perturbation manifested by an elongation of the G2 phase.

Genetic. We have shown that the two genetic complementation groups reported for FA (Duckworth-Rysiecki et al., 1985) correspond to two phenotypic classes identified by measurement of kinetics of DNA semi-conservative synthesis after treatment with 8-methoxypsoralen (8-MOP) plus UVA. This correlation has been confirmed (Digweed et al., 1987) for an earlier set of FA complementation groups also established by cell fusion (Zakrewski and Sperling, 1980). The response to 8-MOP plus UVA is consequently a valid criterion for rapidly classifying FA cells. Psoralen plus UVA mutagenesis at the HGPRT and OUA loci indicate that FA cells from group A are hypomutable. The induction of SCE by the same agent demonstrates an hypersensitivity compared to normal cells but does not allow us to distinguish the two genetic groups (Billardon and Moustacchi, 1986). Hypersensitivity of FA cells to furan-side psoralens photoinduced monoadducts is observed both in terms of cell survival and of SCE induction ; it is however less pronounced, at least for FA group A cells, than that found for crosslinks.

Repair. FA cells from both groups A and B demonstrate an incision capacity of crosslinks, the kinetics and extent of which being, however, different from that of normal cells. The incision is slower and at 24h of post-treatment incubation, the amount of crosslinks incised is lower than that observed in normal cells for group A cells, whereas in group B cells incision approaches the normal level. These results correlate with survival and with rates of DNA semi-conservative synthesis after 8-MOP photoaddition. Also the repair of furan-side monoadducts is impaired in FA cells.

Correction of the defect and gene(s) cloning. The FA phenotype is rescued by transfer of normal human DNA into untransformed fibroblasts. In addition to the normal resistance to mitomycin C, these FA cells had recovered the wild type phenotype for DNA synthesis after treatment with 8-MOP plus UVA. These cells are likely to be true transfectants rather than revertants since homologous FA DNA did not yield the same result. It has been recently confirmed that transfection of normal human and Chinese hamster DNA corrects diepoxybutane-induced hypersensitivity of FA transformed fibroblasts (Shaham et al., 1987). We show that transfection of mouse DNA into FA (A and B) cells also corrects the FA defect. Recombinant phage λ clones containing mouse DNA inserts are analysed.

E 021 THE MOLECULAR BASIS OF RADIOSENSITIVITY IN THE HUMAN DISORDER ATAXIA-TELANGIECTASIA

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Ataxia-telangiectasia (A-T) is an autosomal recessive disorder characterized by immunodeficiency, progressive neuronal degeneration and a high frequency of lymphoreticular cancers. A-T patients (and their cells in culture) are also abnormally sensitive to ionising radiations. Despite many different attempts to show a biochemical defect related to radiosensitivity, there is as yet no consensus on the molecular basis of the disorder.

The DNA double-strand break (dsb) is a potentially important type of damage induced by ionising radiations, but conventional biochemical techniques show that A-T cells are not defective in rejoining dsbs. However, using a 'model' dsb in a transferred plasmid, we have shown that the fidelity of the rejoining process is much reduced in A-T cells (line AT5BIVA) relative to normal cells. We are attempting to extend this finding to cell lines derived from other A-T patients, although these are proving difficult to use in DNA transfer protocols, and by developing an in vitro assay for rejoin fidelity. However, we have observed that one of a new set of X-ray sensitive hamster mutants isolated in this laboratory (irs 1) has a similar defect to A-T cells, relative to the normal line (V79), while two other mutants (irs 2, irs 3) do not show the rejoin fidelity defect. As part of a study of genetic complementation among different X-ray sensitive mutants, we are at present fusing irs 1 and A-T cells to assess their genetic similarity.

An additional feature of A-T cells, shown recently in our laboratory and elsewhere, is their sensitivity to inhibitors of topoisomerase II enzyme. While none of these inhibitors is thought to have an exclusive action on topoisomerase II alone, this observation does correlate with our more extensive data on a new bacterial mutant, rorB. We isolated rorB by its X-ray sensitivity and found (like A-T) that it is competent at dsb rejoining and is sensitive to coumermycin (an inhibitor of the B subunit of DNA gyrase, the bacterial topoisomerase II). Most recently we have used a topoisomerase assay based on measurement of DNA supercoiling by gel electrophoresis to show that plasmid molecules in rorB cells are in a more relaxed state than those in wild type cells. Similar experiments are now in progress with A-T and normal human cells. While it is tempting to speculate that topoisomerase II is involved in repair of radiation damage, it should be noted that the gyrase B gene is dissimilar in size and location to the rorB gene and that it does not complement the X-ray sensitivity of rorB. Rather, it may be that rorB and perhaps A-T cells lack a factor which is involved in processing dsbs which is common to the action of more than one enzyme including topoisomerase II.

Genes and Gene Products Involved in DNA Repair in Eukaryotic Cells: II. Mammalian Cells

E 022 ANALYSIS OF MAMMALIAN EXCISION REPAIR. FROM MUTANTS TO GENES AND GENE PRODUCTS

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To study the mechanism of mammalian excision repair we have set out to clone some of the genes involved. One of the approaches followed relies on the correction of repair deficient mutants by genomic DNA transfection. For excision deficient CHO mutants this strategy has yielded two repair genes: the ERCC-1 gene, correcting mutants of group 2 and very recently at least part of the ERCC-3 gene, located on chromosome 2 and complementing mutant 27-1 (Wood and Burki (1982) Mutat.Res. 95, 505) of group 3. Experiments to clone the gene correcting the UV-sensitive excision proficient CHO mutant UV-61 (Thompson et al. (1987) J.Cell Sci. suppl. 6, 97) are in progress. The incorporation of only very small quantities of exogenous sequences by SV_{40} transformed XP (and other human) cells is one of the main reasons which thusfar have hampered the isolation of XP correcting genes in a similar fashion. As an alternative approach we are utilizing microinjection of poly A^+RNA and proteins in XP fibroblasts, followed by UV-induced UDS to assay for correcting activity. In this way we have purified the XP-A correcting protein from calf thymus to a considerable extend. Furthermore, we have started the isolation of the 1200-1400 b XPA correcting mRNA by cDNA cloning (in collaboration with P. Belt, Leiden). These results should provide the basis for analysis of the molecular defect in XP-A.

Characterization of the cloned ERCC-1 gene has revealed that it encodes a polypeptide of 297 aa (and of 273 aa when the alternatively spliced exon 8 is absent). Its (low) level of expression is not enhanced after UV-irradiation in HeLa cells and is roughly the same in all mouse tissues and stages of differentiation examined. Recently, another gene was discovered within the 3' region of ERCC-1. The 2.6 kb transcript of this gene overlaps with exon 10 of ERCC-1, terminates within intron 9 and harbors antisense ERCC-1 sequence information. Its function is unknown. Within the ERCC-1 aa sequence we have tentatively identified a nuclear location signal (NLS), a helix-turn-helix type of DNA binding domain and a potential site for ADP monoribosylation. The presence of a NLS is supported by the finding that antibodies directed against an oligopeptide corresponding with the putative NLS-region of ERCC-1 specifically react with the nucleus in immunofluorescence.

Computer analysis revealed significant aa-homology between ERCC-1 and the yeast excision repair protein RAD10 and with parts of the E.coli uvrA and C proteins. We are presently investigating whether other repair genes are conserved as well and whether they can be isolated on the basis of nucleotide sequence homology starting from cloned yeast repair genes (in collaboration with Dr. S. Prakash, Rochester and Dr. A. Yasui, Sendai).

E 023 PHENOTYPIC COMPLEMENTATION OF XERODERMA PIGMENTOSUM (XP) CELLS FOLLOWING MICROCELL-MEDIATED CHROMOSOME TRANSFER. Roger A. Schultz, Paul J. Saxon, Chuck C-K. Chao and Errol C. Friedberg, Stanford University, Stanford, CA 94305.

We have examined phenotypic complementation of XP cells following microcell-mediated transfer of single human chromosomes. Screening was facilitated by tagging chromosomes to be transferred with pSV2neo and maintaining these genetically marked chromosomes by selection in human/mouse hybrids. Hybrid chromosome donors were propagated as a limited collection of partially characterized single human chromosome hybrids (in collaboration with Dr. Eric J. Stanbridge, University of California at Irvine), as a series of uncharacterized hybrid pools derived from an aneuploid, rearranged human donor line, and as a series of pools derived from a normal diploid human cell line.

Transfer of human chromosomes from the limited collection of characterized single human chromosome hybrids to XP-A cells failed to reveal phenotypic complementation. However, we identified a rearranged human chromosome (K3) which does complement the UV sensitivity of XP-A cells following transfer from the aneuploid hybrid pools. Complementation is close to wild-type levels for both UV resistance and UDS. Non-complementing, deleted derivatives of K3 have also been identified, permitting sub-chromosomal mapping of the XP-A complementing locus. Further molecular characterization of the K3 chromosome and /or the identification of a complementing normal, non-rearranged human chromosome will permit definitive chromosomal assignment for the XP-A gene.

Transfer of most human chromosomes to XP-F cells results in enhanced sensitization to UV radiation. The mechanism of this sensitization is unknown. However, following transfer of a human chromosome from one partially characterized single human chromosome hybrid (MCH200.3) and independently from a pool (J1) obtained from the aneuploid series, we have observed partial complementation of the UV sensitivity of XP-F cells. Cytogenetic and molecular characterization reveal that the chromosome from both sources is human chromosome 15. To evaluate the significance of the partial complementation observed we have examined whole cell hybrids generated by fusions between XP-F and wild-type cells. The whole cell hybrids yielded partially complemented colonies as well as fully complemented clones capable of segregating partially complemented subclones. These results may reflect gene dosage effects and suggest that transfer of only a single copy of chromosome 15 to XP-F cells results in partial complementation. Non-complementing derivatives of chromosome 15 have also been isolated and are being evaluated to facilitate sub-chromosomal mapping of the putative XP-F gene.

In addition to providing the chromosome location of an XP complementing locus, the availability of non-complementing chromosome derivatives offers the potential of several strategies for cloning these loci. Our progress in this regard for both the XP-A and XP-F complementing genes will be presented.

E 024 RESTORATION OF RODENT CELL RADIATION RESISTANCE BY HUMAN REPAIR GENES, Gary F. Strniste, David J.-C. Chen, Linda S. McCoy, Joyce W. Nickols, Richard T. Okinaka, Judith G. Tesmer, and Mark A. MacInnes, Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

A fundamental problem of human genetics and molecular biology is to understand the mechanisms by which cells prevent the accumulation of deleterious DNA alterations induced by various environmental chemicals and radiation. In an ongoing research project at Los Alamos, we are attempting to isolate and characterize two human genes that mediate repair of DNA damage. This report will summarize our results to date concerning both the human DNA excision repair gene, ERCC-5, and a human DNA repair gene presumably involved in the reparation of ionizing radiation-induced DNA double-strand breaks. Isolation of these two genes is mediated by transfection of restriction enzyme-digested human DNA, which is linked to the plasmid pSV2gpt, into recipient repair-deficient Chinese hamster cells (UV/chemical sensitive mutant line, UV-135; and ionizing radiation sensitive mutant line, xrs-6). For UV-135 cells, isolation of 1°, 2°, and several 3° cotransformants has been accomplished. DNA from several of these 2° and 3° cotransformants, segregants and parental cells are being analyzed by Southern blots for human DNA marker sequences that may show concordant inheritance with the UV resistance phenotype. For xrs-6 cells, pSV2gpt control and cotransformation experiments revealed a significant contribution of spontaneous or induced x-ray resistant revertants in the population of putative 1° DNA cotransformants. Attempts to assess concordant inheritance of human DNA fragments with the x-ray resistance phenotype, as well as attempts to generate several 2° cotransformants are in progress. The favorable genetic evidence for human DNA transformation of UV-135 cells encouraged us to construct cosmid DNA libraries of the 2° and 3° cotransformants. A region of 100 Kbp has been cloned that contains 3 copies of pSV2gpt and flanking human DNA segments. We will report our analyses of cosmid clone functionality for UV resistance and mapping of cloned human DNA sequences putatively associated with the ERCC-5 gene. We will also update work to detect DNA repair gene-specific mRNA expression for the purpose of cDNA library construction. (This research is funded by the U.S. Department of Energy under contract number W-7405-ENG-36.)

E 025 TOWARD THE MOLECULAR CLONING OF THE GENE FOR XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP A BY DNA TRANSFECTION METHOD, Kiyoji Tanaka, Ichiro Satokata, Zenzaburo Ogita and Yoshio Okada, Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka 565, Japan.
In order to clone the gene which complements the defect in DNA excision repair of xeroderma pigmentosum (XP) complementation group A, the XP cells were co-transfected with pSV2gpt and genomic DNA from 14 days ICR mouse embryo. The pSV2gpt-transformed XP colonies were UV-irradiated and UV-resistant XP colonies were selected. In the first transfection experiments, 2 UV-resistant XP clones were obtained by the screening of about 1.6 x 10^5 pSV2gpt-transformed XP colonies. The genomic DNA from UV-resistant XP transformants and pSV2gpt were co-transfected again into XP2OSSV cells. The secondary UV-resistant XP transformant was obtained by the screening of about 3 x 10^5 pSV2gpt-transformed XP colonies. Southern hybridization with mouse repetitive sequences as probe revealed that the secondary UV-resistant XP transformant retained fewer mouse sequences. About 3 kb BamHI fragments retained in the secondary UV-resistant XP transformant were cloned into L47 phage vector using mouse repetitive sequences as probe for plaque hybridization. The mouse repetitive sequences were removed from the cloned fragments, and the unique mouse sequences were used as probe for the screening of the cosmid DNA library which was constructed from the secondary UV-resistant XP transformant.
A cosmid clone with mouse sequences was isolated. Although the introduction of this cosmid DNA into XP2OSSV cells did not confer the UV-resistance on XP2OSSV cells, it was shown that the insertion fragment of this cosmid was retained in both first UV-resistant XP transformants which were isolated independently in the different experiments. These results indicate that the cloned fragment is linked very closely to the gene which can complement the defect of XP2OSSV cells. Gene walking with cosmid and phage vectors are in progress. So far, about 60 kb mouse sequences were cloned from the secondary UV-resistant XP transformant. It is also examined whether the cloned fragments are transcribed in the secondary UV-resistant XP transformant.

E 026 HUMAN DNA REPAIR GENES, L.H. Thompson, K.W. Brookman, C.A.Weber, E.P. Salazar, S.A. Stewart, and A.V. Carrano, Biomedical Sciences Division, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, CA 94550.
A variety of mutations affecting DNA repair and recombination have been isolated in rodent cell lines, providing a supplemental approach to using human cell lines from genetic disorders. Using hybrids and chromosomal mapping, we have studied seven complementation groups that are involved in the UV (ultraviolet radiation) nucleotide-excision-repair pathway. These groups likely correspond to seven different genetic loci. For CHO cell groups 1 through 5, the mutations, which block the incision step as in xeroderma pigmentosum, were corrected by human chromosomes in hybrids derived from human lymphocytes. Complementation group 2 was corrected by chromosome # 19 (1), group 3 by # 2 (2), group 5 by #13 (2), and groups 1 and 4 by # 19 and # 16, respectively (M.J. Siciliano, L.H. Thompson, & A.V. Carrano, in prep.). CHO mutant EM9, which is noted for its hypersensitivity to ionizing radiation, reduced strand-break repair, and greatly elevated sister chromatid exchange (SCE), is also corrected by human chromosome 19 (3).

From a cosmid library made from a tertiary transformant of EM9, we isolated the human gene that corrects this mutant to a phenotype of resistance to CldUrd. This gene was named *XRCC1* (X-ray Repair Complementing Chinese hamster). In both genomic and cosmid transformants, the *XRCC1* gene efficiently restored both resistance to γ-rays and the rate of strand break rejoining. From a pCD2 cDNA expression library provided by H. Okayama, a clone was obtained that has a 2.3 kb insert and that confers stable, but intermediate, CldUrd resistance when transfected into EM9. SCE levels are normal in genomic and cosmid transformants, but intermediate in cDNA transformants.

The human gene designated *ERCC2* (Excision Repair Complementing Chinese hamster) corrects UV5 and other mutants in group 1. We cloned *ERCC2* (4) by first constructing a secondary transformant in which the gene was shown to be closely linked to the bacterial *gpt* dominant-marker as the result of cotransfer of genomic and marker DNAs during primary transfection. Again, a cosmid library was screened to recover the functional gene, which is ~20 kb in size. Transformants of UV5 derived from genomic or cosmid DNA show *efficient* correction of the repair defect by cell survival, mutation induction at the *aprt* locus, and the rate of DNA incision immediately after UV treatment. A cDNA clone from the Okayama library has an insert of 2.8 kb and produced primarily transient UV resistance in UV5. These studies show that rodent mutant lines will play a major role in cataloging and isolating the genes involved in repair and recombination processes in human (and rodent) cells. (Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.)

1. L.H. Thompson et al. Somat. Cell Mol. Genet.11: 87-92, 1985.
2. L.H. Thompson et al. Somat. Cell Mol. Genet.13: 539-551, 1987.
3. M.J. Siciliano et al. Mutat. Res. 174: 303-308, 1986.
4. C. A. Weber et al. Mol. Cell. Biol. in press.

Oxidative Damage to DNA and its Repair

E 027 OXIDATIVE DAMAGE: THE ADAPTIVE RESPONSE IN E. COLI AND A CONSTITUTIVE DNA REPAIR SYSTEM, Bruce Demple, Arlen Johnson and Jean Greenberg, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138.

Escherichia coli bacteria respond to low levels of H_2O_2 by inducing the synthesis of at least 30 proteins in an "adaptive response" to oxidative damage. This induction includes increased levels of scavenging enzymes such as catalase and an alkyl hydroperoxide reductase, controlled with a group of 9 proteins by the oxyR gene. We aim to determine the regulation and function of the oxyR-independent proteins that are induced by H_2O_2. Initially we selected mutations that suppress the H_2O_2-sensitivity of oxyR deletion mutants. This approach led to the isolation of a large number of mutants dominated by strains that overproduce either catalase or alkyl hydroperoxide reductase, evidently via promotor mutations in the kat or ahp genes. Current efforts are directed at obtaining molecular data on the oxyR-independent proteins to help identify their functions and the genes that regulate them.

We have also been investigating the constitutive enzymatic systems that counteract oxidative damage. We have isolated from a variety of sources DNA repair enzymes that remove many small damages from DNA 3' termini. Such damages are caused by various oxidative mutagens including X-rays, hydrogen peroxide and bleomycin. These enzymes, which we have termed the 3'-phosphoglycolaldehyde (PGA) diesterases (from the synthetic substrate used for their purification), evidently perform the critical first step in the repair of oxidative single-strand breaks in DNA - the removal of deoxyribose fragments that block the action of DNA polymerase and ligase. 3'-PGA diesterases have been purified to physical homogeneity from Escherichia coli and yeast, and partly from human cells. The purified bacterial and yeast enzymes remove intact 3'-deoxyribose-5-phosphate and 3-phosphate, in addition to the 3'-PGA moiety used for routine assays. The apparent K_m's for these various substrates are similar for all the enzymes, indicating that these proteins probably operate in a general "clean-up" function to respond to a variety of damages. We are investigating the role of these enzymes in combatting endogenous oxidative damages and the mutations caused by more massive oxidative attack.

E 028 KILLING, STRESS RESPONSES AND MUTAGENESIS INDUCED IN *E. coli* BY HYDROGEN PEROXIDE, Stuart Linn and James. A. Imlay, Dept. of Biochemistry, Univ. of California, Berkeley, CA 94720.

Killing of *Escherichia coli* by hydrogen peroxide proceeds *via* two modes (1). Mode-one killing appears to be due to DNA damage, has a maximum near 1-3 mM H_2O_2, requires active metabolism during exposure, and is inhibited by iron chelators. Strains lacking exonuclease III, DNA polymerase I, or NADH dehydrogenase and cells grown anaerobically are especially sensitive to this mode. Mode-two killing is due to uncharacterized damage, occurs in the absence of metabolism and exhibits a classical multiple-order dose-response curve up to at least 50 mM H_2O_2. It is not inhibited by iron chelators.

H_2O_2 induces the SOS response in proportion to the degree of killing by the mode-one pathway--i.e., induction is maximal after exposure to 1-3 mM H_2O_2 (2). Mutant strains that cannot induce the SOS regulon are hypersensitive to peroxide and analysis of the sensitivities of mutants that are deficient in individual SOS-regulated functions suggests that the SOS-mediated protection is due to the enhanced synthesis of recA protein, which is rate-limiting for recombinational DNA repair. After exposure to H_2O_2, mutagenesis and filamentation also occur with the dose response characteristic of SOS induction and mode-one killing, but these responses are not dependent upon the *lexA*-regulated *umuC* mutagenesis or *sfiA* filamentation functions, respectively.

Exposure of *E. coli* to H_2O_2 also results in the induction of functions under control of the *oxyR* regulon that enhance the scavenging of active oxygen species, thereby reducing the sensitivity to H_2O_2. Catalase levels increase 10-fold during this induction, and *katE katG* mutants, which totally lack catalase, while not abnormally sensitive to killing by H_2O_2 in the naive state, do not exhibit the induced protective response. Protection equal to that observed during *oxyR* induction can be achieved by the addition of catalase to cultures of naive cells in an amount equivalent to that induced by the *oxyR* response. Thus the induction of catalase is necessary and sufficient for the observed *oxyR*-directed resistance to killing by H_2O_2. Although superoxide dismutase appears not to be involved in this enhanced protective response, *sodA sodB* mutants, which totally lack superoxide dismutase, are especially sensitive to mode-one killing by H_2O_2 in the naive state.

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

1. Imlay, J.A. and Linn, S. (1986) *J. Bact. 166,* 519-527.
2. Imlay, J.A. and Linn, S. (1987) *J. Bact. 169,* 2967-2976.

E 029 PROCESSING OF OXIDATIVE DNA BASE DAMAGE IN ESCHERICHIA COLI, Susan S. Wallace, Hiroshi Ide, Yoke Wah Kow, Michael F. Laspia, J. Eugene LeClerc, Robert J. Melamede and Lynn A Petrullo, Dept. of Microbiology, New York Medical College, Valhalla, NY 10595.

In order to assess the biological consequences of DNA bases modified by free radical reactions, we have chosen to study a thymine ring saturation product, thymine glycol, and a thymine ring fragmentation product, urea. Thymine glycols can be selectively introduced into DNA by OsO_4 oxidation and can be both enzymatically and immunologically quantitated. Urea residues are produced by alkali degradation of thymine glycol–containing DNA and can also be enzymatically quantitated. Both products appear to be removed from DNA by excision repair processes. Thymine glycol and urea are cleaved in vitro at the N–glycosylic bond by E. coli endonucleases III and VIII, urea and not thymine glycol by endonuclease IX, and in addition, urea is incised in an endonucleolytic reaction by exonuclease III and endonuclease IV. Further, ϕX RF transfecting DNA containing thymine glycols is inactivated at a greater rate in mutant cells lacking endonuclease III (nth) or both exonuclease III and endonuclease IV (xth nfo) than in wild type hosts, implicating the sequential action of endonuclease III and a class II AP endonuclease in the in vivo repair of this lesion. Similarly, ϕX RF transfecting DNA containing urea residues (or AP sites) is inactivated at a greater rate in xth nfo double mutants in keeping with the in vitro specificities of exonuclease III and endonuclease IV.

Thymine glycols constitute efficient replication blocks to DNA polymerase I in vitro with a base (presumably A) being inserted opposite the lesion before synthesis is arrested. In keeping with this observation, sequencing analysis of OsO_4–induced mutants obtained from M13 lacZ DNA containing thymine glycols showed no mutants derived from T. However, thymine glycols are very efficiently bypassed in UV–irradiated cells with some 63% of the lesions in duplex ϕX DNA being repaired in induced nth hosts. Urea residues are also efficient blocks to DNA polymerase I in vitro and like AP sites, synthesis is arrested one base prior to the lesion. Like AP sites, urea residues also appear to be mutagenic in an SOS–dependent manner. However, in contrast to thymine glycols and thymine dimers, neither urea residues nor AP sites are efficiently bypassed in SOS–induced cells as evidenced by a complete lack of W–reactivation of these lesions in either single stranded or duplex ϕX DNA. Thus SOS–induced lesion bypass appears to be dependent on the nature of the lesions and may not be an efficient repair process for totally non–instructive lesions.

In summary, thymine glycols and urea residues have served as useful models for oxidative thymine damage, and have provided useful information concerning the in vivo processing of two lesions with very different properties. In addition, DNA substrates containing these damages have allowed for the elucidation of the substrate specificities of putative repair enzymes.

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Mutagenesis Detection and Analysis - I

E 030 REGULATION OF ACTIVATION OF RECA PROTEIN IN E. COLI, Raymond DEVORET, Groupe d'Etude de la Mutagenese et de la Cancerogenese, ENZYMOLOGIE, CNRS, F-91190 Gif-sur-Yvette, France.

1- Recent data indicate the need to reevaluate the various factors that control activation of RecA protein. This is the object of this short review. Proper references will be given with the review.
2- RecA protein must be activated in order to act as a coprotease in the cleavage of repressors such as LexA protein or cI protein of a lambdoid phage. Two theories have been proposed in the past: the first postulated that nucleotides or oligonucleotides would suffice to activate RecA protein, the second proposed that RecA protein is activated in a ternary complex formed by single-stranded DNA, RecA protein and dNTPs. There is stronger evidence in favor of the latter theory as shown in vitro and also in vivo. The theory implies that in vivo RecA protein binds to a DNA substrate. Single-stranded DNA may arise from different processes, one example is the formation of gaps during post-replicative recombinational repair. LexA and lambdoid repressors would be cleaved on such sites.
3- Activation of RecA protein is primarily controlled by the protein itself. New mutations in the recA gene can dissociate the recombinase activity from the coprotease activity. Other recA mutations will conversely make RecA protein constitutive for the coprotease activity.
4- The single-strand binding protein (SSB) has been implicated in determining the conversion of RecA protein from an initial coprotease activity to a recombinase activity, this being also due to the property of RecA protein to polymerize on single-strand DNA. RecF protein may favor the conversion of RecA protein to a recombinational activity by antagonizing SSB bound on single-stranded DNA. A new protein called PsiB (12 kDa) has been shown to antagonize the activation of RecA protein, thus preventing SOS induction. The sequence of PsiB protein indicates that it shares homology with IHF-beta, a protein that participates with IHF-alpha in the tertiary-quaternary structure of DNA.
5- In conclusion, RecA protein activation appears to be controlled by a few proteins. Some proteins are involved in DNA metabolism while some others maintain chromosomal structure, the latter proteins may constitute a chromatin-like complex that regulate the access of RecA protein to single-stranded DNA.

E 031 USE OF AN SV40-BASED SHUTTLE VECTOR IN VIVO AND IN VITRO FOR STUDIES ON THE MECHANISMS OF MAMMALIAN MUTAGENESIS, Kathleen Dixon, Janet Hauser, Emmanuel Roilides, and Arthur S. Levine, Section on Viruses and Cellular Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda MD 20892.

We are using the SV40-based shuttle vector, pZ189 (1), to study mechanisms of mutagenesis in mammalian cells. This vector replicates in both bacterial and primate cells, and contains the bacterial *supF* gene as a small, easily sequenced, mutagenesis marker. When the undamaged vector DNA was replicated in monkey cells and then tested in bacterial cells for supF- mutants, a "spontaneous" mutant frequency of about 0.02% was observed (2). When vector DNA was treated with UV radiation or benzo[a]pyrene diolepoxide (BPDE) *in vitro* and then allowed to replicate in monkey cells, the mutant frequency increased at least 10-20 fold and we observed characteristic patterns of mutations which differed from the spontaneous pattern. UV irradiation caused mostly single and tandem double base substitutions within the *supF* target, the majority of which were G·C->A·T transitions (3), suggesting preferential mis-insertion of A opposite UV damage. In contrast, BPDE caused a large proportion of single base deletions at G·C base pairs as well as G·C->T·A transversions. A comparison of the positions of these mutations with the positions of DNA damage caused by these two agents indicates that the mutations are targeted to sites of damage; however, other factors must be important in determining the characteristic (and different) mutagenic hotspots observed with the two agents. To determine whether DNA damage-inducible processes influence mutagenesis (in analogy to the bacterial SOS response), monkey cells were treated with mitomycin C 24 h before introduction of UV-damaged vector DNA. This pretreatment caused a two-fold increase in the frequency of supF- mutants recovered after replication of the vector in monkey cells. Preliminary analysis of the sequence of *supF* in some of these mutants revealed that there was no significant change in the spectrum of UV-induced mutations in pretreated versus untreated cells. Thus, it appears that enhanced mutagenesis in pretreated cells occurs by mechanisms that do not differ from those operative in untreated cells. Moreover, our results suggest the conservation of at least some elements of the SOS response in animal cells. Recently, we have utilized an *in vitro* DNA replication system developed by Li and Kelly (4) to study replication fidelity and mutagenesis at the biochemical level. The pZ189 vector can be completely replicated in an SV40 T antigen-dependent reaction using cytosolic extracts from monkey cells. The fidelity of replication appears to be relatively high (higher than *in vitro* DNA synthesis using purified mammalian DNA polymerases). Introduction of damaged templates should reveal whether the *in vitro* replication system responds to lesions in the DNA in the same way as the cellular replication machinery *in vivo*. We are hopeful that the pZ189 vector system will be as versatile a tool for studying the biochemistry of mutagenesis *in vitro* as it has proven to be for revealing mechanisms of mutagenesis *in vivo*.

(1) Seidman M.M. et al. 1985. Gene 38:233-237; (2) Hauser, J. et al. 1987. EMBO J. 6:63-67; (3) Hauser, J. et al. 1986. Mol. Cell. Biol. 6:277-285. (4) Li, J.J. & T.J. Kelly. 1984. Proc. Nat. Acad. Sci. USA. 81:6973-6977.

E 032 RECA* DEPENDENT PROTEOLYTIC CLEAVAGE OF UMUD AND MUCA PROTEINS IN SOS MUTAGENESIS.

Hideo Shinagawa, Hiroshi Iwasaki, Toshikazu Shiba, and Atsuo Nakata. Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan.

Functions of the recA, umuD and umuC genes are required for the SOS mutagenesis in E. coli. Derepression of these genes in lexA(Def) mutants does not induce mutagenic activity in the cells: In addition activation of RecA protein as a protease (RecA*) is required for the elevation of the mutagenic activity. Since our earlier work (1) suggested a possibility of proteolytic cleavage of UmuD protein, and Perry et al found that UmuD protein contains a homologous sequence to the regions arround the cleavage sites of lambda, P22, 434 and LexA repressors by RecA* (2), we examined whether UmuD is cleaved by RecA*, and that the processed UmuD is the active form for SOS mutagenesis.

Using antiserum against UmuD protein, we examined the fate of the protein in various recA strains following mutagenic treatments of the cells. We found that the 17kDa UmuD protein was proteolytically processed to the 14kDa-protein, and that the cleavage was dependent on RecA*.

Plasmid pKM101 carries mucA and mucB genes that are structurally and functionally homologous to the umuD and umuC genes, respectively. We similarly examined the fate of MucA protein after mutagenic treatment of the cells carrying a plasmid with the mucA gene. We found that MucA protein was proteolytically processed, similarly to UmuD protein.

We constructed several mutants of umuD and mucA that produce alterd amino acids in the putative cleavage sites of these products. The activity of these genes in the SOS mutagenesis and the cleavage of these gene products in the cells after mutagenic treatment were studied. The correlation between the proteolytic cleavage of these proteins and the activity in the SOS mutagenesis was examined. The cleavage of these proteins were observed only in the cells competent for the SOS mutagenesis, suggesting that the processed products are the active forms of these proteins for the mutagenesis.

Reference 1. Shinagawa, H. et al. (1983) Gene 23, 167-174.
2. Perry,K. et al. (1985) Proc. Natl. Acad. Sci. USA 82, 4331-4335.

E 033 RecA-MEDIATED CLEAVAGE ACTIVATES UmuD FOR ITS ROLE IN INDUCED MUTAGENESIS IN E. coli. John R. Battista, Takehiko Nohmi, Lori A. Dodson, and Graham C. Walker, Biology Department, Massachusetts Institute of Technology, Cambridge MA 02139.
The products of the SOS-regulated umuDC operon are required for most UV and chemical mutagenesis in Escherichia coli. We have previously shown that the UmuD protein shares homology with LexA, the repressor of the SOS genes, and with the repressors of bacteriophages lambda, P22, and 434 [Perry, K. L., Elledge, S. J., Mitchell, B. B., and Walker, G. C. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:4331-4335]. We have carried out a series of genetic experiments which indicate that RecA-mediated cleavage of UmuD at its Cys^{24}-Gly^{25} bond activates UmuD for its role in mutagenesis and that the COOH-terminal fragment of UmuD is necessary and sufficient for the role of UmuD in UV mutagenesis. Other genetic experiments have been carried out that support the hypothesis that the primary role of Ser^{60} in UmuD function is to act as a nucleophile in the RecA-mediated cleavage reaction and that indicate that RecA has a third role in UV mutagenesis besides mediating the cleavage of LexA and UmuD. Finally, we present evidence that the cleavage of UmuD at its Cys^{24}-Gly^{25} bond does not increase the spontaneous reversion of the argE3 mutation.

Mutagenesis Detection and Analysis - II

E 034 SHUTTLE VECTORS FOR HUMAN CELLS, Michele P. Calos, Department of Genetics, Stanford University School of Medicine, Stanford, California 94305.
My laboratory has developed shuttle vectors for the analysis of mutation in human cells. The vectors are autonomously replicating plasmids based on Epstein-Barr virus (EBV) and replicate in the nucleus of a wide variety of human cells. Their background mutation frequency is very low (10^{-5}), and they can be rescued to E. coli for rapid scoring and analysis of mutations induced in the human cell. The normal copy number of these vectors is approximately ten per cell. We have developed several methods to raise the vector copy number. Methods involving manipulation of the amount of the EBV EBNA-1 protein and use of SV40 replication will be discussed. We have also used the EBV vectors to isolate human sequences which can mediate vector replication in the absence of any viral sequences. These putative origin of replication sequences and their potential use in more advanced shuttle vectors will be described.

E 035 **REPLICATION ERRORS INDUCED BY DNA ADDUCTS**, John M. Essigmann, Dept. of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.
Repair and replication of viral genomes containing single DNA adducts has been investigated. cis-Thymine glycol isomers (t') are major, stable products of ionizing radiation and oxidative damage to DNA. These lesions block replication in vitro, although certain local sequences allow polymerase bypass. To investigate possible mutagenic processing of t' in vivo, we have prepared an oligonucleotide, d(GCt'AGC), containing this modified base and incorporated it into a duplex M13mp19 phage genome. The single t' residue was positioned in an amber codon in the lacZ α-fragment. The site-specifically modified duplex was converted to single-stranded form and replicated in Escherichia coli. Among progeny phage, ~0.5% were mutant at the site that originally contained t'. DNA sequencing of these mutants showed mainly T→C transitions and, at a lower frequency, transversion mutations. The mutation frequency did not change significantly upon induction of SOS functions. In contrast, no mutants could be detected when the study was performed with t' in duplex DNA. To investigate the possibility of strand-bias in the replication of t' in double-stranded DNA, a duplex genome containing an A/C mismatch was constructed with the t' situated in the (-) strand. Replication in mismatch-repair deficient E. coli showed significant diminution of phage population arising from the (-) strand of the t'-containing DNA, although (-) strand was the preferred substrate for replication when the t' was replaced with the natural base, thymine. Other topics discussed will be O^6-methylguanine mutagenesis and repair in mammalian cells, and mutagenesis in *E. coli* of $1,N^6$-ethenoadenine.

E 036 MUTATIONAL SPECIFICITY STUDIED AT THE ENDOGENOUS *APRT* GENE IN CHO CELLS.
Glickman, B.W., A.J. Grosovsky, J. de Boer, M. Mazur, E.A. Drobetsky. York University, Toronto, Ontario, CANADA M3J 1P3
A knowledge of mutation at the DNA sequence level provides new insights into questions of the role of DNA damage and DNA repair in the production and avoidance of mutation. Here we compare the specificity of spontaneous mutation with that recovered following UV irradiation (254 nm) and treatment with benzo[a]pyrenediolepoxide (BPDE). Each of these spectra is distinct. The spontaneous spectrum is predominated by G:C => A:T transitions that includes hotspots. The G:C => A:T transition also predominates the UV-induced spectrum, but these are now concentrated at dipyrimidine sites i.e., sites of potential cyclobutane or (6-4) pyrimidine-pyrimidone lesions. In many respects the UV-induced spectrum in this mammalian genes is like that seen in bacteria and in mammalian shuttle vector systems. The BPDE-induced spectrum is characterized by a predominance of transversions. These are also not randomly distributed. However, the spectrum obtained in the *aprt* gene is distinct from that recovered in either bacteria and mammalian shuttle vectors as reported to date. These results will be discussed in the context of DNA sequence and chromosomal specific events.

E 037 ANALYSIS OF MUTATIONAL MECHANISMS WITH EUKARYOTIC DNA POLYMERASES, Thomas A. Kunkel, John D. Roberts, Katarzyna Bebenek, Mary P. Smith and Aruna M. Soni, Laboratory of Genetics, NIEHS, Research Triangle Park, NC 27709.

We are investigating the mechanisms by which mutations are produced or avoided during DNA synthesis catalyzed by DNA replication and repair proteins purified from eukaryotic sources. For this purpose we have developed a series of *in vitro* mutagenesis assays based on alpha-complementation of beta-galactosidase activity by several derivatives of bacteriophage M13mp2 (1,2). These assays have been used to define the error frequency and mutational specificity of the four classes of animal cell DNA polymerases (alpha, beta, gamma and delta).

Both DNA polymerases delta (2) and gamma (3,4) are highly accurate enzymes. The high base substitution fidelity of pol-delta (approximately one mistake for each million bases polymerized) is in part due to proofreading of misinserted bases by the 3'→5' exonuclease activity associated with this polymerase. This is based on the observation that both terminal mismatch excision and the fidelity of DNA synthesis decrease using reaction conditions that reduce the exonuclease to polymerase ratio. We have recently identified an exonuclease activity associated with highly purified polymerase gamma as well, one which fulfills the same criteria for a proofreading function. Thus these two animal cell DNA polymerases, like certain prokaryotic DNA polymerases, utilize a proofreading mechanism to improve fidelity.

Analysis of the spectra of base substitution, frameshift and deletion errors produced by DNA polymerases alpha (5) and beta (1), which lack associated exonuclease activity, establishes that the fidelity of the polymerization step depends on at least three parameters: i) the protein(s) involved in the reaction, ii) the template-primer site at which the error is made and iii) the precise nature of the error. The specificity data also permit the formulation of models to explain certain subsets of errors. Two of these models, Streisinger slippage to produce frameshifts within runs of a common base (4) and a transient misalignment mechanism (3) to account for certain base substitutions and complex deletions, have been directly supported by data obtained through the combined use of site-directed mutagenesis, fidelity assays and DNA sequence analysis of mutants.

1) JBC 260,5787 (1985). 2) PNAS 84,4865 (1987). 3) JBC 261, 160 (1986).
4) JBC 261, 13581 (1986). 5) JBC 260, 12866 (1985).

E 038 MOLECULAR MECHANISMS OF MUTAGENESIS IN MAMMALIAN CELLS : USE OF SIMIAN VIRUS 40 AND SHUTTLE VIRUS AS PROBES, A. Sarasin, F. Bourre and C.F.M. Menck, Laboratory of Molecular Mutagenesis, I.R.S.C., B.P. 8, 94802, Villejuif (France).

The analysis of induced mutations in mammalian cells has become a major goal for the understanding of the carcinogenesis initiation. Due to the complexity of the cellular genome, the use of small and easily manipulatable DNA probes, depending on cellular enzymic machinery, is highly desirable.

Simian virus 40 (SV40) has been used as a molecular probe after being treated in vitro with mutagens. Our mutation assay is based upon the reversion of a temperature-sensitive growth at 41° C to a wild-type growth phenotype. UV irradiation of ts SV40 DNA leads to the induction of temperature-independent phenotypic revertants which are due to single-base substitutions located opposite potential UV-induced DNA lesions. This mutagenesis appears therefore to be targeted opposite lesions. Treatment of UV-irradiated DNA with E. coli photolyase increased virus survival and strongly decreased virus mutagenesis. The spectrum of mutations is different after photoreactivation with more transversions than transitions. This result implies that pyrimidine dimers and Py(6-4)Py photoproducts are both premutagenic lesion.

SV40-based shuttle vectors have been used to rapidly analyse mutagenesis in mammalian cells. These vectors are however, introduced by DNA transfection which is inefficient and toxic to some cells. Transfection of naked DNA could also be mutagenic per se due to the production of DNA lesions during DNA transfer to the nucleus. Therefore, we have constructed SV40-based E. coli shuttle vectors which can be packaged as pseudovirions without excision of plasmid sequences and can be rescued in bacteria. These vectors replicate and are transmitted as virus in monkey COS cells without requiring a helper virus. Extrachromosomal vector DNA isolated from infected cells can be rescued in E. coli, so that DNA alterations can be easily screened (1). The spontaneous mutation fequency measured in bacteria, on the lacO target, is around 5×10^{-4}. UV irradiation of shuttle virus gives rise to single base or insertion mutations which are located either on the lacO sequence or in the bacterial replication origin. Pretreatment of host mammalian cells with UV light increases the mutation rate of both unirradiated and UV-irradiated shuttle virus suggesting the existence of an inducible repair pathway as shown with SV40 (2). The shuttle virus combines the convenience of plasmid rescue and analysis in bacteria, with the advantages of infectious virus.

(1) C.F.M. Menck, A. Sarasin and M.R. James, Gene, 53, 1987, 21-29.
(2) A. Sarasin and A. Benoit. Mol. Cell. Biol., 6, 1986, 1102-1107.

DNA Damage Tolerance: Recombinational Mechanisms and Lesion Bypass

E 039 DNA POLYMERASE, RECA AND MUTATION RATE. H. Echols. University of California, Berkeley, California 94720

The error frequency of normal genome duplication is about 10^{-10} per base replicated; the DNA polymerase itself accounts for about 10^{-8}. The high fidelity of prokaryotic (and possibly eukaryotic) DNA polymerases depends on a two-stage process: precise base selection in the initial 5' → 3' incorporation; and exonucleolytic 3' → 5' editing of a misincorporated base. Both stages likely depend on the geometric identity of the A-T and G-C base pairs. For *E.coli* PolIII, the polymerase and editing functions reside on separate subunits. In the presence of helix-distorting lesions in the DNA, such as pyrimidine dimers, replication is inhibited. This inhibition probably occurs because the geometry of the correct base pair is lost; as a consequence, base selection is difficult and even a correctly incorporated base will be efficiently edited. For *E.coli*, the damage-induced inhibition of replication triggers the SOS response, which includes an induced mutagenesis dependent on the UmuD/C proteins and RecA. Mutations presumably derive from relaxed-specificity replication past the DNA lesion. RecA has a multi-functional role in the SOS response: (i) derepression of SOS genes; (ii) point mutation mutagenesis; (iii) repair by homologous recombination; (iv) restart of DNA replication; (v) enhanced formation of large duplications. The derepression function is cleavage of LexA. The mutagenesis function involves cleavage of UmuD. Thus, SOS mutagenesis is regulated by two protein cleavage reactions. In addition, mutagenesis may involve a direct participation by RecA in the replicative bypass of DNA lesions; this function might derive from the capacity of RecA to bind to the site of the lesion because of its partially unwound nature and thereby inhibit the editing activity of PolIII. The ability of RecA to bind to a lesion site might also relieve the inhibition of DNA replication by a daughter-strand copying mechanism. Homologous recombination and induced duplication formation also presumably depend on the ability of RecA to associate with damaged double-strand or damage-produced single-strand DNA.

E 040 THE GENETIC CONTROL OF RECOMBINATION BETWEEN REPEATED SEQUENCES, Rodney Rothstein, John Wallis*, Elaine Hiller and Lane Arthur, Dept. of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Recombination between repeated sequences can cause deleterious chromosome rearrangements including deletions, inversions and translocations. We are studying recombination between yeast repeated sequences to examine how these events may be controlled genetically. In yeast, the two major families of repetitive sequences are the retrotransposons, Ty, repeated approximately 35 times in a haploid genome and their 330 bp LTR's called delta sequences. Direct repeat recombination between the two deltas flanking a Ty element results in loss of the element leaving a solo delta in the genome. There are approximately 150 solo deltas in common lab strains. We have been investigating the SUP4 region in which recombination between solo deltas results in deletions and/or inversions. To search for genes that may control delta and Ty-mediated rearrangements, we screened mutagenized colonies for mutations that alter the frequency of genomic rearrangements in this region. One recessive mutation was identified that increased the frequency of recombination between delta sequences in the SUP4 region and between Ty elements in other regions without affecting non-delta recombination events. We call this mutation edr1-1 for enhanced delta recombination. The wild-type copy, EDR1+, was cloned and genetic analysis of gene disruptions revealed that the null mutation (edr1::null) causes an even greater increase in delta and Ty recombination. The disruption also causes an increase in chromosome non-disjunction. The edr1::null disruption causes a three- to four-fold increase in generation time and causes a shift in the cell cycle distribution in log phase with cells accumulating as large budded cells with a single nucleus. Although edr1::null strains form diploids at normal frequencies when mated to wild type strains, homozygous edr1::null diploids are not efficiently formed. Diploid edr1::null homozygotes can be constructed using tetraploid genetics. These diploids grow even more slowly than haploid edr1::null strains. Finally, suppressor studies of the slow growth phenotype have uncovered a bypass mutation that suppresses slow growth, the abnormal morphology and the altered cell cycle distribution, but fails to suppress the recombination phenotype. Interestingly, this suppressor in a wild type EDR1$^+$ background increases delta recombination. We hypothesize that the wild type EDR1 function is to repress potentially deleterious recombination events between delta and Ty elements.

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E 041 THE MULTIPLE PATHWAYS OF POSTREPLICATION REPAIR, Kendric C. Smith and Tzu-chien V. Wang, Department of Therapeutic Radiology, Stanford University School of Medicine, Stanford CA 94305.

Postreplication repair (PRR), as defined here, involves two processes: (i) the repair of daughter-strand gaps (secondary lesions) that arise when replication skips past a primary lesion in DNA (e.g., pyrimidine dimer) and reinitiates downstream from the primary lesion, and (ii) the repair of DNA double-strand breaks (tertiary lesions) that arise at unrepaired daughter-strand gaps. PRR is recA-dependent, but not all of PRR requires SOS induction. About 50% of the daughter-strand gaps are repaired by a process that is recF dependent (1). The remaining daughter-strand gaps are repaired by a process(es) that is independent of the recF and recB genes, and appears to be constitutive (2). DNA polymerase I, especially its 5'——>3' exonuclease activity, appears to be essential for this recF-independent process (3). A small fraction of the recF-independent repair of daughter-strand gaps is umuC dependent (4). This process may represent the mutagenic pathway of PRR. The repair of DNA double-strand breaks that arise at unrepaired daughter-strand gaps normally proceeds via a recBC dependent process (1). However, under certain circumstances (i.e., in recBC sbcB cells), the repair of DNA double-strand breaks proceeds via a recF-dependent process (5). It is postulated that DNA double-strand breaks that have blunt or nearly blunt ends are repaired by the recBC-dependent process, while those double-strand breaks that contain long single stranded tails are repaired by the recF-dependent process. recA-dependent nucleotide excision repair, which only occurs in the portion of the chromosome that was replicated prior to UV irradiation, appears to proceed by the two processes that are responsible for PRR, the major difference being the manner in which the gaps are formed in the DNA (6).

(1) T.V. Wang and K.C. Smith, J. Bacteriol. 156, 1093-1098 (1983).
(2) R.C. Sharma and K.C. Smith, Mutation Res. 146, 169-176 (1985).
(3) R.C. Sharma and K.C. Smith, J. Bacteriol. 169, No. 10 (1987).
(4) T.V. Wang and K.C. Smith, Mutation Res. 145, 107-112 (1985).
(5) T.V. Wang and K.C. Smith, Mol. Gen. Genet. 201, 186-191 (1985).
(6) K.C. Smith and R.C. Sharma, Mutation Res. 183, 1-9 (1987).

E 042 ATP-INDEPENDENT DNA STRAND TRANSFER CATALYZED BY PROTEINS FROM THE YEAST *SACCAROMYCES CEREVISIAE*, Christine C. Dykstra[1], John Nitiss[2], Michael Resnick[2], and Akio Sugino[1]. [1]Laboratory of Genetics and [2] Cellular and Genetic Toxicology Branch. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

In order to investigate the molecular basis of homologous genetic recombination in eukaryotes, we have been attempting to identify and purify components in yeast that might be involved in the process(es). An activity that catalyzes the transfer of a strand from a duplex linear molecule of DNA to a complementary circular single strand has been identified and purified from meiotic and mitotic yeast cell extracts. The apparent molecular weight of the meiotic protein is 38,000 under denatured conditions. The molecular weight of the less abundant mitotic protein is not yet assigned. Both activities require homologous DNA and Mg^{2+} but no nucleotide cofactor. This activity is stimulated greater than 10-fold by the addition of yeast single-stranded DNA binding proteins (ySSBs). In the presence of ySSBs, very small amounts of the protein catalyzes strand transfer efficiently, contrasting with the ATP requirement and stoichiometric amounts of protein needed for strand transfer in other systems (e.g. T4, *E. coli*, and *Ustilago*). The mitotic specific activity increases 2-3-fold after treating growing cells with UV light or MMS. The strand transfer activity increases greater than 15-fold during meiosis specifically in *MATα/MATa* diploids but not in *MATa/MATa* or *MATα/MATα* diploids which do not undergo meiosis. This increase is not seen in *rad50* or *rad52* mutants. We are in the process of identifying the gene responsible for the meiotic activity. Both the meiotic and mitotic activities catalyze a number of different DNA interactions. The activities require homologous DNA, Mg^{2+}, and are stimulated by ySSBs. Strand transfer can occur with linear double-stranded DNA + linear single-stranded DNA, linear double-stranded DNA + single-stranded circular DNA, nicked circular double-stranded DNA + single-stranded circular DNA, supercoiled double-stranded DNA + single-stranded circular DNA, and supercoiled double-stranded DNA + linear single-stranded DNA. We suggest based on the results with various mutants and the time of increased activity that is specific to meiosis that the protein identified in meiosis is implicated in recombination. The relevance of the mitotic activity and its relation to the meiotic activity is under investigation.

Biological Consequences of Genomic Alterations

E 043 GENE REARRANGEMENTS IN TRYPANOSOMES, Piet Borst, Jan M. Kooter, Bart W. Swinkels, Sylvia M. Le Blancq, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

African trypanosomes, like Trypanosoma brucei, evade the immune response of their mammalian host by repeatedly switching the composition of their surface coat, which mainly consists of a single protein species, the Variant-specific Surface Glycoprotein (VSG). There are some 10^3 different VSG genes per trypanosome and these genes and associated repeats occupy about 20% of the genome. Actively transcribed VSG genes invariably reside near a chromosome telomere. Switching of the VSG gene transcribed can occur in two ways (reviewed in 1 and 2):
1. A new VSG gene is slotted into an active expression site (ES), displacing the old one. Usually this occurs by duplicative transposition, but it may also occur by a reciprocal recombination in which a silent and an active telomeric VSG gene exchange position by a cross-over between gene and transcription start. In both cases the new gene is activated by promoter addition. The duplicative transposition has the characteristics of a gene conversion and resembles analogous gene transpositions involved in antigenic switching in bacteria, such as Neisseria and Borrelia. As the switching rate is low (10^{-6}/trypanosome division), it may not require a specific enzyme, but depend on genetic background noise.
2. An active VSG gene expression site is shut off and a silent one is activated. How this is done, is not known yet. As different expression sites can be simultaneously active, a single mobile promoter unit is ruled out, but gene conversion between active and inactive promoters is not (3, 4). Activation might also involve loss of an unusual form of DNA modification which only affects telomeric VSG genes and which waxes and wanes with the growth and contraction of the telomere (5).

We have also observed apparent gene conversion events between the three tandemly arranged genes for phosphoglycerate kinase. The possible involvement of sequences resembling the chi sequence of E.coli in these conversions will be discussed.

1. P. Borst, Ann. Rev. Biochem. 55 (1986) 701-732.
2. P. Borst and D. Greaves, Science 235 (1987) 658-668.
3. J.M. Kooter, H.J. Van der Spek, R. Wagter, C.E. d'Oliveira, F. Van der Hoeven, P.J. Johnson and P. Borst, Cell (1987) in press.
4. P.J. Johnson, J.M. Kooter and P. Borst, Cell (1987) in press.
5. K.A. Osinga, B.W. Swinkels, W.C. Gibson, P. Borst, G.H. Veeneman, J.H. Van Boom, P.A.M. Michels and F.R. Opperdoes, EMBO J. 4 (1985) 3811-3817.

E 044 THE UV INDUCED SIGNAL TRANSDUCTION PATHWAY TO SPECIFIC GENES, Peter Herrlich, Bernd Stein, Marita Büscher and Hans Jobst Rahmsdorf, Kernforschungszentrum Karlsruhe, Institute for Genetics and Toxicology, P.O. Box 3640, D-7500 Karlsruhe 1, F.R.G.

Ultraviolet irradiation induces the expression of a large number of human genes in cultured primary cells or cell lines (1). We have isolated cDNA and genomic clones of UV responsive genes. Chimeric or mutant constructs containing portions of the regulatory DNA sequences of the human cfos gene, of HIV-1, of the gene coding for human collagenase and of the human metallothionein II A gene were used to delimit the cis acting DNA sequences required for UV induction and to define the transcription factors that recognize these sequences. These factors are at the receiving ends of UV induced signal transduction pathways. From the ends we can try to trace back to the components of the signalling cascade. The action spectrum for the UV induced gene activation and the comparison of the UV doses required in normal human cells and in cells from a patient with Xeroderma pigmentosum (group A) suggest that absorption of UV by DNA is the first step in the signal chain to most if not all UV inducible genes. Because of the rapid turnover of c-fos mRNA, we can measure how fast the signal transduction pathways become reexcitable. We have also compared kinetics and protein kinase involvements in c-fos induction by various inducing agents. Most parameters are very similar irrespective of the inducer. The signal transduction pathways pass through a period of refractoriness. When restimulated by the same agent (UV, phorbol ester or cAMP) at 3 or 24 hours, no transcription is detectable. Alternating the agents, however, leads to full responses indicating that limiting components of the signal transduction pathways must be different. Further, saturating doses of these agents were at least additive in their effect on c-fos transcription. Beside UV, several other non-physiologic and physiologic pathways activate the same genes and converge onto the same transcription factors. Evidence will be presented to show that the activities of these transcription factors in fact reflect the activity state of the intracellular communication network of oncogene products. The activities of the transcription factors are modulated by posttranslational modification.

1. Michael Karin and Peter Herrlich: Cis- and Trans-acting Genetic Elements Responsible for induction of Specific Genes by Tumor Promoters, Serum Factors and Stress. In: "Genes and Signal Transduction in Multistage Carcinogenesis", Ed. N.H. Colburn, Marcel Dekker, Inc., New York, in press

E 045 GENE AMPLIFICATION AND TUMORIGENICITY Thea D. Tlsty, Susan McCord, David Sorscher and Edward Otto, University of N. Carolina at Chapel Hill, Chapel Hill, N.C.27599
Genomic alterations or genomic rearrangements, hallmarks of neoplastic populations, exemplify the genomic instability of tumorigenic cells. The acquisition of genomic instability has been hypothesized to be an important component in the genesis of neoplasia. One of the most studied types of genomic rearrangement is gene amplification. In this study we have asked if the ability of cells to form tumors is correlated with their ability to amplify genomic sequences.
Our model system consists of rat liver epithilial cell lines which have been clonally derived from carcinogen-treated cells and vary in their ability to form tumors after injection into day-old isogenic rats. For several of these lines, we have determined their ability to amplify the gene coding for the multifunctional enzyme CAD by exposing cells to N-(phosphonoacetyl)-L-aspartate (PALA), a drug inhibiting the aspartate transcarbamylase activity of this enzyme. From our studies, we have found a striking parallel between the ability of a cell to form tumors and its ability to amplify the CAD gene. We hope that this system will help provide insight into the cause/effect relationships between genomic instability and carcinogenesis and possibly provide us with an early indication of the tumorigenic potential of a cell.

Concluding Comments

E 046 BIOLOGICAL CONTEXT OF DNA REPAIR, Robert H. Haynes, Dept. of Biology, York University, Toronto, Canada M3J 1P3.
Thirty years ago it was widely believed that the genetic material was intrinsically very stable and stood 'isolated' from the routine metabolism of the cell. Genes were not thought to be subject to any sort of non-replicative biochemical 'turnover'. Any suggested process which entailed the breakdown and resynthesis of segments of chromosomal DNA ran counter to an established orthodoxy. Thus, the discovery of excision repair played a key role in the formulation of our contemporary picture of genetic stability and change which is based on biochemical dynamics rather than molecular statics. Replication errors, spontaneous physicochemical decay of DNA subunits, and damage by many mutagenic agents, are ever-present sources of genetic noise in cells. If these had free rein, genomes would quickly erode toward destruction; they, could hardly be expected to increase greatly in length over evolutionary time. Countervailing mechanisms of replicational fidelity, DNA protection and repair are therefore essential, not only for the proper development and genetic integrity of the individual, but also for longevity and evolution of species. The genetic machinery of cells is the best known example of a highly reliable, dynamic system built from vulnerable and unreliable parts. That such genomes contain many loci necessary for DNA repair and other processes which contribute to genetic stability is an important illustration of Dancoff's 'principle of maximum error': that is, if very great fidelity is to be achieved with equipment of poor precision, extensive checking procedures must be built into the system so as to reduce the error rate to a tolerable level. Thus, it is ironic that the 'genetic noise' which is tolerated, and indeed, which never can be eliminated entirely, is the ultimate source of the genetic variation upon which natural selection feeds in evolution.

Damage Recognition and Repair Enzymology

E 100 BASE SEQUENCE DAMAGE IN DNA FROM X-IRRADIATED MONKEY CV-1 CELLS, Robert Bases, Department of Radiology, Albert Einstein College of Medicine, Bronx, NY. 10461.

Two kinds of 3'-ends were detected in DNA scission fragments of highly repetitive primate component αDNA isolated from irradiated monkey CV-1 cells. The fragments' 3'-ends were characterized by 5'-^{32}P-end-labeling the DNA, followed by examination in high resolution polyacrylamide gels. Hydrolysis of the labeled fragments' termini with exonuclease III of E. coli or by the 3' phosphatase activity of T4 polynucleotide kinase generated a third (slowest migrating)species in each mobility size class. Reference to mobility size class standards make it highly probable that the fragment ends generated by x-rays in cells are 3'-phosphoryl and 3'-phosphoglycolate which can be converted to slower migrating fragments with 3' OH ends. Densitometer measurements of gel autoradiograms showed that x-ray induction of DNA fragments with 3'-phosphoryl and 3'-phosphoglycolate ends was dose dependent over the range 100 Gy to 900 Gy. The frequency of single-strand breaks in αDNA was 8.6×10^{-7} breaks/nt/Gy. The two kinds of ends disappeared in post-radiation incubation with half time of 1.6 hrs. 5'-^{32}P-end-labeled αDNA was x-irradiated in water and introduced into unirradiated cells by transfection. Its repair and that of αDNA residing in x-irradiated plasmids could also be studied after transfection. These results provide new means to study x-ray damage and repair of specific DNA sequences in animal cells.

E 101 CELL SPECIFIC DIFFERENCES IN O^6-METHYLGUANINE-DNA METHYLTRANSFERASE (O^6MGMT) ACTIVITY AND REMOVAL OF O^6-METHYLGUANINE (O^6-MG) IN RAT PULMONARY CELLS,

S.A. Belinsky[1], M.E. Dolan[2], C.M. White[1], A.E. Pegg[2], M.W. Anderson[1], NIEHS[1], RTP, NC 27709 and Penn. State Univ.[2], Hershey, PA 17033

Previous studies have demonstrated that cell specificity exists for the alkylation of DNA from lung cells following treatment of rats with the tobacco specific carcinogen 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The concentration of the promutagenic adduct O^6MG was greatest in Clara cells followed by macrophages, type II cells and alveolar small cells. The purpose of this study was to measure the activity of the repair protein O^6MGMT and to determine whether differences exist for the removal of O^6MG among pulmonary cell types. Constitutive activity of O^6MGMT was 2-fold greater in macrophages and type II cells than alveolar small cells and Clara cells. Treatment for 4 days with NNK (10 mg/kg/day) had no effect on O^6MGMT activity in macrophages, but decreased activity in alveolar small cells and type II cells by 57 and 84%, respectively. O^6MGMT activity was below limits of detection in Clara cells. The effect of NNK on O^6MGMT activity paralleled rates of removal of O^6MG in macrophages and Clara cells. The loss of O^6MG from DNA from macrophages followed 1st order kinetics ($t_{1/2}$ = 48 hours) while virtually no removal of this adduct was observed in Clara cells over an 8 day period following cessation of carcinogen treatment. O^6MG was removed from DNA from alveolar small cells and type II cells, however the loss of this adduct was biphasic suggesting that the initial higher rates of loss may result from cell turnover rather than repair. The high concentration of O^6MG in Clara cells and the persistence of this adduct may be important factors in the strong carcinogenicity of NNK in the rat lung.

E 103 THE MECHANISM OF EXCISION REPAIR IN E.COLI: DISSECTION OF FUNCTIONAL DOMAINS OF THE UVRA PROTEIN BY MUTAGENESIS STUDIES OF THE GENE, Jourica A. Brandsma, Martina de Ruijter, Judith G. Tasseron, Jaap Brouwer and Pieter van de Putte, University of Leiden, 2333 AL Leiden, The Netherlands.

The UvrA, UvrB and UvrC proteins together form the ABC excinuclease, an enzyme which plays a major role in the repair of many different DNA damages. The domains within each subunit of the enzyme, responsible for the different functions of the complex are still unknown. Mutagenesis of the uvrA, B and C genes is a suitable tool to study the roles of the individual subunits and their domains.

We used different techniques to create and/or analyse mutations in the uvrA and uvrB genes. By linker mutagenesis, a collection of new uvrA mutations has been gathered, delivering UV sensitive as well as UV resistent phenotypes. Furthermore, formerly isolated chromosomal uvrA mutations have been cloned and identified by using a technique that allows rapid analysis of every chromosomal mutation in uvrA. By this method we identified the widely used uvrA6 mutation as a frame shift mutation that leads to a stopcodon at residue 67. The latter result implies that in a UvrA6 mutant a truncated UvrA protein with a residual activity might be present.

The cloned uvrA mutations are used to investigate possible domains in the UvrA protein. To be able to study the mutant genes and their products in a real UvrA$^-$ background we constructed an E.coli mutant carrying a deletion of uvrA.

E 104 THE NATURE OF PRECURSORS OF DNA CROSS-LINKS INDUCED BY CHLOROETHYLATING AGENTS, Thomas P. Brent, St. Jude Children's Research Hospital, Memphis, TN 38101.
The O^6-alkylguanine-DNA alkyltransfer (GAT) activity from human cells can repair the precursors of DNA interstrand cross-links induced by chloroethylnitrosoureas (CENUs). Cells with this activity are thus relatively resistant to these antitumor drugs. We have shown recently that DNA treated briefly with one such drug (BCNU), such that it contains cross-link precursors, becomes covalently bound to the purified GAT protein, suggesting the presence of an ethano diadduct that remains attached to DNA after cleavage of one of its bonds at O^6-guanine and acceptance by the GAT protein. We have sought to determine whether the substrate in DNA for formation of this complex is the same as the precursor of interstrand cross-linking by comparing the kinetics of formation of the adducts that lead to the complex, with those leading to cross-links. Initial studies showed that a variety of chloroethylating drugs other than BCNU (i.e. CCNU, methylCCNU, CNU, chlorozotocin, mitozolomide and clomesome) all generated adducts that led to complex formation with GAT protein. The rates and extents of such adduct formation varied over a wide range that paralleled rates and extents of DNA cross-linking induced by each of these drugs. Preliminary analysis of the data is consistent with the hypothesis that the complex forming adduct is a cross-link precursor, although the possibility that it is a co-incidental non-precursor adduct cannot yet be excluded. Supported by grants CA-14799, CA-36888 and CA-21765 from the NIH and by ALSAC.

E 105 ANALYSIS OF AN ENDODEOXYRIBONUCLEASE OF S. CEREVISIAE REQUIRED FOR DOUBLE STRAND BREAK REPAIR. **Dave Burbee, Judy Campbell, and Fred Heffron. Scripps Clinic and Research Foundation, La Jolla, Ca. 92037. California Institute of Technology, Pasadena, Ca. 91125.**
We have purified to near homogeneity a 140 Kd endonuclease from the yeast Saccharomyces that appears to be one of the major nuclease activities of the yeast cell. This enzyme requires a divalent cation for the degradation of both double and single stranded DNAs. Antibodies were prepared against this nuclease and seven clones were selected by lambda gt11 screening. One of these clones was modified by integration of URA3 or LEU2 genes and transplaced back into yeast. These disruptions of the nuclease gene, tentatively labelled NUC1, render the cell highly sensitive to MMS and to the presence of a persistent double strand break produced by HO endonuclease. These cells are also deficient in sporulation. However, the cells are completely resistant to UV irradiation. Thus, this mutation appears to fit in the rad52 epistasis group. We have located NUC1 on chromosome II; this chromosome bears no known genes with products that are involved in radiation damage repair.

E 106 CHARACTERIZATION OF A STRAND EXCHANGE PROTEIN FROM HUMAN CELLS, Peggy Hsieh, Carol S. Camerini-Otero and R. Daniel Camerini-Otero, NIH, Bethesda, MD 20892.
To date, the ability to carry out strand exchange between homologous DNA is unique to proteins involved in recombination and repair such as *E. coli* RecA protein. We have partially purified and characterized a strand exchange activity from the human lymphoblastoid cell line RPMI 1788 (Hsieh, *et al.*, 1986, *Cell 44*, 885-894) and from HeLa cells and are currently examining several aspects of strand exchange by this protein. The product of strand exchange between a linear duplex DNA and homologous single-strand circular DNA is a joint molecule in which the two parental substrates are held together by a region of heteroduplex DNA. Using DNA substrates containing very short double-strand regions, we have demonstrated that the human strand exchange protein has no detectable helicase or DNA melting activity. In addition, we have determined that as few as 22 bp of homology is efficiently recognized by the human protein in a strand exchange assay. We are examining the effects of DNA mismatches, deletions and insertions on branch migration rates and the possible role of the strand exchange protein in promoting branch migration.

E 107 POTENTIAL ROLE OF PROTEOLYSIS IN THE CONTROL OF UVRABC ENDONUCLEASE ACTIVITY, Paul R. Caron and Lawrence Grossman, The Johns Hopkins University, Baltimore, MD

The incision of damaged DNA by the *Escherichia coli uvr* system requires three separate proteins which in the presence of ATP participate in a dual catalytic incision event. The UvrAB protein complexes are responsible for imposing damage specificity as well as preparing the site for incision; however, at high concentrations of UvrAB there is a loss of specificity. The UvrC protein can bind to the UvrAB nucleoprotein complex leading to endonucleolytic hydrolysis. The incision product generated with undamaged DNA behaves as a nanonucleotide (9mer) in contrast to the 12-13mer generated with damaged DNA. High concentrations of pure UvrC protein has demonstrable endonuclease activity which also generates a 9mer independent of the UvrAB proteins.

From the sequence data of the *uvrB* gene [Arikan, Kulkarni, Thomas and Sancar, (1986) Nucleic Acids Res. 14: 2638] there is a putative ATP binding site as well as an Ada protease cleavage sequence. In support of these sequence homologies the UvrB protein can be proteolyzed either with crude *E. coli* extracts or with trypsin to yield to yield a 70 kDa fragment, UvrB*. UvrB* can form a stable UvrAB*-nucleoprotein complex, has an ATPase activity which is similar to UvrAB but is defective in oligonucleotide displacement (helicase) activity and in dual incision events. Thus, proteolysis of UvrB leads to functional inactivation of the UvrABC endonuclease. The protease has been partially purified and it has been shown to be capable of cleaving both the UvrB and the Ada protein. Analysis of this protease suggests that it is a "heat-shock" protein which by molecular weight, pH optimum, and spectrum of inhibitors is distinct from the Lon protease.

E 109 POLARITY OF STRAND EXCHANGE PROMOTED BY PARTIALLY PURIFIED HUMAN TRANSFERASE, Era Cassuto and Stanka Romac, Yale University, New Haven, CT 06511.

A protein fraction partially purified from HeLa cells has been previously shown to promote strand transfer between circular single strand DNA and homologous linear duplex DNA. To determine the polarity of the strand transfer reaction, we used circular duplex DNA with a defined gap and homologous linear duplexes with ends at various positions relative to the gap. We found that whilst the strand exchange reaction could proceed when either the 3' or the 5' end of the linear DNA was complementary to the gap, a strong preference for the 3' end was observed, suggesting that the human tranferase activity has the same polarity as the RecA protein of *E. coli*.

E 110 USE OF TRIPLE-STRANDED DNA COMPLEXES TO STUDY CROSSLINK REPAIR, Suzanne Cheng, Bennett Van Houten*, Aziz Sancar*, and John E. Hearst, Department of Chemistry and the Division of Chemical Biodynamics, Lawrence Berkeley Laboratory, Berkeley, CA 94720 and the *Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, NC 27514.

Triple-stranded DNA complexes that are site-specifically stabilized by a psoralen crosslink have been prepared by using recA protein and synthetic monoadducted oligonucleotides of fewer than 110 residues. These triple-stranded complexes have been used to test the second excision step in a recently proposed model for crosslink repair by ABC excinuclease (Van Houten, B., Gamper, H., Holbrook, S. R., Hearst, J. E., and Sancar, A. **1986**. PNAS 83: 8077-8081). This model, based upon that of R. S. Cole (**1973**. PNAS 70: 1064-1068), involves two excision steps separated by a recA-mediated recombination event. As predicted by the model, ABC excinuclease does cut on the pyrone-side of the crosslink in the triple-stranded intermediate, releasing a crosslinked excision product. Addition of polymerase I, helicase II, ligase, and deoxynucleoside triphosphates completes an *in vitro* system capable of fully repairing the crosslink. This work was supported by NIH grants GM11277, GM11180 and GM32833, and by DOE, Office of Health and Environmental Research Contract DE-AC03-76F0093.

E 111 DELETION ANALYSIS OF THE STRUCTURAL DETERMINANTS OF THE ACTIVITIES OF THE E. coli UVRA PROTEIN, Lark Claassen and Lawerence Grossman, Biochemistry Department, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205.

UvrA, a component of the UvrABC damage-specific endonuclease, is a multi-functional protein. It is an ATPase and a DNA-binding protein that prefers damaged sites. It can locally unwind DNA, and in association with UvrB exhibits a limited helicase activity.

Based on amino acid sequence homologies, UvrA can be divided into two tandemly arranged segments, each containing a putative ATP binding site and a metal-coordinating domain. Between these two segments lies a region of the protein that may be able to form a helix-turn-helix DNA-binding motif.

The structure of this protein raises several questions: if these sequences represent actual functional regions, to what extent are they interdependent, to which partial activities of UvrA do they contribute, and at what level is the symmetry of this protein manifested in the reaction mechanism. In an effort to understand how each of these regions contribute to the functions of this protein, a set of defined deletion mutants has been constructed. Progress in characterizing these mutants will be described.

E 112 RADIATION-INDUCED DAMAGE TO HUMAN INTERPHASE CHROMOSOMES: BREAK REJOINING AND ITS RELATIONSHIP TO CELLULAR RECOVERY, Michael N. Cornforth, Los Alamos National Laboratory, Los Alamos, NM 87545.
The technique of premature chromosome condensation (PCC) was used to visualize radiation-induced breaks in the chromosomes of density-inhibited normal human cells, and the rejoining of these lesions with time. For X-rays, the initial break frequency was linear, approximately 6 breaks per cell per Gy. For 5.5 MeV α particles (^{238}Pu) the initial break frequency was also linear, but the yield of breaks was more than twice that of X-rays per unit dose. X-ray-induced breaks disappeared (rejoined) with a half-time of 1.6 hours, to ultimately yield a frequency of residual (unrejoined or misrejoined) lesions that was equal to the frequency of aberrations scored when cells eventually reached mitosis. The kinetics of break rejoining in X-irradiated cells can be used to predict the increase in survival associated with post-irradiation delayed plating (i.e., PLDR), and split dose recovery (SLDR). The rejoining kinetics of breaks in prematurely condensed chromosomes produced by 5.5 MeV α particles will be discussed in connection with cellular recovery. This work supported by grant CA 45141-01 from the National Cancer Institute.

E 113 INITIAL CHARACTERIZATION OF A YEAST DNA REPAIR ENDONUCLEASE SIMILAR TO E. COLI ENDONUCLEASE III, Paul W. Doetsch*, Jill Gossett*, Richard P. Cunningham+, and Keunmyoung Lee*, Dept. of Biochemistry, Emory University, Atlanta, GA 30322* and Dept. of Biological Sciences, SUNY, Albany, Albany, NY 12222+.
A DNA repair endonuclease was isolated from baker's yeast (S. cerevisiae). The yeast enzyme has been partially purified, is active in the absence of divalent cations, and has an apparent molecular size of 38-42,000 daltons. The base specificity and mode of phosphodiester bond cleavage appear to be identical to that of E. coli endonuclease III when thymine glycol-containing end-labelled DNA fragments of defined sequence are employed as substrates. The yeast enzyme also cleaves heavily UV-irradiated DNA at sites of cytosine, thymine, and guanine photoproducts. The identification of such an enzyme in yeast may provide insight into the biochemistry of the radiation repair pathways in S. cerevisiae.

E 114 MITOCHONDRIAL URACIL-DNA GLYCOSYLASE FROM RAT LIVER: IDENTIFICATION, PURIFICATION AND COMPARISON OF THE BIOCHEMICAL PROPERTIES OF TWO MOLECULAR FORMS, John D. Domena, Richard T. Timmer, Sherry A. Dicharry and Dale W. Mosbaugh, Clayton Foundation Biochemical Institute and Dept. of Chemistry, The University of Texas, Austin, TX, 78712. We have highly purified and characterized two uracil-DNA glycosylases from rat liver mitochondria. Two distinct activities which have been designated form I and II were resolved during the penultimate purification step, phosphocellulose chromatography. Uracil-DNA glycosylase I was the predominant form, constituting about 85% of the total activity. The two forms are associated with different-sized polypeptides (Form I, MW=24,000 and Form II, MW=27,500) as determined by activity recovered after extracting and renaturing the enzymes from an SDS-polyacrylamide gel. By a similar method, both forms have also been shown to exist in mitochondrial extracts. Both species have similar catalytic properties and exhibit: (i) a Km value of about 1 μM for uracil residues in DNA; (ii) product-inhibition by free uracil (Ki≈0.5 mM); (iii) competitive inhibition by apyrimidinic sites in DNA (Ki≈1.5 μM); (iv) no detectable inhibition by 2'-deoxyribose and 2'-deoxyribose-5'-phosphate; and (v) similar cleavage specificity for various uracil residues located in different nucleotide sequence contexts in M13mp2 lacZα DNA. Synthetic oligonucleotides have also been constructed which contain uracil residues at unique positions to assess the relative substrate specificity of mitochondrial uracil-DNA glycosylase I and II. (Supported by NIH grant GM32823)

E 115 SITE-DIRECTED MUTAGENESIS OF THE T4 ENDONUCLEASE V GENE: ROLE OF ARGININE-3 IN DNA BINDING, Diane R. Dowd and R. Stephen Lloyd, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232. Endonuclease V, a pyrimidine dimer-specific endonuclease in T4 bacteriophage, is able to scan DNA, recognize pyrimidine dimer photoproducts produced by ultraviolet light, and effectively incise DNA through a two-step mechanism at the damaged bases. Endonuclease V may bear some structural resemblance to the DNA-interactive proteins λ repressor or EcoRI which both contain an N-terminal flexible "arm." The arms contain one or more basic amino acids involved in the recognition and interaction of the protein with DNA. By analogy, endonuclease V is proposed to have an N-terminal arm containing the positively charged amino acid arginine at the third residue. This arm, and particularly Arg-3, appear to be important in the interaction of the enzyme with DNA. To assess the role of this arginine, site-directed mutagenesis was performed changing Arg-3 to Lys, Asp, Glu, and Gly. The mutated proteins were characterized with regard to 1) cellular resistance to ultraviolet light; 2) pyrimidine dimer-specific nicking activity; and 3) binding affinity to UV-irradiated DNA.

The conservative change from Arg-3 to Lys-3 resulted in a protein which retained all of the activities which are characteristic of the wild type enzyme, while the other mutants lost all activity. These results suggest that Arg-3 of endonuclease V is important in dimer-specific binding or in nonspecific electrostatic protein-DNA interactions. Supported by Vanderbilt University Research Council Award, NIH ES 04091 and NIH ES 00267.

E 116 DNA POLYMERASE δ PARTICIPATES IN BOTH UV-INDUCED DNA REPAIR SYNTHESIS AND DNA REPLICATION IN PERMEABLE HUMAN FIBROBLASTS, Steven L. Dresler, Mark G. Frattini and Kevin Sean Kimbro, Washington University School of Medicine, St. Louis, MO 63110. In mammalian cells, both UV-induced DNA repair synthesis and replicative DNA synthesis are inhibited by aphidicolin; these processes are thus mediated by one or both of the aphidicolin-sensitive DNA polymerases, α and/or δ. In permeable human fibroblasts, we have examined the sensitivity of UV-induced repair synthesis and replicative DNA synthesis to N^2-(p-n-butylphenyl)-2'-deoxyguanosine-5'-triphosphate (BuPh-dGTP), which inhibits polymerase α more strongly than it inhibits polymerase δ, and 2',3'-dideoxythymidine-5'-triphosphate (ddTTP), which inhibits polymerase δ more strongly than it inhibits polymerase α. Replicative DNA synthesis and UV-induced DNA repair synthesis show very similar sensitivities to both BuPh-dGTP and ddTTP; both processes are much less sensitive to BuPh-dGTP and much more sensitive to ddTTP than is DNA polymerase α. These data are consistent with the involvement of DNA polymerase δ in both DNA replication and UV-induced DNA repair synthesis in human cells. We have also studied the K_m's of replication and UV-induced repair synthesis for substrate dNTP's. The K_m's for dCTP, dGTP, and dTTP of repair synthesis are 7 to 28-fold lower than those of replication. We propose that UV-induced DNA repair synthesis and semiconservative DNA replication are mediated by complexes containing the same DNA polymerase modified in different ways by association with accessory proteins or other factors. (Supported by PHS Grant CA37261 and by the Life and Health Insurance Medical Research Fund.)

E 117 ENHANCED DNA REPAIR AS A MECHANISM OF RESISTANCE TO CIS-DIAMMINEDICHLORO-PLATINUM(II), Alan Eastman, Nader Sheibani and Nancy Schulte, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE 68105.
Murine leukemia L1210 cells, either sensitive or resistant to the toxic action of the cancer chemotherapeutic agent cis-diamminedichloroplatinum(II) (cis-DDP), have been studied for potential differences in DNA repair. The plasmid expression vector pRSVcat was damaged with cis-DDP and transfected into cells. The cell lysate was subsequently assayed for chloramphenicol acetyltransferase (cat) activity. In the sensitive cells, one cis-DDP adduct per cat gene was sufficient to inhibit expression. Eight times the amount of damage was required to inhibit cat expression in the resistant cells. This suggested that the resistant cells were able to repair the plasmid DNA permitting cat expression. In parallel experiments, the formation and repair of specific DNA-bound adducts was followed. Cells were incubated with the analogue [^{3}H]-cis-dichloro(ethylenediamine)platinum(II). The DNA was purified, digested and the adducts were separated by HPLC. Both cell lines demonstrated intrastrand cross-links at GG, AG and GNG sequences. Repair of these cross-links occurred in a biphasic manner, rapid for the first 6 h then much slower. The resistant cells removed four times as many adducts during the rapid phase of repair. Hence, both assays suggested that enhanced DNA repair contributed to cellular resistance to cis-DDP. (Supported by the National Cancer Institute, CA36039.)

E 118 DETECTION OF A PROTEIN FROM HUMAN CELL EXTRACTS THAT BINDS TO SYNTHETIC HOLLIDAY JUNCTIONS. Kieran Elborough and Stephen. C. West , Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts., EN6 3LD, U.K.
A central intermediate in genetic recombination is a structure in which two double-stranded DNA molecules are linked by single-stranded crossovers that form a Holliday junction. Using a gel retardation assay, a protein has been detected that binds to synthetic X- and Y-junctions in DNA. The binding is structure specific, since linear duplex DNA containing the same sequences were not retarded in the assay. Maximum binding occurs at 150mM NaCl and does not require Mg^{++}. This activity has been found in all human cell lines looked at so far including two with inheritable diseases thought to be due to defects in DNA repair (Bloom's syndrome and xeroderma pigmentosum).

Using a glycerol gradient, whole cell extracts were partially fractionated and the molecular weight of the activity determined to be approximately 20 kD. A nuclease activity was also found in the same fraction as the binding protein. We are at present studying its action upon other synthetic Holliday junctions.

E 119 RELATIONSHIP BETWEEN TOPOISOMERASE II ACTIVITY AND DNA REPAIR IN L5178Y MOUSE LYMPHOMA CELLS, Helen H. Evans, Thomas E. Evans, Min-fen Horng, and Marlene Ricanati, Case Western Reserve University, Cleveland, OH 44106.
Mouse lymphoma strain L5178Y-S (LY-S) is sensitive to the cytotoxic effects of ionizing radiation and deficient in the repair of DNA double-strand breaks in comparison to the parental strain L5178Y-R (LY-R) (Evans et al, Proc. Natl. Acad. Sci. **84**, in press). In comparison to strain LY-R, we have found strain LY-S to be sensitive to the cytotoxic effects of m-AMSA and ellipticine (inhibitors of topoisomerase II which bind to DNA and inhibit the breaking-reunion activity of the enzyme), and to etoposide (which inhbits the same reaction but which is thought to bind to topoisomerase II rather than to DNA). Two independent radiation-resistant variants of strain LY-S, isolated following selection during protracted low dose-rate irradiation, were found to have regained resistance to all of these inhibitors. In contrast, strain LY-S and the radiation-resistant variants are more resistant than strain LY-R to novobiocin (a competitaor for the topoisomerase II ATP site) and campothecin (an inhibitor of topoisomerase I). Treatment with m-AMSA and etoposide resulted in more DNA damage in strain LY-S and the radiation-resistant variants than in strain LY-R, as measured by filter elution at pH 9.6. Repair of this m-AMSA induced damage was more extensive in the radiation-resistant variants than in either strain LY-S or LY-R, while repair of etoposide-induced damage was similar in all four strains. The results suggest that the repair deficiency and radiation sensitivity of strain LY-S is related to an alteration in topoisomerase II or in the reaction of this enzyme with DNA. (Supported by grants R37CA15901 and P30CA45703 from the U.S. Public Health Service.)

E 120 PURIFICATION AND PROPERTIES OF O^6-METHYLGUANINE-DNA METHYLTRANSFERASE FROM HUMAN PLACENTA. D. Bhattacharyya, R. S. Foote, A. M. Boulden, and S. Mitra*, University of Tennessee Graduate School of Biomedical Sciences and *Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

O^6-Methylguanine-DNA methyltransferase (MGMT) has been partially purified from human placenta by a series of steps to a purity of about 2%. The molecular weight of the protein is 22-24 kDa as determined by gel electophoresis, gel filtration and sedimentation. Gel filtration profiles indicate that the human protein is similar in size to that of mouse liver MGMT, but is somewhat larger than the corresponding protein from rat liver. The methyltransferase reacts with apparent second-order kinetics with O^6-methylguanine in either double- or single-stranded calf thymus DNA with rate constants of 2.20×10^8 and 0.067×10^8 lit mol^{-1} min^{-1}, respectively, at 37°. The human MGMT has a pI of 6.2 and reacts optimally at pH 8.0. The reaction is strongly dependent on salt concentration and is reversibly inhibited by excess DNA and certain polyvalent metal ions. Methylation of the protein by reaction with substrate causes a slight downward shift in its pI, but does not affect its binding to DNA-cellulose columns. (Research supported by NCI grant CA 31721 and by U. S. Dept. of Energy contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.)

E 121 CHARACTERIZATION OF DEOXYRIBOSEPHOSPHATASE, A NOVEL ENZYME IN THE BASE-EXCISION REPAIR PATHWAY OF *E. coli*, **William A. Franklin and Tomas Lindahl, Imperial Cancer Research Fund, Clare Hall Laboratories, Potters Bar, Herts. EN6 3LD United Kingdom**

We have isolated an activity in *E. coli* that removes 2-deoxyribose-5-phosphate (dRp) following the cleavage of DNA containing apurinic/apyrimidinic (AP) sites by a 5' AP endonuclease. The enzyme responsible for this activity has been purified more than 200 fold, is free from AP endonuclease activity, and has an absolute requirement for Mg^{2+} cation. The enzyme has a molecular weight of 60000 daltons based on gel filtration chromatography and sucrose gradient centrifugation. This activity appears novel, as it is found in *E. coli* mutants deficient in either exonuclease I, exonuclease III, endonuclease III, exonuclease VII, or DNA polymerase I. The enzyme does not catalyze the release of mononucleotides from termini of linear double- or single-stranded DNA. Furthermore, purified preparations of endonuclease III, endonuclease IV, exonuclease III, and DNA polymerase I do not catalyze the release of the dRp residue. We believe the enzyme is part of the base-excision repair pathway in *E. coli* responsible for the repair of AP sites. We are currently investigating the presence of this activity in a series of human cell lines.

E 122 THE EFFECT OF A SPECIFICALLY LOCALIZED DAMAGED NUCLEOTIDE ON THE *ESCHERICHIA COLI* UVRAB HELICASE. Gilberto Fronza [1,2] and Lawrence Grossman [1], [1]Dept. of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, USA, [2]Istituto Nazionale per la Ricerca sul Cancro, Laboratorio di Mutagenesi, Genova, Italy.

One of the distinctive features of the E. coli UvrAB "helicase" [Oh E.Y. and Grossman,L. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 3638] is its sensitivity to the presence of UV-damage in the DNA substrate, which may be required of a repair complex in order to discriminate between damaged and undamaged sites in DNA. However in that study quantitation and characterization was limited because the localization of the "damaged DNA" was random and, hence, uncharacterized. To analyze the UvrAB protein complex associated strand displacement activity more definitively specifically AAF-damaged DNA of suitable length, with the AAF-G adduct located at a single specific locus was synthesized. An oligonucleotide (15mer) with the following structure 5'-CACACGTTAACACAC-3' referred to as the "target sequence" was treated in vitro with N-Acetoxy- N-Acetyl-2-Amino (ring-^{3}H-G) fluorene (AAAF). The "target sequence" has only one guanine target site located at the last position (5') of an Hinc II sequence. The modified fragment was resolved from the unreacted population by RP-HPLC and the AAF-modified 15mer characterized by determining 1) its UV spectrum, 2) the ratio of 15mer molecules / ^{3}H-AAF and 3) after hybridization with ss M13mpGF (M13mpGF is derived from M13mp19 cloning in its Hinc II site the complement of the target sequence) the Hinc II sensitivity was assessed. The results were consistent with the presence of a single AAF-G adduct per fragment molecule located in the Hinc II site. Taking advantage of the sequence downstream from the site of hybridization of the 15mer, the AAF-G 15mer was elongated to a 17mer or to a mixture of 19mer, 27mer or 34 mer and simultaneously 3'labeled with ^{32}P-dATP . The response of the UvrAB helicase to these substrates will be discussed.

E 123 INTERACTION OF CLINICALLY USED ANTI-TUMOR AGENTS WITH ACTIVATED ONCOGENE STRUCTURE AND FUNCTION. Bernard W. Futscher, Nicholas S. Vlahos, Russell O. Pieper, and Leonard C. Erickson. Section of Hematology/Oncology, Loyola University Medical Center, Maywood, IL 60153.

The goal of this study is to determine if DNA damage produced by clinically used alkylating anti-tumor agents can alter the expression of activated oncogenes which are critical for the maintenance of the tumorigenic phenotype. Using Maxam-Gilbert DNA sequencing methodology in initial studies we have found that nitrogen mustard (HN-2), L-phenylalanine mustard (L-PAM), and a cyclophosphamide derivative (C2) have unique DNA sequence preferences for attack in cloned coding regions from the c-myc and N-myc oncogenes. These drugs were then studied for DNA damaging properties as measured by DNA alkaline elution, cytotoxicity as measured by colony formation assays, and oncogene mRNA transcription inhibition in human tumor cell lines having these activated myc oncogenes. We have found that at the periods of peak DNA interstrand crosslinking, these agents produce significant depression in the levels of oncogene mRNA transcripts as measured by Northern blot analysis. We are currently comparing the responses of cell lines having single copy, over-expressed oncogenes to cell lines having amplified copies of the same oncogene, to determine if the anti-tumor agents are more, or less, effective at oncogene inactivation and cell killing depending on the oncogene copy number.

E 124 CHARACTERIZATION OF PSORALEN-DAMAGED DNA, Francis P. Gasparro[1] and Regina M. Santella[2]. [1]Department of Dermatology, Yale University, New Haven CT 06510 and Institute for Cancer Research, Columbia University, NY NY 10032.

Psoralens are tricyclic furocoumarins which intercalate with DNA in the dark. Photoactivation of the dark complex with long wavelength ultraviolet light (UVA, 320-400 nm) results in the formation of three psoralen-thymine photoadducts (2 monoaddition products and a diadduct or interstrand crosslink). To determine the frequency of occurrence of these photoadducts in lymphocytes treated with psoralen and UVA light, we have developed a panel of monoclonal antibodies that recognize one of the monoadducts and the crosslink. In addition a monclonal antibody has been prepared that recognizes the monoaddition produuct of the angular psoralen, 6,4,4'-trimethylangelicin, which cannot form crosslinks. The specificity and sensitivity of these monoclonal antibodies have been characterized using competitive ELISA (Nucleic Acids Research 13: 2533 [1985]). These antibodies are capable of detecting femtomoles of psoralen-damaged DNA. For example, leukemic phase lymphocytes treated by extracorporeal photochemotherapy (8-methoxypsoralen and UVA) contained ~3000 psoralen adducts per cell (or 1 photoadduct per million base pairs). These antibodies are now being used to characterize the repair of psoralen-damaged DNA.

E 125 PROCESSIVE NICKING ACTIVITY OF T4 ENDONUCLEASE V ON UV-IRRADIATED PLASMID MINICHROMOSOMES, Elliott A. Gruskin and R. Stephen Lloyd, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

T4 endonuclease V is a pyrimidine dimer-specific endonuclease which generates single-strand incisors in DNA at the sites of pyrimidine dimers. In vitro studies have shown that endonuclease V acts by a processive mechanism on plasmid DNA. This processive mechanism is the result of one-dimensional diffusion of the enzyme on DNA. In vivo studies have shown that plasmid DNA repair initiated by endonuclease V within UV-irradiated E. coli occurs by a processive mechanism. In order to investigate the possibility that the overall processive mechanism is due to endonuclease V, plasmid minichromosomes were isolated from E. coli. The complexes were visualized by electron microscopy and appeared as condensed plectonemic branched structures bound by a heterogeneous population of protein particles. Endonuclease V incised the DNA dimers at sites on UV-irradiated plasmid minichromosomes by a processive mechanism. This demonstrates that protein particles bound to DNA do not inhibit the one-dimensional diffusion of endonuclease V on DNA and the overall processive pathway in vivo could be due to the processive activity of endonuclease V.

E 126 PURIFICATION AND CHARACTERIZATION OF AN ACTIVITY THAT CATALYZES DNA HETERODUPLEX FORMATION FROM SACCHAROMYCES CEREVISIAE, James Halbrook and Kevin McEntee, UCLA School of Medicine, Los Angeles, CA 90024.

An activity that catalyzes the formation of heteroduplex DNA has been fractionated from extracts of Saccharomyces cerevisiae. A protein of Mr 120,000 has been purified to 90% as judged by silver staining of fractions separated by polyacrylamide gels and is deficient in endo or exonuclease activities. The highest purity fractions do not carry out strand displacement reactions with circular ssM13 DNA and RFIII. Joint molecules are observed however, when circular ssM13 DNA and linear duplex M13 DNA possessing short single-stranded termini are employed as substrates. Using heat denatured [3H] P22 DNA as a substrate, this activity catalyzes DNA reannealing at a rate approximately 100-fold that of the RecA protein of Escherichia coli. Optimal activity occurs at pH 7.5 in the absence of any nucleoside triphosphate and either Mg^{++} or Ca^{++} satisfies the divalent cation requirement for the protein. Both the rate and extent of reannealing are dependent on the amount of protein in the reaction. Rapid reannealing is detected at a stoichiometry of 1 protein monomer per 100 nucleotides. Filter binding studies indicate that protein-DNA complexes can be formed with both single- and double-stranded DNA. Protein can be dissociated from duplex DNA or single-stranded viral M13 DNA with 1% SDS or 6 M guanidine HCl. Despite differences in the energetics of the two proteins, this yeast enzyme and RecA protein promote DNA pairing by similar mechanisms.

E 127 THE DOUBLE STRAND DNASE ACTIVITIES OF THE NEUROSPORA RECOMBINASE. Zafer Hatahet and Murray Fraser, McGill University, Montreal, Canada. H3G 1Y6.

Endo-exonuclease (EE) of Neurospora has been implicated in DNA-repair. EE was shown previously to possess distributive endonuclease activity with high specificity for single-strand (ss) DNA and highly processive exonuclease activity with double strand (ds) DNA which generates long ss-tails and ss-gaps in linear ds-DNA. Superhelical DNA was randomly nicked and then the relaxed DNA linearized and exonucleolytically degraded. Electron microscopy of EE-treated PM2 DNA revealed that the ends of the ds-DNA often remained juxtaposed as if EE remained bound at the ds-breaks. When restriction enzyme linearized pBR322 ds-DNA was treated briefly with EE and then electrophoresed in agarose gels, "ladders" of ds-DNA fragments were detected on a background smear of DNA, indicating that frequent site-specific ds-breaks were made. Sequencing of end-labelled EE-treated ds-DNAs has revealed some homology at the breaks sites including two sites 163 bps apart on opposite strands of pBR322 DNA which are identical for 6 bps at the break: 5'-p..AG , CACT..OH-3'. It was found that EE removed 5'-p-label about 100 times faster than 3'-nucleotides. Dephosphorylation of the ends of linear ds-DNA reduced the rate of its exo-nucleolytic degradation. Limited nicking of this DNA with pancreatic DNase I greatly stimulated the degradation, but nicking with Micrococcal nuclease had no effect. It is suggested that the EE can either track from the ends of linear ds-DNA and sense specific nucleotide sequences in which nicks are made to initiate exonucleolytic degradation of one strand, or enter the duplex at 3'-OH, 5'p nicks to do the same. An alternative action at these nicks is to cleave the other strand to generate a ds-break. (Supported by MRC Can.)

E 128 CELL CYCLE DEPENDENT REGULATION OF MAMMALIAN DNA REPAIR ENZYMES. Dag E. Helland and Lisbeth C. Olsen, Laboratorium of Biotechnology, University of Bergen, BERGEN, Norway.

The goal of this study is to determine the activity of mammalien DNA repair enzymes at different phases in the cell cycle and in different tissues in order to understand the regulation of these enzymes. Mouse L cells were grown in suspension cultures and separated according to size by centrifugal elutriation. The cell cycle status of the fractions obtained was monitored by flow cytometry and coulter volume histograms. Cells in the early G_1 phase were recultured and the growth of these cells was followed with regard to cell number and macromolecule synthesis. At various intervals high salt extracts were made from nuclei. Extracts were also made from different mouse tissues. These extracts were used for measuring the activities of the redoxyendonuclease, AP-endonuclease, 3-methyladenine DNA glycosylase and uracil DNA glycosylase.

The conclusions which can be drawn from these experiments are: The activities of mammalien DNA repair enzymes increase several folds in late G_1 phase prior to the start of DNA synthesis and that their synthesis are controlled in the same way as the other enzymes involved in the DNA metabolism. In tissues a positive correlation was observed between the mitotic index and the enzyme activities. This implies that the cell has to go through the G_1 phase in order to increase its DNA exision repair capacity.

E 129 CHARACTERIZATION OF A STRAND EXCHANGE PROTEIN FROM HUMAN CELLS, Peggy Hsieh, Carol S. Camerini-Otero and R. Daniel Camerini-Otero, NIH, Bethesda, MD 20892.

To date, the ability to carry out strand exchange between homologous DNA is unique to proteins involved in recombination and repair such as *E. coli* RecA protein. We have partially purified and characterized a strand exchange activity from the human lymphoblastoid cell line RPMI 1788 (Hsieh, *et al.*, 1986, *Cell 44*, 885-894) and from HeLa cells and are currently examining several aspects of strand exchange by this protein. The product of strand exchange between a linear duplex DNA and homologous single-strand circular DNA is a joint molecule in which the two parental substrates are held together by a region of heteroduplex DNA. Using DNA substrates containing very short double-strand regions, we have demonstrated that the human strand exchange protein has no detectable helicase or DNA melting activity. In addition, we have determined that as few as 22 bp of homology is efficiently recognized by the human protein in a strand exchange assay. We are examining the effects of DNA mismatches, deletions and insertions on branch migration rates and the possible role of the strand exchange protein in promoting branch migration.

E 130 IMMUNOLOGICAL QUANTIFICATION OF THYMINE RADIOLYSIS PRODUCTS IN DNA X-IRRADIATED IN VITRO, Karen Hubbard, Yoke W.Kow, Hiroshi Ide, and Susan.S.Wallace, New York Medical College, Valhalla, NY 10595.

Antibodies specific for lesions produced in X-irradiated DNA have proved to be sensitive probes for the detection and measurement of DNA radiolysis products. In order to determine the contribution of thymine glycols to the spectrum of damages produced by X-rays, antibodies raised to thymine glycol were used to quantitate this lesion in X-irradiated DNA, and the number observed were compared to the number of acetol fragments and endonuclease III-sensitive sites. PM2 DNA was X-irradiated under a variety of conditions and in all cases, the majority of endonuclease III-sensitive sites measured were thymine ring-saturation products. Thymine glycols were detectable, using the direct ELISA assay, only when the DNA was irradiated at high concentration. In phosphate buffer, thymine glycols represented a small proportion of thymine ring-saturation products formed, while in Tris, all thymine ring-saturation products were thymine glycols. An hydroxyl radical scavenger, KI, more efficiently reduced the number of thymine ring-saturation products than endonuclease III-sensitive sites. Further, antibodies raised towards dihydrothymine, a major anaerobic radiolysis product, were used to quantitate this product in X-irradiated PM2, calf thymus, and M13 DNA, as well as poly dT. For these susbtrates, the presence of sodium formate, which increases the concentration of reducing radicals, caused an increase in the production of dihydrothymine. This work was supported by DHHS fellowship 7 F32 GM10567 and DOE grant DE-FGOZ-87ER60510.

E 131 QUANTITATION OF NON-RANDOMLY DISTRIBUTED DNA STRAND-BREAKS, Darel J. Hunting, Erno Keszei and Bonnie J. Gowans. U. of Sherbrooke, Sherbrooke, Quebec, J1H 5N4.

Alkaline elution is a sensitive method for quantitation of strand-break frequencies in DNA containing a random distribution of strand-breaks or alkali-labile sites. To date, it has not been possible to quantitate DNA strand-break frequencies induced by DNA damaging agents or DNA repair processes which produce non-random distributions of strand-breaks. We have developed a method, involving a non-linear least squares estimation of alkaline elution parameters, which permits the quantification of DNA strand-break frequencies and population sizes for cells containing a non-random distribution of strand-breaks. This method has been applied to two problems: DNA damage by bleomycin and the genomic distribution of excision repair events in Xeroderma pigmentosum group C cells. Bleomycin treatment of cells produced two populations of DNA, one heavily damaged and the other lightly damaged. The size of the heavily damaged population increased as a function of bleomycin dose which indicates that the two populations of DNA do not correspond to physical parameters but is consistant with a cooperative mechanism of damage by bleomycin. To analyse excision repair events in XP-C cells following UV irradiation, cells were incubated with hydroxyurea and aphidicolin, which permited incision but prevented repair patch ligation. During the first 30 min of repair, incision occurred randomly but after 1 hr two populations of DNA were present: ca. 8% of the DNA contained the majority (>90%) of incised sites while after 2 hr, this population of DNA had expanded to 20%. Funded by the MRC (Canada) and the FRSQ.

E 132 THYMINE DIMER INDUCED BENDING IN DNA, Intisar Husain, Jack Griffith and Aziz Sancar, Department of Biochemistry and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514.

Pyrimidine dimers are the major UV photoproducts. In order to understand the mechanism by which various enzymes recognize and repair these photoproducts, it is important to know the conformational changes induced by these lesions. We have constructed a 48 bp fragment with a thymine dimer in the center, BamHI site near one terminus and BglII site near the other. A 32 mer generated after digestion with these enzymes was ligated head to tail in their presence to produce multimers of the above sequence. The distance between dimers in these ligated products was 32 bp (~ 3 helix turns). Ligation products from dimer and non-dimer fragments were analyzed by one dimension and 2-dimension polyacrylamide gel electrophoresis as well by electron microscopy to detect any structural deformities in DNA helix. Dimer containing ligated multimers showed slow migration on acrylamide gel compared to their non-dimer counterparts indicating dimer induced bending. This effect was abolished after repairing the dimers with photolyase. To find out whether the dimer induced bend facilitated circle formation, samples were subjected to 2D-gel electrophoresis. Circles as small as 96 bp length were observed. The frequency of small circles was drastically higher with modified DNA compared to unmodified. The results from 2D gel electrophoresis were confirmed by direct electron microscopic visualization of modified and unmodified ligated products. These results suggest that the presence of dimers causes significant bend in the helix, facilitating the formation of smaller circles. From the size distribution of circles with modified and unmodified DNA we calculate a 30° bend by thymine dimer.

E 133 SPECIFICITY OF DNA N–GLYCOSYLASE ACTIVITIES OF ESCHERICHIA COLI ENDONUCLEASES III, VIII AND IX, Hiroshi Ide, Yoke Wah Kow, Robert J. Melamede and Susan S. Wallace, Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595.

DNA lesions produced by ionizing radiation and/or free radical reactions can be removed and the DNA restored to its initial state by a series of enzymatic reactions. One of these repair processes is initiated by cleavage of the N–glycosylic bond of the damaged base, thereby removing it from the DNA backbone. Endonuclease III from E. coli incises X–irradiated DNA and is known to have N–glycosylase activity on damaged bases such as thymine glycol, urea, as well as other ring saturation and contraction products of thymine. In addition, two new activities that incise X–irradiated DNA, endonucleases VIII and IX, have been recently isolated from E. coli. However, the complete spectrum of base damages recognized by these enzymes, including endonuclease III, remains to be elucidated. We are addressing this question by analyzing the released products after N–glycosylase action. In addition to an AP endonuclease activity, endonuclease VIII releases urea, thymine glycol, dihydrothymine and while endonuclease IX has thus far been shown to release urea from DNA or poly (dA·dT) containing respective unique base damages.

X–irradiated or osmium tetroxide–treated poly (dA·[^{3}H,^{14}C]dT) and poly (dG·[^{3}H]dC) containing multiple base damages have also been used as substrates and the released products derived from thymine or cytosine analyzed by HPLC. In preliminary experiments, we have found that all three enzymes (endonucleases III, VIII and IX) catalyze the release not only of thymine products but also of cytosine products from X–irradiated polynucleotides. The results based on the release assay will be presented. This work was supported by DHHS grant CA 33657 and GM 37216 and DOE grant DE–FGOZ–87ER60510.

E 134 THE PURIFICATION OF A G/T MISMATCH BINDING PROTEIN FROM HeLa CELLS. Josef Jiricny, Friedrich Miescher-Institut, PO Box 2543, 4002 Basel

The results of our recent studies, concerned with the investigation of the repair of a series of base-base mismatches in vivo, indicated that G/T mispairs were the sole efficiently- and directionally-corrected lesions (Brown and Jiricny, Cell, Sept. 1987). These data were substantiated by our in vitro DNA-protein binding studies. Band-shift assays, using labeled mismatch-containing oligonucleotide duplexes, identified a protein factor in HeLa whole cell extracts, which binds selectively to DNA substrates with G/T mismatches. UV cross-linking experiments, using 5-bromodeoxyuridine-containing oligonucleotide substrates, indicated that the G/T binding factor is a protein of approximately 190 kilodaltons in size, which is degraded to two fragments of 115 and 80 kd. The 190 kd protein was partially-purified (approximately 500-fold) by a series of chromatographic steps, involving Sephacryl S-300, Heparin-Sepharose and Mono-Q FPLC. Further purification is being accomplished by means of DNA-affinity chromatography. As G/T mismatches can arise in "resting" DNA as the result of 5-methyl cytosine deamination, correction of these lesions is of utmost importance if the methylation pattern, and with it the mode of transcription, of the DNA is to be preserved. It is anticipated that this protein may provide us with the first insight into the mammalian pathway of mismatch correction.

E 135 BIOCHEMICAL CHARACTERIZATION OF HUMAN CELL UV-ENDODEOXYRIBONUCLEASE ACTIVITIES.
W.K. Kaufmann and L.P. Briley, University of North Carolina at Chapel Hill.
Two endodeoxyribonuclease activities have been identified in human fibroblasts which act on UV-irradiated cellular DNA (UV-endo's). One activity is present in normal and xeroderma pigmentosum (XP) variant fibroblasts but missing from XP cells from complementation group A. This activity appears to represent the UV-endo that operates within the nucleotidyl DNA excision repair pathway and cuts DNA at sites of pyrimidine dimers. The second activity is equally well expressed in normal, XPV and XPA strains and probably corresponds to the redoxy UV-endo that cuts DNA at sites of ring-saturated pyrimidines. These two activities were initially distinguished in permeable cells based upon their fluence-responses and requirement for ATP. The pyrimidine dimer-directed activity was saturated at fluences above 10 J/m^2 and displayed a strong requirement for ATP. The putative redoxy activity exhibited a linear response to fluences in the range of 10-100 J/m^2 and was independent of ATP. These activities were further characterized by the biochemical conditions that permitted maximal incision of damaged DNA. The pyrimidine dimer-directed UV-endo operated with greatest efficiency within permeable XPV in an incubation mixture containing 2.5 mM ATP, 5 mM $MgCl_2$, 12 mM KCl, and 0.5 M sucrose at pH 8.0. Under these conditions this activity incised DNA within permeable cells at 30% of the initial rate seen in intact cells. The redoxy UV-endo in XPA did not require ATP, $MgCl_2$, KCl or sucrose, and during incubation in the absence of ATP it cut DNA in permeable cells at 100% of the rate seen in intact cells. These observations are relavent to the design and operation of an assay for in vitro complementation of XP. Supported by PHS grant CA36906

E 136 REPAIR OF HETERODUPLEX-LOOPS IS INITIATED BY ENDONUCLEASE VII IN VITRO
Susanne Kleff and Börries Kemper, University of Cologne, 5000 Köln 41, Weyertal 121, Institute of Genetics, Federal Republic of Germany.
Heteroduplex DNA is generated in vivo when two genetically distinct molecules recombine and exchange strands within a region of heterology. In the case of deletion or insertion-mutations, *heteroduplex loops are formed. These are sub* ject to repair processes in pro- and eukaryotes; an enzyme was not assigned to this repair, yet. From genetic evidence it was suggested that endonuclease VII (EndoVII=gp49) of bacteriophage T4 attacks heteroduplex loops in vivo because it prevents them from being packaged into progeny phage, causing a dras tic loss in heterozygotes (HETs;1.).-We have purified Endo VII and previously shown that the enzyme specifically cleaves at DNA-structures like Holliday-junctions, cruciforms and Y-branches (2). Here we report the construction of heteroduplex-loop DNAs in vitro and show that EndoVII intoduces staggered nicks into both strands flanking the loop-structure, irrespective of loopsize (51n and 8n were tested) and orientation (plus- and minus-strand). As a result double-strand breaks with 5'-recessive ends are produced and the entire loop-sequence protrudes as a 3'-OH terminated single-stranded tail from one fragment. This fragment appears highly recombinogenic and may be rescued in vivo during further rounds of recombination.- Ref.:1.Mosig,G.& Powell, D. 1985 American Society for Microbiology, 85th annual meeting, M4:209 (Abstr); 2.Jensch, F. & Kemper, B. (1986),The EMBO Journal 5:181 - 189.

E 137 PURIFICATION AND CHARACTERIZATION OF THREE "UV ENDONUCLEASE" ACTIVITIES FROM MAMMALIAN CELLS, Joon Kim, Soo-Young Choi and Stuart Linn, Univ. of Calif., Berkeley, CA 94720.

Three "UV endonuclease" activities from mouse plasmacytoma cells which nick heavily UV-irradiated and OsO_4-treated DNA were resolved by chromatography upon Sephacryl S-200. The three activities also subsequently behaved differently during chromatography upon phosphocellulose and DEAE-cellulose.

Physical characterization of the purified enzymes showed the following:

UV endonuclease	$S_{20,w}$ (corresp. MW)	SDS gel M.W.
I	3.5 (47 kD)	43 kD
II*	2.9 (36 kD)	28 kD
III	3.1 (40 kD)	N.D.

Each of these enzymes appears to act both as a DNA glycosylase and as a class I AP endonuclease. UV endonucleases I and III (and possibly II) catalyze the latter reaction by a β-elimination mechanism. These enzymes differ in their requirements for DNA secondary structure and they appear to have overlapping, but not identical substrate specificities. They are each active in EDTA, but have different requirements for salt, pH and Triton X-100. All of these enzymes appear also to be present in non-transformed human fibroblasts. UV endonuclease III appears to be identical to AP endonuclease I described by Kuhnlein *et al.* (NAR(1978) 5:951). It is missing from xeroderma pigmentosum (XP) group D cells and both the human and mouse enzyme are able to restore repair DNA synthesis to these cells. We are also investigating alterations of UV endonucleases I and II in XP cells.

*This activity is probably that described by Nes (EJB(1980) 112:161) and by Hollstein *et al.* (PNAS (1984) 81:4003).

E 138 COMPARATIVE STUDY OF THE MECHANISMS OF ACTION OF THE APURINIC ENDONUCLEASES OF ESCHERICHIA COLI, Yoke Wah Kow, Hiroshi Ide, Robert J. Melamede and Susan S. Wallace, Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595.

A comparative kinetic study of the apurinic (AP) endonuclease activities of endonucleases III, IV, VIII and IX, and exonuclease III of E. coli were performed using different O–alkyl hydroxylamine–modified apurinic PM2 DNA substrates. O–methyl, O–ethyl, O–allyl, O–benzyl and O–4–nitrobenzyl hydroxylamine were used for the preparation of modified AP sites so that substrates containing O–alkyl hydroxylamine residues with increasing size could be examined for their susceptibility to cleavage by the E. coli AP endonucleases. In contrast to the UV dimer DNA–glycosylases from T4 phage and Micrococcus luteus, as well as the AP endonucleases from calf thymus and rat liver, the AP endonucleases from E. coli recognize the O–alkyl hydroxylamine–modified AP sites. For exonuclease III and endonuclease IX, the rate of incision of substrates containing O–alkyl hydroxylamine residues were affected by the size of the residue. As the size of the residue was increased, a slower rate of incision was observed. Based on the relative kinetic rates of reaction with the various substrates, a mechanism(s) of action for the AP endonucleases from E. coli will be presented.

E 139 QUANTITATION OF THYMINE DAMAGES IN THE DNA OF X–IRRADIATED PHAGE PM2, Kihei Kubo, Yoke W. Kow, and Susan S. Wallace, New York Medical College,Valhalla, N.Y. 10595.

Neither the spectrum nor the frequency of DNA base damages produced in X–irradiated phages has been well defined. In order to determine the contribution of particular damages to X–ray–induced phage inactivation, we quantitated the yield of classes of damage in PM2 phage using chemical, enzymatic and immunological methods. Using the acetol fragment assay, the yield of thymine ring saturation products was found to be about 0.7/kGy/DNA molecule. Using the direct ELISA assay with anti–thymine glycol antibody, we were unable to immunologically detect thymine glycol, possibly because of the reduced binding efficiency of the DNA to the ELISA plate at 10 kGy or more. We are presently developing new techniques to improve the binding efficiency. The number of E. coli endonuclease III–sensitive sites was determined by the fluorometric assay, thus only alkali–stable sites were detected. About 0.1 sites/kGy/DNA molecule were observed. These data suggest that a substantial fraction of the thymine ring saturation products produced under these conditions are not recognized by endonuclease III, and further, that they are not thymine glycols. To eliminate the potential effect of the background of frank strand breaks on the measurement of enzyme–sensitive sites, form I DNA was purified from X–irradiated phage and used as a substrate. The results were essentially the same. Since these results were surprising in light of the known specificity of endonuclease III, we are currently examining the production of endonuclease–sensitive sites using endonucleases IV, VIII and IX and exonuclease III. This work was supported by DOE grant DE–FGOZ–87ER60510.

E 140 DEFICIENT DNA BINDING OF AN APURINIC/APYRIMIDINIC DNA ENDONUCLEASE ACTIVITY FROM XERODERMA PIGMENTOSUM CELLS, Muriel W. Lambert and Lynn K. Bickley, UMDNJ–New Jersey Medical School, Newark, NJ 07103

A chromatin–associated apurinic/apyrimidinic (AP) DNA endonuclease activity (Endo) from two normal human and two xeroderma pigmentosum, complementation group A (XPA), lymphoblastoid cell lines was examined for its ability to bind to partially AP DNA. We have previously shown that this Endo, which has a pI of 9.8, has 71% of the normal human Endo activity on AP DNA and also differs from the normal human Endoin activity on AP nucleosomal DNA. The AP Endo was incubated with purified pWT830/pBR322 plasmid DNA (a clone of the entire SV40 and pBR322 genomes), which had been labeled with [^{3}H]-thymidine and linearized. DNA was rendered partially AP by first alkylating the DNA with methyl methanesulfonate (12.5 mM), followed by heat. Protein:DNA binding was measured using a filter binding assay. Filter retention of DNA-Endo complexes was measured using a range of concentrations of normal and XPA Endos. Normal Endo, incubated with AP DNA, produced significantly greater filter binding (60+2%) than when incubated with untreated DNA, but this increase in binding was not observed when the XPA Endo was incubated with AP versus untreated DNA. The XPA Endo showed only 6.8+0.7% increased binding to AP DNA (approximately 11% of normal). These results indicate that the XPA AP Endo is deficient in its ability to bind to AP DNA. Further purification and characterization of this Endo is in progress to determine whether the binding activity and the enzyme activity are identical or whether they reside in different proteins. Supported by Grants AM-35148 from the NIH and 86-490-CCR from the New Jersey State Commission on Cancer Research.

E 141 NEUTRAL FILTER ELUTION (pH 9.6) OF KNOWN MOLECULAR WEIGHT DNAS DEMONSTRATES BEHAVIOR DEPENDENT ON PORE SIZE, NOT PORE DENSITY, Peter J. Mayer and Christopher S. Lange, SUNY Health Science Center, Brooklyn, NY 11203

In attempting to validate and calibrate the neutral filter elution (NFE) technique, we used intact genomes of known molecular weight, i.e., E. coli (M_r=2.7x10^9) and coliphage T4c (M_r=1.15x10^8) in conjunction with polycarbonate filters of varying pore size (0.03-3.0 um). T4c or E. coli containing radiolabelled DNA (^{14}C-thymidine) were mixed with a tritiated sucrose (5%) solution, gently layered onto the filters and lysed. Released DNA was eluted (pH 9.6) for 120-180 or 960 min and radioactivity per elution fraction determined by scintillation counting; sucrose eluted prior to DNA.

NFE at all pore sizes consistently discriminates the two genomes as measured by %DNA retained on the filter. However semi-log plots of %DNA retained on the filter vs. time reveal a discontinuity: as pore size decreases the expected linear slope becomes an anomalous two-component curve. This discontinuity occurs between pore size 2.0 and 1.0 um for E. coli and between pore size 0.1 and 0.08 um for T4c. For E. coli DNA rate of elution increases slightly with increased pore size from 0.05 to 1.0 um and substantially (47%) for pore size 2.0 and 3.0 um; for T4c DNA rate of elution is constant for pore sizes 3.0 to 0.2 um and decreases as pore size decreases from 0.2 to 0.03 um. Elution rate of intact DNA of M_r<10^{10} appears related to pore size per se and not to pore density or "open area" per filter; we are currently investigating larger DNA molecules. This work was funded by the Mathers Foundation.

E 142 FORMAMIDO RING-OPENED PYRIMIDINES ARE A BLOCK TO DNA SYNTHESIS, T.R. O'Connor, S. Boiteux and J. Laval. UA147 CNRS, U140 INSERM, Institut Gustave Roussy, 94805 Villejuif Cédex, France.

Guanine modified at the N7 position is the major base product of DNA treated with alkylating agents. These residues do not appear to cause mutations and do not result in cell death. If the DNA is treated with alkali the modified N7 guanine undergoes a ring-opening to form a formamido ring-opened pyrimidine (fapy). At this stage, since polymers of poly(dG-dC) which contain fapy residues do not present a good template for DNA synthesis and blocks to DNA synthesis present lesions which may be lethal, we investigated the efficiency of fapy lesions to act as replicative blocks. Single-stranded M13 DNA used as a template was modified with dimethylsulfate. The level of modification was estimated using ^{3}H labeled dimethylsulfate. The N7 guanines in the modified DNA were converted to fapy residues by treatment with alkali. The DNA containing fapy residues and DMS residues were then primed and treated with DNA polymerase I (Klenow fragment) in a reaction mixture containing [α ^{32}P]-dATP and the DNA electrophoresed on sequencing gels. This analysis showed that the DNA synthesis stops 1 base before the lesion. In addition we have also investigated the processivity of other enzymes.

E 143 **THE FORMATION OF A PROTEIN-DNA COMPLEX BETWEEN MutH AND MutS AND THE BACTERIOPHAGE Mμ mom PROMOTER. Brian A. Learn and Robert H. Grafstrom. Thomas Jefferson University, Philadelphia, PA 19107. In E. coli, deoxynucleotide mispairs which result from errors during DNA replication are corrected via a methyl-directed, DNA mismatch repair pathway. The repair complex is directed to the incorrect base through the transient undermethylation of the daughter strand. Both in vivo and in vitro studies have implicated the gene products of mutH, mutL, mutS, and dam in this DNA repair process. In addition to their function in mismatch correction, both Dam and MutH regulate the expression of the bacteriophage Mμ mom gene. The mom promoter contains a cluster of three GATC sites. The methylation of these sites by Dam is required for mom transcription. Genetic experiments have suggested that MutH is a negative regulator of mom expression and binds to the promoter when these three GATC sequences are unmethylated. Both mutH and mutS have been cloned and their gene products purified. MutH protein binds to the mom promoter in vitro. As reported previously, MutS is a non-specific, single stranded DNA binding activity, which does not bind to the double-stranded mom promoter alone. However, MutH and MutS together form a complex with this GATC rich DNA sequence. This is the first report of a physical interaction between MutH, MutS, and DNA and suggests that these proteins function as a complex in methyl-directed DNA mismatch repair. (This work was supported by NSF Grant No. DMB-8517662.)**

E 144 USE OF SNAKE VENOM PHOSPHODIESTERASE HYDROLYSIS TO ASSAY UV-DAMAGED DNA CONTAINING CYCLOBUTANE PYRIMIDINE DIMERS WITH CLEAVED AND INTACT INTRADIMER PHOSPHODIESTER BONDS, Michel Liuzzi and Malcolm C. Paterson, Cross Cancer Institute, Edmonton, Alberta T6G 1Z2, Canada.
Recently we have reported the existence of cyclobutane pyrimidine dimers with a cleaved internal phosphodiester linkage in DNA from post UV-incubated cells belonging to xeroderma pigmentosum complementation groups A and D, and in excised dimer-containing oligonucleotides recovered from similarly treated normal human fibroblasts. In order to investigate fully the metabolic fate of dimers in UV-irradiated human cells, a simple assay is required that permits detection of cleaved versus intact dimers. Here we show that snake venom phosphodiesterase (SVP) digestion can serve this purpose. Using specifically 3'-endlabeled, UV-photoligated decanucleotides as a model substrate containing hydrolyzed intradimer phosphodiester bonds, we demonstrate that upon exhaustive treatment with SVP and alkaline phosphatase, modified dimer sites are retrieved as nuclease-resistant trinucleotides containing the altered photodimer at the 3'terminus (TpT^dT). We also show that this latter molecule, like its unmodified analogue containing an intact dimer (TpT$\hat{}$pT), can be 5'-phosphorylated by T4 polynucleotide kinase. This peculiar feature, coupled with the availability of [γ-^{32}P] ATP of high specific activity, provides the basis for a sensitive method in which to assay DNA for cyclobutane pyrimidine dimer sites with a severed intradimer phosphodiester bond.

E 145 DNA POLYMERASE β OVER-PRODUCTION AND ITS INVOLVEMENT IN THE REPAIR OF UV DAMAGED DNA IN E. COLI A. Matsukage[1], T. Ohnishi[2], M. Yamaguchi[1], F. Hirose[1] and T. Date[3] ([1]Laboratory of Cell Biology, Aichi Cancer Center Research Institute, Nagoya, Japan; [2]Department of Biology, Nara Medical University, Kashiwara, Nara, Japan; [3]Laboratory of Biochemistry, Kanazawa Medical University, Ishikawa, Japan)
A recombinant plasmid for expression of rat DNA polymerase β was constructed in a plasmid/phage chimeric vector, pUC118 and by an oligonucleotide-directed mutagenesis technique. The insert contained a 1,005 bp coding for the whole rat DNA polymerase β. The recombinant plasmid was designed to use the regulatory sequence of E. coli lac operon and the initiation ATG codon for β-galactosidase as those for DNA polymerase β. The recombinant clone, JMpβ5, obtained by transfection of E. coli JM109 with the plasmid produced high level of DNA polymerase activity and 40 kDa polypeptide which were not detected in JM109 cell extract. By inducing this recombinant E. coli with IPTG, the amount of 40 kDa polypeptide reached as high as 19.3 % of total protein. The DNA polymerase was purified to homogeneity from the IPTG-induced JMpβ5 cells. The properties of this enzyme in specific activity, chromatographic behaviors, size, antigenicity and also lack of the associated nuclease activity were indistinguishable from DNA polymerase β purified from rat cells, indicating the identity of the over-produced DNA polymerase in the JMpβ5 and the rat DNA polymerase β.
By using the recombinant plasmid from JMpβ5, we obtained transformants of E. coli mutants which are carrying various defects in DNA polymerase I gene and compared the UV-sensitivities of these transformants and non-transformants. The transformants got resistancy to UV-irradiation only when the pol I-associated 5'-3' exonuclease was alive. Results suggest that rat DNA polymerase β can compensate pol I-defect and requires 5'-3' exonuclease for its function in the DNA repair.

E 146 **THE MECHANISTIC INPLICATIONS OF THE ASSOCIATION KINETICS OF UVRA AND UVRB TO ULTRAVIOLET LIGHT DAMAGED DNA**, Sharlyn J. Mazur and Lawrence Grossman, Department of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St. Baltimore MD 21205

In *E. coli* , the repair of DNA damaged by ultraviolet light or by a variety of agents producing bulky adducts is initiated by the UvrA,B and C proteins. Equilibrium constants determined in vitro for UvrA binding to undamaged and to uv-damaged sites only differ by a factor of 10^4. The discrimination between undamaged and damaged sites is only slightly increased in the UvrA/B complex. The small magnitude of this ratio and the report of a helicase activity of the UvrA/B complex suggest that the discrimination between damaged and undamaged sites may be a kinetic rather than an equilibrium effect. We have measured the association and dissociation kinetics of the UvrA and UvrA/B proteins with DNA damaged by ultraviolet light as a function of DNA molecular weight, dose and solution conditions using the nitrocellulose filter binding assay. The data are analyzed using a randomly located specific sites model. The association and dissociation kinetics of UvrA binding to uv-damaged DNA in the absence of ATP support a mechanism involving one-dimensional diffusion along the DNA. The effects of ATP and UvrB on the kinetics are presented. Alternative models are also discussed.

E 147 ESCHERICHIA COLI ENDONUCLEASE VIII AND ENDONUCLEASE IX: SUBSTRATE SPECIFICITY AND IDENTIFICATION OF CLASSES OF X–RAY–INDUCED DNA DAMAGES, Robert J. Melamede, Yoke Wah Kow and Susan S. Wallace, New York Medical College, Valhalla, NY 10595

Using FPLC, we have identified and purified two new endonuclease activities from Escherichia coli and have tentatively called them endonuclease VIII and endonuclease IX. Endonuclease VIII has both N–glycosylase and class II AP endonuclease activities. The enzyme recognizes DNA containing thymine glycol, dihydrothymine, methoxyamine–modified AP sites, β–ureidoisobutylic acid and urea. Endonuclease IX incises at AP sites, methoxyamine modified AP sites and at urea residues. However, in contrast to endonuclease VIII, endonuclease IX does not incise DNA containing the other above thymine modifications. Because of their differences in substrate specificities, endonucleases VIII and IX, as well as endonucleases III and IV, were used to probe the subclasses of lesions produced in DNA by X rays. Different classes of alkali stable X–ray induced DNA lesions were defined that depended on the conditions of irradiation. These in vitro studies suggest that endonuclease VIII and endonuclease IX may be important enzymes for repairing X–ray induced DNA damages. This work was supported by National Institutes of Health Grants CA33657 and GM37216.

E 148 O^6-METHYLGUANINE-DNA METHYLTRANSFERASE AND 3-METHYLADENINE-DNA GLYCOSYLASE IN VARIOUS TISSUES OF INBRED AND HYBRID MOUSE STOCKS, W.J. Washington*, R.S. Foote+, W.C. Dunn, W.M. Generoso and S. Mitra, *Biol. Dept., Central State Univ., Wilberforce, OH 45384; +Univ. Tenn. Grad. Sch. Biomed. Sci., Biol. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831.

Cell-free extracts from liver, lung, kidney, spleen, stomach, brain and ovary of several stocks of inbred and hybrid female mice (6-8 wks old) were assayed for levels of O^6-methylguanine-DNA methyltransferase (MGMT) and 3-methyladenine-DNA glycosylase (MAG) per µg DNA. Additionally, the MGMT and MAG levels in liver, brain, lung and ovary of C57Bl and C3H mice were measured as a function of age. The results could be summarized as follows: (1) Different mouse stocks vary by 2-fold or more in their MGMT and MAG activities for a given tissue. Some stocks (e.g. C3H) have consistently higher enzyme levels than others (e.g. Balb/C). (2) Both enzymes vary widely in their tissue-specific levels. The MGMT level is generally the highest in the liver and ovary, with lung and brain and particularly spleen having low activity. In contrast, the stomach generally contains the highest MAG level, with 1/2 to 1/3 as much in liver. (3) Both MGMT and MAG levels are higher in young adults (6-8 weeks old) than in 9 day-old mice. However, the MGMT level does not change significantly further in 15-17 month-old animals while the MAG levels in these animals are comparable to that of the young pups. (Research supported by NCI grant CA31721 and by the U. S. Dept. of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems. WJW was supported by a MARC (NIGMS) Faculty Fellowship.)

E 149 DNA POLYMERASE δ PLUS HELA- OR HUMAN FIBROBLAST CELL-FREE EXTRACTS COMPLEMENT PERMEABILIZED XERODERMA PIGMENTOSUM (XP) FIBROBLASTS: APPLICATION FOR PURIFICATION OF XP CORRECTING FACTORS, Craig Nishida, Soo-Young Choi and Stuart Linn, University of California, Berkeley, CA 94720.

Previous studies (Nishida et al., *J. Biol. Chem.* **263**, in press) have shown that if UV-irradiated cultured diploid human fibroblasts are permeabilized with Brij-58 then soluble material removed by centrifugation, conservative DNA repair synthesis can be restored by a soluble factor obtained from the supernate of similarly treated HeLa cells. Extensive purification of this factor yields a 10.2S, 220,000-dalton polypeptide with the DNA polymerase and 3'→5' exonuclease activities of DNA polymerase δ. Moreover, if monoclonal antibodies to DNA polymerase α, BuPdGTP, or BuAdATP are added to the reconstituted system, there is no significant inhibition. Thus, it appears that the major portion of UV-induced DNA repair synthesis is catalyzed by DNA polymerase δ, not α.

When permeabilized XP human diploid fibroblasts were utilized in the system, however, DNA repair synthesis dependent upon ultraviolet light could be restored by addition of DNA polymerase δ plus T4 endonuclease V, but not by either of these enzymes alone. More significantly, a positive response was also observed with XP group A, D, and E fibroblasts by the addition of DNA polymerase δ plus cell-free extracts prepared either from HeLa cells or from normal human fibroblasts. This response has been exploited to partially purify XP correcting factors.

E 150 DEVELOPMENT OF A NEW METHODOLOGY TO INTRODUCE MODIFIED NUCLEOTIDES INTO DNA, Seyma Ogut, Yoke W.Kow, Hiroshi Ide and Susan Wallace, New York Medical College, Valhalla NY 10595.

T4 RNA ligase catalyzes the ligation of single 2' deoxyribonucleoside 3'5' diphosphates to the 3' end of oligoribonucleotides or DNA in the presence of ATP. First, the enzyme is adenylated with ATP forming a covalent enzyme–AMP intermediate; then the adenyl group is transferred to the 5' end of the deoxyribonucleoside 3'5'diphosphate to form a 5'–5' phosphoanhydride linkage. This intermediate can be isolated and be used as either starting material or donor for a second ligation reaction either to a polynucleotide or DNA. The purpose of this study is to develop a methodology to ligate modified nucleotides to restriction fragments of PM2 or other phage DNA, and to use these as substrates for repair endonucleases/glycosylases or for the study of the biological consequences of unique modified bases. The advantage of this approach is that specific modified nucleotides that are not amenable to oligonucleotide synthesis, such as pyrimidine ring saturation products, can be inserted.

We have prepared the adenylated intermediate by using ^{3}H labeled thymidine 3'5' diphosphate as a substrate in the presence of ATP and RNA ligase. The ligation intermediate was extracted from PEI plates with 1 M triethylammonium bicarbonate, pH.8.0, after chromatography with 1 M LiCl saturated with boric acid, pH.8.0. This compound was used for ligation to poly [dA–dT]·poly [dA–dT] and PM2 DNA. We are currently preparing ring saturation products of thymine for ligation to PM2 DNA for use as repair enzyme substrates. This work was supported by DHHS Grants CA33657 and GM37216.

E 151 SUBUNITS OF ABC EXCINUCLEASE INTERACT IN SOLUTION IN THE ABSENCE OF DNA. David K. Orren and Aziz Sancar, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

ABC excinuclease is an *Escherichia coli* DNA repair enzyme which acts on a wide variety of lesions in double-stranded DNA. The enzyme removes DNA adducts by incising on both the 5' and 3' sides of damaged nucleotides; this occurs via the concerted action of the UvrA, UvrB, and UvrC subunits. Previous experiments have suggested assembly of the three subunits on DNA at the site of damage; the UvrA and UvrB subunits are involved in damage recognition while UvrC triggers the incision events. *In vitro* experiments in the absence of DNA utilizing gel permeation chromatography and glycerol density gradient ultracentrifugation indicate that UvrA dimerizes in an ATP-stimulated reaction and that UvrB interacts with UvrA in an ATP-dependent reaction. ATP hydrolysis is apparently necessary for the formation of the UvrA-UvrB complex, as neither ATP-γ-S, nor ADP, promote this interaction. Upon addition of UvrC and UV-irradiated plasmid DNA to the UvrA-UvrB complex, incision activity characteristic of ABC excinuclease was observed, even though UvrC failed to form a ternary complex with the UvrA-UvrB complex in the absence of DNA. These results suggest the possibility of pre-assembly of a UvrA-UvrB complex which is involved in specific recognition of DNA damage, while UvrC (having no affinity for the other subunits) associates with the UvrA-UvrB-DNA complex only at the damaged site.

E 152 ENDONUCLEASE FROM HELA CELLS THAT ACTS ON 4NQO MODIFIED DNA, G.B. Panigrahi and I.G. Walker, Dept. of Biochemistry, Univ. of Western Ont., London, Canada, N6A 5C1.

4-nitroquinoline-1-oxide (4NQO) produces a wide variety of tumors when administered to experimental animals. 4NQO is metabolically activated and predominantly reacts with the purines of DNA. The modifications induced in DNA by 4NQO and other large molecules like N-acetoxy AAF have been termed "bulky lesions" and these modifications are considered to be recognized by a "bulky lesion repair complex". Several endonuclease activities present in mammalian cells and other eukaryotic cells that recognize and cleave DNA altered by oxidizing agents, ionizing radiation or high doses of UV light have been described. To date no such mammalian enzyme has been isolated which is responsible for the excision of 4NQO adduct from DNA. We consider it worthwhile to search for "a bulky adduct" specific endonucleases using DNA containing AQO adducts as a substrate. The substrate was prepared by treating 500ng of colE$_1$ supercoiled plasmid DNA with 100ng of the ultimate carcinogen of 4NQO to give generally 10-20 adducts per DNA molecules. Endonucleolytic activity was assayed by the conversion of colE$_1$ supercoiled plasmid DNA to nicked circles. The 20µl assay mixture routinely contained 50 mM Tris-HCl, pH 8.00, 5 mM MgCl2, 10% glycerol, 2 mM DTT and 0.5 mM PMSF. After 30 minutes incubation at 37°C with a DEAE Sephadex fractionated enzyme prepared from HeLa cells, the amount of nicked circles was determined by gel electrophoresis. All enzyme samples were tested in parallel on 4NQO-treated and untreated supercoiled plasmid DNA. The 4NQO-specific activity elutes at 500 mM NaCl fraction whereas the non-specific activity elutes at a lower NaCl concentration.

E 153 STRUCTURAL MODIFICATIONS INDUCED BY BULKY ADDUCTS TO THE DOUBLE HELIX, A.M. Pedrini S. Tornaletti, Ist. Genetica Biochimica ed Evoluzionistica C.N.R., Pavia, Italy, G. Fronza, P. Menichini, A. Abbondandolo, Ist. Nazionale Cancro, Genova, Italy.

The conformational changes caused by UV photodamage and by 4-NQO adducts have been investigated by analyzing the electrophoretic behaviour on agarose gel of DNA topoisomers with different degree of supercoiling. Irradiation at 254 nm caused a change in electrophoretic mobility of DNA bands corresponding to a topological unwinding angle per pyrimidine dimer of 11.4°. This value had to be further reduced to 8.8°, because photoreactivation experiments have shown that topoisomer shift is caused for about 20% by other kinds of photodamage. The role played by dimers in conferring a single-stranded character to UV irradiated DNA was tested by comparing the number of UV induced S1-sensitive sites before and after photoreactivation. We found that these sites are present after photoreactivation, suggesting that they are due to rare UV photoproducts. An analogous study performed with 4-NQO adducts allowed us to calculated, after a correction due to the contribution of AP sites to the variation in electrophoretic mobility of damaged molecules, an unwinding angle per adduct of 9.3°. These results suggest that UV-like damage cause a local unwinding of duplex DNA inconsistent with the actual disruption of the hydrogen bonds and introduce distortion of the helix axis which imposes a positive and negative write in the negatively and positiviely supercoiled topoisomers respectively.

E 154 INVESTIGATION OF SEQUENCE SPECIFICITY IN FORMATION OF O^6-METHYLGUANINE AND ITS REPAIR USING OLIGODEOXYNUCLEOTIDE SUBSTRATES, Anthony E. Pegg, Michele Oplinger and M. Eileen Dolan, Pennsylvania State University College of Medicine, Hershey, PA 17033.

Self-complementary dodecadeoxynucleotides containing one or two guanine residues were reacted at pH 7.4 with 0.15mM N-[^{3}H-methyl]-N-nitrosourea (5.6 Ci/mmol) and the extent of total alkylation, formation of 7-methylguanine, O^6-methylguanine and 3-methyladenine was determined. Comparison of the dodecamers 5'-TATACGCGTATA-3' and 5'-TATACCGGTATA-3' indicated that the formation of O^6-methylguanine and 7-methylguanine was 1.6 fold greater in the latter substrate whereas the formation of 3-methyladenine was not different. Comparison of the alkylation of 5'-TATACTAGTATA-3' and 5'-TATACATGTATA-3' indicated that the formation of O^6-methylguanine was 2.9 times greater when the preceding base was A rather than T. These results suggest that the extent of production of O^6-methylguanine may be considerably greater when the methylated G is preceded by a purine instead of a pyrimidine. However, studies of the repair of these methylated oligodeoxynucleotides by the alkyltransferase protein isolated from human colon carcinoma HT-29 cells indicated that the repair of O^6-methylguanine was slower in the sequences which were alkylated more extensively. The combined effects of a greater interaction and a slower rate of repair would be expected to render such sequences more vulnerable to mutagenesis by methylating agents and this has been found to be the case by Burns et al.[J. Mol. Biol. 194, 385-390, 1987]. The use of oligodeoxynucleotides of defined sequence containing O^6-methylguanine provides a useful tool to study the role of sequence in DNA repair by the alkyltransferase. Labeling such substrates with ^{32}P by reaction with [γ-^{32}P]ATP and polynucleotide kinase provides an ultrasensitive method for the assay of alkyltransferase activity which can be applied to samples when only limited amounts of material are available. Supported by CA-18137.

E 155 JOINING OF NONHOMOLOGOUS DNA TERMINI IN EXTRACTS FROM XENOPUS LAEVIS EGGS, Petra Pfeiffer and Walter Vielmetter, Institute of Genetics, University of Cologne, Weyertal 121, D-5000 Cologne 41, FRG.

Extracts from Xenopus laevis eggs circularize linear DNA molecules with dissimilar ends. For an analysis of this end-to-end joining process, plasmid DNA substrates were prepared by duplicate cuts in the polylinker region with different restriction enzymes to produce dissimilar termini. These include blunt ends as well as 4 nucleotide long 3'- and 5'-protruding single-strand ends (referred to as PSS). After incubation in the egg extract, circularized plasmid was shuttled back into E.coli for analysis. Any tested combination of such termini was found to be efficiently joined in vitro. Sequence analyses of numerous junctions obtained from a set of 7 terminus combinations reveals that (i) apart from rare deletions (11 to 18 basepairs long) repair corrections are restricted to the immediate junctional region. (ii) Combinations between PSS and blunt ends or between PSS of opposite polarity preserve PSS sequences by fill-in synthesis. Occasional nucleotide loss or gain was noticed. (iii) A unique repair mode was observed in a joining of noncomplementary PSS of equal polarity (i.e. both ends 3' or 5' protruding). These ends align in a register which may be set by even a single fortuitously matching basepair. This setting then determines the final pattern of mismatch and fill-in repair at the junction. Since matching of a single basepair within otherwise noncomplementary single-strands appears energetically unfavourable, we postulate that a yet unknown DNA binding protein(s) is involved in the establishment of this unusual structural relation.

E 156 RECOGNITION AND REPAIR OF AMINOFLUORENE AND ACETYL-AMINOFLUORENE DNA ADDUCTS BY UVRABC NUCLEASE, J. Pierce, R, Case and M.-S. Tang, The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX 78957.

Excision repair of N-hydroxy-aminofluorene (N-OH-AF), N-Acetoxy-2-acetylaminofluorene (NAAAF) and ultraviolet light (UV) induced DNA damage by UVRABC nucleases was investigated. DNA fragments, (HaeIII-EcoRI)$_{174}$ and (EcoRI-BstNI)$_{129}$, isolataed from plasmid pBR322 were ^{32}P labeled at either the 5' or 3' end and subsequently treated with N-OH-AF, NAAAF, or UV. Using a piperidine cleavage method, we have found that only guanine residues are modified by N-OH-AF . We have previously demonstrated that N-OH-AF and NAAAF treatment produce (deoxyguanosine-8-yl)-2-aminofluorene (dG-C8-AF) and (deoxyganosine-8-yl)-2-acetylaminofluorene (dG-C8-AAF) respectively in DNA . These two kinds of adducts have different impacts on the DNA helix structure; while dG-C8-AF maintains the anti configuration, dG-C8-AAF is in the syn form. Although DNA transfection results indicate that while the uvrA, uvrB, and uvrC gene products are needed to repair dG-C8-AAF, the uvrC, but not the uvrA or uvrB gene products, is needed for repair of dG-C8-AF. However, we have found that *in vitro* UVRA, UVRB, and UVRC products must work in concert in repair of both dG-C8-AF and dG-C8-AAF. In general, the reactions of UVRABC toward dG-C8-AF are similar to that of dG-C8-AAF; it incises 7 nucleotides from the 5' side and 4 nucleotides from the 3' side of the DNA adduct. However in some sequences dG-C8-AF was poorly recognized compared to dG-C8-AAF indicating there may be some sequence dependence on the efficiency of recognition of UVRABC. With respect to the overall activity of UVRABC, evidence will be presented that suggests that hydrolysis on the 3' and the 5' sides of the damaged base is not simultaneous and that at least occasionally hydrolysis occurs only on the 3' side of the damage site.

E 157 NEOCARZINOSTATIN-INDUCED AP SITES ARE ACCOMPANIED BY A CLOSELY OPPOSED BREAK IN THE COMPLEMENTARY STRAND Lawrence F. Povirk, Medical College of Virginia, Richmond, VA 23298

Treatment of supercoiled colE1 DNA with neocarzinostatin (NCS), in the presence of 2-mercaptoethanol cofactor, produced numerous single-strand breaks, while double-strand breaks were virtually undetectable. However, when glutathione was used as a cofactor, a significant fraction of double-strand breaks (6-8%) was produced. Furthermore, subsequent treatment with endonuclease IV or with putrescine produced a dramatic (3- to 4-fold) increase in double-strand breaks, but little if any increase in total breaks. It is proposed that this conversion of a substantial fraction (20-25%) of the NCS-induced single-strand breaks to double-strand breaks represents cleavage of NCS-induced AP sites, the majority of which are accompanied by a closely opposed break in the complementary strand. Several properties of these closely opposed AP site/strand break lesions suggest that they correspond to NCS-induced AP sites previously detected at certain cytosine residues on sequencing gels; namely, they are slightly resistant to endonuclease IV but profoundly resistant to endonuclease III and exonuclease III (probably due to the presence of the closely opposed break), and they are formed only when glutathione is used as a cofactor. These lesions are strongly implicated in NCS-induced mutagenesis.

E 158 REPAIR OF CLOSELY OPPOSED PYRIMIDINE DIMERS IN UV-IRRADIATED CHINESE HAMSTER OVARY CELLS, Richard J. Reynolds, Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

A bifilar enzyme-sensitive site assay has been used to examine the fate of closely opposed dimers induced in Chinese hamster ovary (CHO) cells. It has previously been demonstrated that closely opposed dimers are subject to repair in normal human fibroblasts, that this repair is dependent upon normal excision-repair capacity and that it is independent of semi-conservative DNA synthesis (Lam and Reynolds, 1986, Mutation Res. 166:187-198). It has also been demonstrated that the disappearance of bifilar enzyme sensitive sites in excision-proficient human fibroblasts begins soon after irradiation and proceeds with kinetics similar to those observed for the majority of dimers at isolated sites. Recent results with Chinese hamster ovary cells differ markedly from those with human fibroblasts. "Repair" of bifilar enzyme-sensitive sites in CHO cells exhibits a marked delay, differs significantly from the rate of repair at isolated dimers and is independent to excision-repair capacity. The possibility that closely opposed dimers are "repaired" primarily during semiconservative DNA synthesis in CHO cells is currently under investigation. Results with both CHO and human cells will be presented. This research is supported by research grant CA-42390 from the U.S. National Institutes of Health and is conducted under the auspices of the U.S. Department of Energy.

E 159 *SEQUENCE SPECIFICITY IN PSORALEN-DNA PHOTOBINDING AND ITS BIOLOGICAL ROLE,* *Evelyne Sage, Véronique Boyer and Ethel Moustacchi, Institut Curie, Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.*

Psoralens intercalate in the DNA double helix and form cycloaddition products with pyrimidine bases (mainly thymine) upon UVA irradiation. The photoproducts result in furan-side and/or pyrone-side monoadducts. Furan-side monoadducts can give rise to interstrand crosslinks by absorbing UVA light.

Using DNA sequencing technology, we demonstrate that psoralen derivatives photoreact preferentially at 5'-TpA crosslinkable sites, and more strongly in $(AT)_n$ repeated sequences. This is a general rule for bifunctional compounds such as 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4'-(hydroxymethyl)-4,5',8 trimethylpsoralen (HMT), or monofunctional compounds such as angelicin, pyridopsoralens, 3-carbethoxypsoralen (3-CPs). These derivatives produce photoadducts of the same cis-syn configuration but induce different conformational changes of the double helix. This has an implication in their differential cytotoxic and mutagenic effects observed in vivo. For example, 3-CPs photoadducts which are clustered in $(AT)_n$ regions, induce nuclease S1-sensitive sites, whereas pyridopsoralens which are more equally distributed along DNA, are strongly stabilized by stacking interaction with adjacent bases. The former are well recognized by repair enzymes, whereas the repair of the later is delayed and uncomplete in yeast and mammalian cells. The first compound is much less toxic and mutagenic than the last one. This highlights the role of DNA sequence and structure on biological events.

E 160 **Roles of *Escherichia coli* UvrB ATPase homology as investigated via oligonucleotide-directed mutagenesis,** Todd W. Seeley and L. Grossman, Department of Biochemistry, Johns Hopkins University, Baltimore, MD 21205.

The UvrA, B and C proteins are required for incision of damaged DNA as carried out by the excision repair pathway of *Escherichia coli*. This reaction is dependent on ATP hydrolysis. Of these three proteins, only UvrA possesses a Mg^{++}-dependent ATPase activity with no other cofactor requirements. Addition of either DNA or UvrB tends to inhibit ATPase activity. Addition of DNA to a UvrAB mixture, however, results in a marked increase in ATPase activity. Interestingly, UvrAB complexes possess a unidirectional 5'→3' ATP-dependent helix unwinding activity (E. Oh and L. Grossman, PNAS USA 84:3638-3642). Although purified UvrB preparations have no measurable ATPase activity, UvrB possesses a sequence motif common to many known ATPases, including UvrA, suggesting a cryptic ATPase may reside within the UvrB polypeptide. Indeed, proteolysis of UvrB has been used to activate a cryptic ATPase (P. Caron, pers. comm.). To further investigate this activity, eight point mutants possessing specific amino acid changes within the region of UvrB corresponding to ATPase homology have been constructed via oligonucleotide-directed mutagenesis. Potential roles of this region of UvrB are investigated by assay of *in vivo* complementation in a uvrB deletion mutant and *in vitro* complementation of purified excision repair proteins.

E 161 CHARACTERIZATION AND PARTIAL PURIFICATION OF A NOVEL ACTIVITY FROM *E. COLI* THAT ACTS ON PSORALEN MONOADDUCTS, Frances M. Sladek, Brian Dynlacht and Paul Howard-Flanders, Yale University New Haven, CT 06511.

In *E. coli* psoralen monoadducts can be accurately repaired via the UvrABCD excision pathway; they can also lead to mutations by some unknown mechanism. In order to investigate the latter, we looked at the processing of monoadducted plasmid DNA in crude extracts under conditions such that the UvrABC system would be inoperative. A Mg^{++}-independent activity was found that releases acid soluble counts from plasmid DNA treated with tritium-labeled psoralen and that converts psoralen-treated supercoiled DNA into nicked circles. The activity appears to act only on psoralen (and angelicin) monoadducts and not on psoralen interstrand crosslinks. The activity has been purified several 100-fold and partially characterized. It appears to be different from any known DNA repair activity in *E. coli*.

E 162 ASSESSMENT OF HISTONE DAMAGE BY CARCINOGENS. Pamela C. Stacks*, Joseph Mazrimas, Michele Corzett, and Rod Balhorn, *San Jose State University, San Jose CA 95192, and Lawrence Livermore National Laboratory, Livermore CA 94550.
Quantitation of carcinogen exposure by analysis of DNA damage is complicated by individual variation in DNA repair capacities. Although it is thought that the primary lesions are associated with DNA, other macromolecules also may be modified. Thus, assessment of histone damage as a stable measure of DNA carcinogen exposure may be especially beneficial since histones and DNA are closely associated in chromatin. It is also of interest to distinguish between intrinsic chemical reactivity and accessibility of different target sites in native chromatin by a comparison of both in vivo and in vitro reactivity of the histones.

The alkylation of histones by the direct-acting carcinogen 7-bromomethylbenz(a)-anthracene occurred both in vivo and in vitro. The relative molar reactivity for mouse liver histones in vivo was H3 > H1 > H2b > H4 > H2a. A detailed analysis of the adducts formed in vitro focused on the two histones H3 and H1. Those amino acid adducts stable to acid hydrolysis were characterized following acetic anhydride derivatization and subsequent separation by reverse-phase high pressure liquid chromatography. The acetylated adducts were identified by their UV absorbance spectra and by comparison with synthetic amino acid standards. The extent of in vitro modification of H1 was greater than that of H3. Histone H1 modification yielded a major adduct whereas histone H3 yielded at least four distinct products. Comparison of these results with the relative reactivity of isolated N-acetylated amino acids with 7-bromomethylbenz(a)anthracene indicated that the protein environment limited the accessibility or altered the reactivity of the various amino acids.

E 163 RECOGNITION AND REPAIR OF THE CC-1065-(N3-ADENINE)-DNA ADDUCT BY THE UVRABC NUCLEASES, Moon-shong Tang, Chong-Soon Lee, Richard Doisy, Donald R. Needham-VanDevanter, and Laurence H. Hurley, University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX., 78957.

The recognition and repair of CC-1065-(N3-adenine)-DNA adduct by the UVRABC nuclease has been investigated both *in vivo* using ϕX174RFI DNA with a transfection assay, and *in vitro* using a site-directed adduct in a 117 base pair (bp) fragment from M13mp1. CC-1065 is a potent antitumor-antibiotic which binds within the minor groove of DNA through N3 of adenine. In contrast to the helix destablilizing and distortive modifications of DNA caused by NAAAF, CC-1065 increases the melting point of DNA and decreases the S1 nuclease activity. The transfection results show that the uvrA, uvrB, and uvrC genes, are involved in the repair of CC-1065-DNA adducts. In contrast, the uvrD gene product has no effect in repairing CC-1065-DNA adducts. Purified UVRA, UVRB, and UVRC proteins must work in concert to incise the drug-modified ϕX174RFI DNA. Using a site-directed and multiple CC-1065 modified (MspI-BstNI) 117 bp fragment we have found the UVRABC nuclease incises at the 8th phosphodiester bond on the 5' side of the CC-1065 DNA adduct on the drug modified strand. The enzymes do not cut the non-covalently modified strand. At low drug binding ratios, of the four CC-1065 binding sites identified in the (Msp1-BstN1) 117 bp fragment only the adduct at the high affinity binding site -GATTA* is incised by the UVRABC nucleases. No difference in the effect of CC-1065 on local DNA structure, as determined by the DNase I cleavage pattern was evident between these sites. At high drug binding ratios, UVRABC nucleases are unable to incise any of the CC-1065-DNA adducts. DNA sequence and/or helix stabilizing effect of multiple adducts may determine the recognition and/or incision of drug-DNA adduct by UVRABC nuclease.

E 164 SITE-DIRECTED MUTAGENESIS OF E. COLI ADA PROTEIN (O^6-METHYLGUANINE-DNA METHYLTRANSFERASE). K. Tano#, R. S. Foote#, D. Bhattacharyya# and S. Mitra, #Univ. Tenn. Grad. Sch. Biomed. Sci., Biol. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831.

The 39 kDa E. coli Ada protein repairs O-methyl adducts in DNA by accepting methyl groups in suicide methyltransferase reactions. Its Cys_{321} is the methyl acceptor for O^6-methylguanine (MGMT activity) and Cys_{69} accepts methyl groups from methylphosphotriesters (PMT activity). Methylation of Cys_{69} activates transcription of the ada gene and several other genes involved in alkyl lesion repair by binding of the methylated protein to their 5'-upstream regulatory sequences. We obtained high level expression of Ada protein from a recombinant plasmid in which the regulatory sequence is deleted. This gene is not inducible by alkylating agents. Using site-directed mutagenesis, we also created mutant Ada proteins with His_{321} His_{322} (I) and His_{321} Cys_{322} (II) and compared with the wild type (Cys_{321} His_{322}) protein for inducibility and enzyme activities. Both (I) and (II) have no MGMT activity. However, (I) also has no PMT activity and is noninducible while (II) is active as a PMT and is inducible by alkylating agents, although not as efficiently as the wild type. The lack of PMT activity of (I) may be due to its unfolding as a result of the bulky substitution and in vivo degradation or His_{321} may affect the PMT active site by its close proximity. (Research supported by U. S. Dept. of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems and by NCI Grant CA 31721.)

E 165 STRUCTURE AND BIOLOGICAL IMPLICATIONS OF THE FURTHER PHOTOPRODUCT OF THE (6-4) PHOTOPRODUCT. John-Stephen A. Taylor, Daniel S. Garrett and Michael Cohrs
Department of Chemistry, Washington University, St. Louis, MO 63130.

We report the remarkable structure of TpT3, the further photoproduct of the (6-4) photoproduct of thymidylyl-(3'->5')- thymidine, which has remained unknown since it was first discovered by Johns et al. in 1964. They had shown that the (6-4) product of TpT, known to them only as TpT4, could be quantitatively converted to TpT3 by photolysis at 313 nm. We have determined that TpT3 results from the photoisomerization of the pyrimidinone ring of the (6-4) product of TpT to its Dewar valence isomer. A biological role for this photochemical transformation comes from reports in the early 1970s of Type III photoreactivation of the lethal effects of 254 nm light in a variety of bacteria. This type of photoreactivation was shown to be maximal at 313 nm and to proceed through an uncharacterized, though non-enzymatic, pathway which was correlated with the disappearance of (6-4) photoproducts. More recently Mitchell and Rosenstein have produced antibodies to the further photoproduct of the (6-4) lesion which suggests that the Dewar product is indeed formed in native DNA. We also report that direct photolysis of TpT with pyrex filtered medium pressure mercury arc light leads to the formation of TpT3 and none of the (6-4) photoproduct. This suggests that the (6-4) photoproducts produced during exposure of DNA to sunlight might be converted primarily, if not exclusively, to their Dewar valence isomers. We propose that the Dewar photoproducts and not the (6-4) photoproducts are the ultimate lesion with biological significance in mutagenesis by sunlight.

E 166 ALLOSTERIC INTERACTIONS BETWEEN NUCLEOTIDE AND DNA BINDING SITES IN THE *E.COLI* **UVRA PROTEIN**, S. Thiagalingam[1], E. Y. Oh[2], S. J. Mazur[1] and L. Grossman[1],
[1]Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore MD 21205; [2]Department of Biochemistry and Biophysics,University of California-San Francisco, San Francisco CA 94143.

The incision of damaged DNA by the *E.coli* UvrABC proteins requires the hydrolysis of ATP. An analysis of the sequence of UvrA has identified two potential ATP binding sites. In addition, UvrA binds to uv-damaged sites as a dimer. Since excision repair is complex, we have examined allosteric interactions between nucleotide binding sites as well as between nucleotide binding sites and DNA binding sites. In the absence of effectors, the ATPase activity of UvrA follows simple Michaelis-Menton kinetics. The Km is 200 μM and the turnover number is 200 min^{-1}. However, the ATPase activity in the presence of ADP indicates positive cooperativity. The binding of ATP-γ-S to UvrA in the presence of DNA also exhibits positive cooperativity. The kinetics of dissociation of ATP-γ-S from UvrA/DNA complexes supports interaction between sites. At 0.1 μM ATP-γ-S, $k=5.1x10^{-4}$ s^{-1} while at intermediate concentrations, the dissociation profile is biphasic. At high concentrations (>3μM), all of the bound ATP-γ-S dissociates with $k=1.7x10^{-3}$ s^{-1}. Double stranded DNA is a noncompetitive inhibitor which both increases Km and decreases Vmax. At high concentrations, because of spurious damage or single-stranded regions, supercoiled DNA is an activator of the ATPase activity. The data are analyzed in terms of detailed models for the coupling between nucleotide binding sites and DNA binding sites. Implications for the mechanism of excision repair are discussed.

E 167 MITOCHONDRIAL DNA REPAIR ENZYMES FROM MOUSE PLASMACYTOMA CELLS, Alan E. Tomkinson, Thomas Bonk, Neil Bartfeld and Stuart Linn, University of California, Berkeley, CA 94720.

Since nucleotide excision repair and post replication recombinational repair have not been observed in mammalian mitochondria and the rate of mutation of the mitochondrial genome in somatic cells is very low, it has been proposed that DNA repair does not occur and that damaged genomes are degraded. Mitochondria are the site of electron transport and oxidative phosphorylation, both of which generate active free radical species that can cause DNA damage. As this type of damage appears to be mainly repaired by base excision repair, we have examined mitochondrial extracts for base excision repair enzyme activities. We detected and purified several such enzymes, showed that they co-sedimented with intact mitochondria and compared these activities with their extramitochondrial counterparts. A mitochondrial uracil DNA glycosylase had similar native size (3.2S) and enzymatic properties, to the nuclear enzyme but was less sensitive to NaCl concentration and dilution. Mitochondria also contain two AP endonuclease activities, which exhibit small but significant differences in enzymatic properties. These enzymes have the same size (3.84S[63kd] by sedimentation and 60kd by immunoblot of SDS polyacrylamide gels using antibody to the major HeLa AP endonuclease) but differ significantly from the mouse nuclear AP endonuclease (molecular weight 28kd). These mitochondrial AP endonucleases appear not to be associated with DNA glycosylases. They cleave AP sites by a class II mechanism, generating 3'OH termini which can prime DNA synthesis. Mitochondria also contain an endonuclease activity, which acts at lesions caused by high UV doses (presumably free radical type lesions) and at AP sites. This enzyme appears to be similar to E. coli endonuclease III and UV endonucleases from several mammalian sources, since it acts both as a DNA glycosylase and a class I AP endonuclease. No significant hydroxymethyl uracil DNA glycosylase activity was detected in mitochondrial extracts. The presence in mitochondria of these base excision repair enzymes in addition to DNA polymerase γ and DNA ligase suggests that base excision repair can occur. Alternatively it is possible that these are not DNA repair enzymes but that they function instead to initiate degradation of a damaged mitochondrial genome.

E 168 FACTORS INFLUENCING THE ASSEMBLY AND DISASSEMBLY OF ABC EXCISION NUCLEASE. Bennett Van Houten[1], Howard Gamper[2], John Hearst[2], and Aziz Sancar[1]. [1]University of North Carolina, Chapel Hill, NC 27514; [2]University of California, Berkeley, CA 99720.

The formation of the ABC excision nuclease complex on DNA containing a single psoralen adduct at a unique position, and the disassembly of the complex following incisions, has been studied by DNAse I footprinting. The UvrA subunit is the damage recognition subunit and binds to the DNA specifically in the absence of ATP. Addition of ATP increases the binding affinity 5-fold. ATP-γ-S, the nonhydrolyzable ATP analog, inhibits specific binding, while ADP has no effect. ATP hydrolysis is required for the formation of the ternary AB-DNA complex. It has been shown that the concerted action of helicase II (hel II) and DNA polymerase I (pol I) is required for the dissociation of the ABC complex. The present study reveals that hel II does not bind specifically to the damaged DNA substrate, nor does it increase the binding affinity of the UvrA and UvrB subunits. Hel II appears to interact with the 5' region of the post incision ABC-DNA complex. Pol I, in the absence of hel II, can fill in the excision gap with patch sizes of 15-25 nucleotides; gap filling is concommitant with the disappearance of the ABC footprint. A model for the action of pol I and hel II in the turnover of ABC excision nuclease will be presented. Work supported by NIH grants GM11277, GM11180, and GM32833.

E 169 CELL SURVIVAL AND O^6-METHYLGUANINE DNA METHYLTRANSFERASE (MT)LEVELS IN MNNG-TREATED HUMAN CELLS HARBORING A REGULATABLE MT GENE, Evelyn Waldstein, Tel Aviv University, Tel Aviv, Israel.

O^6-methylguanine (O^6MeG) DNA adducts are regarded to be mutagenic and carcinogenic but their role in cell death is still disputed. We investigated their effect on cell survival by varying O^6MeG DNA methyltransferase (MT) levels in the cell. This repair enzyme stoichiometrically removes O^6MeG from the DNA. MT-deficient HeLa SMR cells were stably transformed by the E. coli ada gene put under the control of the mmtv promoter that responds to glucocorticoids. The expression of the MT gene was induced by dexamethasone (Dex) or Dex and TPA, since according to our findings TPA considerably enhanced the effect of Dex. Cell survival and MT levels were measured in cells that have been exposed to dexamethasone at different time points: before (24 h), during and after (24 h) their treatment with different doses of MNNG. It appeared that cell survival was significantly enhanced not only if Dex and TPA were added before MNNG but also if applied up to 24 h after the MNNG treatment. Cell survival correlated best with the rate at which the cells were able to resynthesize the MT-activity lost during the first hours after the treatment with MNNG. Our data suggest that O^6 MeG adducts are lethal to the cell and that their repair may be considerably delayed in time.

E 170 THE ANTHRAMYCIN-N2-GUANINE DNA ADDUCT IS RECOGNIZED BY THE UVRABC NUCLEASE IN VITRO, R.B. Walter, J.R. Pierce, R. Case, L. Hurley, and M-S. Tang. The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX 78957.

The binding of the antitumor antibiotic anthramycin to a defined linear DNA fragment was investigated using both exonuclease III and lambda exonuclease. We show that virtually all guanine residues are reactive toward anthramycin, however several guanine residues showed preferential reactivity for the drug when the lambda exonuclease analysis was performed. Using purified UVRA, UVRC, and UVRC proteins we present evidence that these three proteins in concert are able to recognize and produce specific strand cleavage flanking anthramycin-DNA adducts. The cleavage of anthramycin adducts by UVRABC nuclease results in strand breaks at six or seven bases 5' and/or three or four bases 3' flanking the adduct. The 5' strand breaks observed often occured as doublet bands on sequencing gels indicating plasticity in the site of 5' cleavage whereas the 3' cleavage did not show this effect. When elevated levels of anthramycin were used for the modification of the DNA fragment the activity of the UVRABC nuclease toward the anthramycin adducts decreased. The recognition and specific cleavage of the non-distortive anthramycin adduct by the UVRABC nuclease and the inhibitory effect of high-level anthramycin modification on the activity of UVRABC nuclease implies that alteration of the pitch of DNA may be important for adduct recognition by the UVRABC nuclease.

E 171 IMMUNODETECTION OF SPECIFIC DNA DAMAGE AND REPAIR IN HUMAN CELLS, Altaf A. Wani and Steven M. D'Ambrosio. Department of Radiology, The Ohio State University Medical center, Columbus, Ohio 43210.

Sensitive immunoassays (competitive and non-competitive) have been utilized for the quantitation of specific DNA base alterations, induced by chemical (ENU) and physical (260 nm UV) agents. Antibodies specific for mutagenic miscoding base O^4-ethylthymidine (O^4-EtThd), exhibited an inhibition of antigen-antibody binding proportional to both the concentration of ENU and alkylated DNA in competitive enzyme-linked immunosorbant assay (ELISA). However, the sensitivity of detection is greatly enhanced to fmol adduct in ng amounts of alkylated DNA using non-competitive ELISA and nitrocellulose filter based immunoslot blot assays. DNA alkylated in vitro at an O^4-EtThd/Thd molar ratio of 2.6×10^{-6} was used to immunodetect 0.12 fmol of modified base O^4-EtThd. Thus, O^4-EtThy was quantitated in cultured human skin fibroblast and kidney epithelial cells immediately following treatment with ENU and subsequently at intervals upto 72 h. The initial O^4-EtThy/Thy molar ratio of 5.6×10^{-7} in the DNA of both cell types decreased approximately 20 to 30 percent within 8 h postincubation at 37 °C. This initial rapid loss of O^4-EtThy was followed by a slower rate of removal with about 50% eliminated at 72 h post-treatment. This repair could be due to very low constitutive levels of an alkyltransferase or by an alternative mechanism of repair. In addition to the detection of chemical damage, non-competitive immunoslot blot assay could afford the detection of one thymine dimer per 10 megabase UV irradiated DNA and determination of repair of thymine dimers in human cells irradiated with 0.5 $J.m^2$. Currently, these assays are being applied to the quantitation of genotoxin induced DNA base damage in defined gene fragments subsequent to electrophoretic separation, transfer to filters and detection by DNA damage specific antibodies. (Supported by ES2388, ES3101 and EPA812865).

E 172 SYMMETRICAL CLEAVAGE OF HOLLIDAY JUNCTIONS BY AN ENDONUCLEASE FROM YEAST IS DEPENDENT UPON HOMOLOGOUS DNA SEQUENCES. Stephen C. West, Carol A. Parsons and Stephen M. Picksley, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K.

The specificity of a nuclease from *Saccharomyces cerevisiae* that cleaves Holliday junctions *in vitro* has been studied using a variety of DNA structures. Using plasmids that extrude inverted repeat sequences into X-junctions, we found that nicks were introduced into strands of like polarity approximately 6-8 nucleotides from the base of the junction. In all cases, cleavage occurred within homologous sequences, and with precise symmetry across the junction. In contrast, a junction that contained four arms of unrelated DNA sequence was cleaved asymmetrically. This relationship between homology and symmetrical cleavage establishes the enzyme as a true junction-specific nuclease.

A general mechanism for resolution is suggested. Resolution of a target Holliday junction occurs by the formation of a complex between the nuclease and two homologous strands. Cleavage occurs in a concerted manner as nicks are introduced into identical sequences. A consequence of nicking at symmetrically related positions in the two duplexes, followed by strand realignment, is the separation of recombinant chromosomes each containing a ligatable nick.

E 173 ^{32}P ENDLABELING OF DNA ADDUCTS FORMED BY STREPTOZOTOCIN AND METHYLNITROSOUREA IN INSULIN SECRETING PANCREATIC BETA CELLS. Glenn L. Wilson, Steven E. Woodley and Susan P. LeDoux, University of South Alabama, Mobile, Alabama 36688

Specific nitrosoamides, like streptozotocin (STZ), are of interest in diabetes because of their ability to selectively interact with insulin secreting cells and cause their functional impairment or destruction. Since a prime target for alkylation by these toxins is DNA, ^{32}P endlabeling techniques were adapted to identify the major adducts that are formed in beta cells so that specific DNA damage and repair mechanisms could be evaluated. The two adducts that have been identified to date are N-7 methylguanine and O-6 methylguanine. To isolate the altered bases, primary cultures of beta cells were exposed for one hour to varying doses (0.1-10 mM) of STZ or the aglycone methylnitrosourea (MNU). The DNA then was extracted from the cells, digested to nucleotides and labeled with ^{32}P. N-7 methylguanine was separated from the normal nucleotides using 2-directional thin layer chromatography. However, to isolate O-6 methylguanine it was necessary to chromatograph the digest in four directions. The formation of either type of base adduct was found to be dose dependent. Interestingly, a nontoxic concentration of MNU and an equimolar concentration of STZ, which is toxic, alkylated the DNA of beta cells to the same extent. These results suggest that although alkylation of DNA in beta cells may account for the mutagenic and carcinogenic properties of some nitrosoamides in these cells, it is not the sole factor responsible for the selective toxic effects of these chemicals. (This work is supported by NIH Grant ES03456).

E 174 SUBSTRATE SPECIFICITY OF REPAIR REPLICATION IN HUMAN CELL-FREE EXTRACTS, Richard D. Wood, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, United Kingdom.
Gently prepared soluble extracts from cultured human cells can perform repair replication of damaged exogenous plasmid DNA. All repair reactions take place in the extract, including nicking at damage, gap filling, and ligation. The substrate specificity of this repair is being studied. A few apurinic sites per molecule, or a few DNase-I sensitive sites, induce little repair replication. However, significant repair replication of ultraviolet light-irradiated DNA takes place. Repair activity is still present when minor photoproducts (such as pyrimidine hydrates) are removed by E. coli endonuclease III. Repair of UV-irradiated DNA can be further enhanced in extracts by supplementing them with UV-endonuclease from Micrococcus luteus. Photoreactivation of the UV-irradiated substrate with E. coli photolyase suggests that (6-4) dipyrimidine photoproducts may be better substrates for initiation of repair than cyclobutyl pyrimidine dimers. Repair replication is also observed after treatment of plasmids with 8-methoxypsoralen and 360 nm light.

E 175 ANTIBODIES AGAINST HUMAN AND E. COLI O6-METHYLGUANINE-DNA METHYLTRANSFERASE Daniel B. Yarosh, Nestor Rosales and Joe Ceccoli, Applied Genetics Inc., 205 Buffalo Ave., Freeport NY 11520.
Polyclonal antibodies (Ab) were raised in mice against purified human O6-methylguanine-DNA methyltransferase (O6MT) and in rabbits against purified *E. coli* O6MT. Neither Ab was inactivating nor precipitating. No cross-reactivity between human and *E. coli* O6MT and Ab was observed. The anti-human O6MT Ab detected enzyme in human liver and Mer+ cell extracts, but no reacting protein was observed in Mer- cell extracts. This is the first report that the O6MT activity deficiency in Mer- cells is due to a lack of O6MT peptide. The anti-*E. coli* O6MT cross-reacted with O6MT from adapted *M. luteus*, but not adapted *B. subtilis*, *B. thuringiensis* or *S. fradiae*. An adaptive response with induction of O6MT has been found in *P. aeroginosa* which has little effect on post-MNNG survival but reduces MNNG mutagenesis. The anti-*E. coli* O6MT Ab did not react with the *P. aeroginosa* O6MT. The dual role of the *E. coli ada* protein in lesion reversal and induction of gene expression may account for the failure of Ab directed against it to recognize other O6MT.

E 176 REPAIR OF 4,5',8-TRIMETHYLPSORALEN MONOADDUCTS AND CROSSLINKS BY THE ESCHERICHIA COLI UVRABC ENDONUCLEASE, Anthony T. Yeung and Beverly K. Jones, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

We have used an oligonucleotide model system to analyze the UvrABC endonuclease repair of 4,5',8-trimethylpsoralen monoadducts and crosslinks. The use of homogeneous substrates revealed several novel UvrABC endonuclease mechanisms. For one of the psoralen monoadducts, UvrABC endonuclease, besides incising the psoralen-containing DNA strand once at the eighth phosphodiester bond on the 5' side of the adduct and once at the fifth phosphodiester bond on the 3' side of the adduct, produces an alternate incision on the undamaged complementary strand as though the adduct was on the 5'TpA site across from the damaged strand. Such erroneous repair is potentially mutagenic. The UvrABC endonuclease incises the crosslinked DNA on either the furan side strand or the pyrone side strand. The incisions are at the ninth phosphodiester bond on the 5' side of the modified thymine and at the second, or third, or fourth phosphodiester bond on the 3' side of the modified thymine. The relative efficiency of the incisions appeared to be partly a function of the DNA sequence and it sometimes favors the furan side of the psoralen crosslink.

Repair Genes, Genetic Control of Repair, Intragenomic and Structural Effects-I

E 200 UV Damage Increases Outcrossing and Recombinational Repair in Phage T4, Stephen T. Abedon, Department of Microbiology and Immunology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Phage T4 prevents oucrossing and the sharing of host cell resources by exluding from infection secondarily adsorbing homologous phage (Genetic Exclusion). Thus fitness is optimized. However, genetic exclusion would result in a reduction in fitness when a phage's DNA is damaged since the maintenance of genetic exclusion would block outcrossing, prevent recombinational repair and ultimately result in phage death. I have shown that the presence of moderate levels of UV damage relaxes the expression of the genetic exclusion phenotype. Fitness is therefore optimized both when DNA damage by UV irradiation is and is not present, the former via an increase in outcrossing and recombinational repair.

E 201 *REPAIR OF DNA LESIONS PHOTOINDUCED BY BIFUNCTIONAL PSORALENS IN NORMAL AND FA CELLS. CORRELATION WITH SENSITIVITY,* *Dietrich Averbeck, Dora Papadopoulo and Ethel Moustacchi, Institut Curie, Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.*

Fanconi's anemia (FA) cells are known to be more sensitive than normal human cells to DNA crosslinking agents. Since these agents usually induce a mixture of different lesions the contribution of each type of lesion needs to be clarified. The use of bifunctional psoralens (Ps) in combination with a reirradiation protocol allows to do this. We studied the contribution of Ps photoinduced monoadducts (MA) and crosslinks (CL) to the particular sensitivity of genetically well defined FA cell lines (complementation groups A and B) and to which extent this sensitivity is related to their repair capacity. As a function of total photoadducts induced by either 8-methoxypsoralen (8-MOP) or 4,5',8-trimethylpsoralen (TMP) FA group A cells are, respectively 2 and 4 times more sensitive than normal cells. Using the alkaline elution technique, we show that, after 8-MOP photoaddition, FA group A cells incise about half as efficiently CL than normal cells in 24 hours. FA group B cells show an intermediate response. As a function of TMP induced MA alone (405 nm irradiation), the following order of sensitivity was obtained : FA "B" > FA "A" > normal. Both FA cell lines are deficient in the repair of TMP photoinduced crosslinkable MA.

The differential response of group A and B FA cells suggest that subtle differences may exist in the recognition and/or incision step(s) for each type of lesion, i.e. MA and CL.

E 202 THE ABILITY OF *DCM* AND *ECO*RII METHYLASES TO METHYLATE CC(A/T)GG SEQUENCES IS NOT SUFFICIENT FOR THEIR PARTICIPATION IN VERY SHORT PATCH REPAIR AT THE SAME SITES. Ashok S. Bhagwat, Anjum Sohail and Margaret Lieb*, P. O. Box 100, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, *University of Southern California, Los Angeles, CA 90033.

E. coli has the ability to correct mismatches such as

—CTAGG— —CTTGG—
—GGTCC— —GGACC—

to restore CC(A/T)GG sites by a mechanism called very short patch (VSP) repair. It also codes for a DNA cytosine methylase, Dcm, that methylates the second cytosine within CC(A/T)GG sequence at its C5 position. Several mutations in *dcm* that lack ability to methylate are defective in VSP repair as well, suggesting a role for *dcm* in VSP repair. We have isolated a deletion mutant of *dcm* that is active in methylation, but inactive in VSP repair. Therefore, the ability of Dcm to methylate is not sufficient for its role in VSP repair. Another strain of *E. coli* carries restriction-modification system *Eco*RII that includes a DNA methylase with the same sequence specificity as Dcm. The two enzymes methylate the same cytosine in the sequence at the identical position, but *Eco*RII methylase cannot complement *dcm* mutations defective in VSP repair. This is despite the fact that the two methylases share significant sequence homology. It appears that the two methylases have evolved to perform separate functions in the cell.

E 203 A CORRELATION BETWEEN TRANSCRIPTIONAL ACTIVITY AND DNA REPAIR EFFICIENCY.
Diane S. Okumoto and Vilhelm A. Bohr, Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

Metallothionein I (MT I) gene expression can be regulated by the presence of heavy metals in the medium. We have studied the DNA repair efficiency in the metallothionein gene in CHO cells carrying about 12 copies of the gene under conditions where the genes are transcriptionally active or inactive. The methodology for measuring repair has been described in a number of communications (1,2). Cells are UV-irradiated and the frequency of pyrimidine dimers in specific genomic sequences is measured using T4 endonuclease V combined with Southern hybridisation with specific DNA probes. The MT I is about 1 kb long, but repair was examined in 6 or 18 kb fragments containing the gene.

We consistently found a considerable (50%) increase in the DNA repair efficiency in the MT I gene upon transcriptional activation. As a control, the repair efficiency in the dihydrofolate reductase (DHFR) gene was not affected by heavy metal. The overall genome repair as measured by repair replication was also unaffected by the presence of heavy metal. We conclude that DNA repair efficiency is correlated to gene activity of the MT I gene. Furthermore, since repair was detected in a much larger restriction fragment than the size of the gene, a region larger than the gene must be repaired upon gene activation. It may resemble the situation in the DHFR locus, where we have demonstrated the existence of a DNA repair domain (3) with a size of 60-80 kb. DNA repair may be regulated by chromatin structural changes within domains or loops extending from the nuclear matrix. Interestingly, Hildebrandt et al. have demonstrated that the CHO MT I gene is associated with the nuclear matrix when it is activated (pers. comm.).

(1) Bohr et al., Cell **40**: 359-369, (2) Bohr et al., Proc. Nat. Acad. Sci. **83**: 3830-3833, (3) Bohr et al., J. Biol. Chem. **261**: 16666-16672.

E 204 THE denV GENE OF BACTERIOPHAGE T4 COMPLEMENTS DNA EXCISION DEFECTS IN mei-9 AND mus201 MUTANTS OF DROSOPHILA, Satnam S. Banga, James B. Boyd, Kristoffer Valerie, Paul V. Harris, Eva M. Kurz and Jon K. de Riel, Department of Genetics, University of California, Davis, CA 95616 and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140. We have subcloned the denV gene of phage T4 into a transformation vector which places that gene under control of the Drosophila hsp70 heat-shock promoter. This construct was inserted into the Drosophila germ line by P-mediated transformation. After genetic crosses revealed the chromosomal position of the insertions, they were introduced into stocks homozgous for excision deficient mutants at either the mei-9 or the mus201 locus. The mus201 mutant is typical of excision deficient mutants in other organisms whereas the mei-9 mutant is also deficient in meiotic recombination and mismatch repair. Both stocks containing the denV gene display strong incision activity following heat treatment and UV-irradiation. These transformants will permit an analysis of the mutagenic and killing effects of pyrimidine dimers on the germ line of higher eukaryotes. In addition, this study opens the possiblity for investigating the influence of other well charaterized prokaryotic repair genes on DNA damage in both the somatic and germ cells of an intact metazoan.

E 205 FORMATION AND FATE OF INTERSTRAND CROSSLINKS INDUCED BY HN2, PUVA, CIS-DDP, AND TRANS-DDP IN S. CEREVISIAE: NO EVIDENCE FOR DNA DOUBLE-STRAND BREAKS DURING REPAIR, Martin Brendel and Freimut Wilborn, Johann Wolfgang Goethe-Universität, 6000 Frankfurt/Main, Federal Republic of Germany.

The number of interstrand crosslinks (ICL) was determined in DNA from WT and in excision-deficient repair mutants rad1, rad2, and snm1 by de- and re-naturation of DNA coupled with isopycnic and sucrose centrifugation analysis. Removal of ICL in log cells, regardless of inducing agent, was observed in WT but not in excision-deficient mutants rad1 and rad2. Mutant snm1, highly sensitive to bi- and polyfunctionally acting mutagens (allelic with pso2), was able to undo ICL, however at a rate slower than WT. The molecular weight of treated DNA, as determined in sucrose gradients, gave no indication that removal of ICL is correlated with formation of DNA double-strand breaks within 6 h of post-incubation. In the absence of an external energy source HN2- or PUVA-treated mutant rad1-18 but not mutants rad2 and snm1 showed significant degradation of DNA. At equal exposure concentrations trans-DDP was much less toxic than cis-DDP but formed higher numbers of ICL.

E 206 OVEREXPRESSION OF THE Rad1, Rad2, AND Rad10 EXCISION REPAIR PROTEINS IN *E. coli* AND IN YEAST, Helmut J. Burtscher, A. Jane Cooper, William A. Weiss, Charles M. Nicolet and Errol C. Friedberg, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305. The *RAD1, RAD2, RAD3, RAD4* and *RAD10* genes of *S. cerevisiae* are required for the incision of DNA during nucleotide excision repair. To facilitate purification of these proteins for biochemical studies we have tailored the cloned genes into expression vectors for overexpression in yeast. We have also overexpressed these genes in *E. coli* to generate proteins as immunogens to raise specific antibodies. In *E. coli* the Rad1, Rad2 and Rad10 proteins are highly expressed from a variety of regulatable promoters. However, in all cases the majority of the proteins visualized in Coomassie blue-stained gels are associated with a fraction of the cell extracts that can only be solubilized with detergents. Affinity-purified polyclonal antisera raised against the insoluble proteins react specifically with the appropriate Rad proteins and were used to monitor expression of Rad proteins in extracts of yeast cells. The *RAD1* and *RAD10* genes can be overexpressed in yeast when driven by the yeast *GAL1* promoter activated by the *GAL4* gene. This results in constitutive overexpression of Rad1 and Rad10 proteins, considerable amounts of which are detected in the soluble fraction of cell extracts. Overexpression of soluble Rad2 protein requires induction of the *GAL1* promoter by galactose. Both Rad1 and Rad2 proteins migrate in SDS polyacrylamide gels with apparent molecular weights of ~150 kDa. These values are considerably larger than those calculated from the coding regions of the cloned genes (~126 and ~118 kDa respectively). The anomalous electrophoretic mobility of these proteins is not an artefact of overexpression since the phenomenon is also observed with Rad1 and Rad2 proteins expressed from untransformed cells containing single copy chromosomal alleles. Fractionation of cell extracts is facilitated by dot blot assays using specific antisera. The Rad10 protein has been extensively purified and is under investigation for possible catalytic and structural activities.

E 207 Yeast Rec Gene Rad52 Can Functionally Substitute for Phage T4 Rec Genes 46 and 47, Davis S. Chen and Harris Bernstein, Department of Microbiology and Immunology, College of Medicine, University of Arizona, Tucson, AZ 85724.
A plasmid containing the Rad52 gene from Saccharomyces cerevisiae was placed under the control of either a LacZ or λP_L promoter and expressed in E. coli. The Rad52 gene complemented three deficiencies of bacteriophage T4 gene 46 and 47 mutants. First, expression of Rad52 allowed T4 gene 46 and 47 temperature sensitive mutants to grow and form plaques under conditions that normally restrict their growth. Second, expression of Rad52 restored the ability of gene 46 and 47 phage mutants to undergo recombination of genetic markers. Third, expression of Rad52 substantially increased the ability of UV irradiated 46 and 47 mutants to undergo multiplicity reactivation, a form of recombinational repair. These results indicate that there are common elements in the mechanisms of recombination and recombinational repair in procaryotic viruses and eucaryotes. A computer comparison of the 514 amino acid sequence of S. cerevisiae of Rad52 with the 540 amino acid sequence of T4 gene 46 and the 339 amino acid sequence of T4 gene 47 showed significant homology. There was a match of 88 identical amino acids between the products of Rad52 and gene 46 and 79 identical amino acids between the products of Rad52 and gene 47. These results suggest that recombination arose as an early monophylogenetic event and that sexual reproduction evolved in an ancestor common to both procaryotes and eucaryotes.

E 208 CLONING AND GENETIC ANALYSIS OF AN ENDO-EXONUCLEASE GENE THAT REQUIRES A FUNCTIONAL RAD52 GENE. Terry Y.-K. Chow[1] and Michael A. Resnick[2]. [1]NRCC-Biotech. Research Inst., Montreal, Quebec, Canada, H4P 2R2, and [2]Natl. Inst. Envir. Health Sc., Research Triangle Park, NC. 27709.

We have previously identified and characterized an endo-exonuclease (yNucR) in Saccharomyces cerevisiae that immunocross reacts with an antiserum raised against a nuclease from Neurospora crassa (in press). Expression of normal level activity of yNucR requires a functional Rad52 gene. The activity and CRM is detected in logarithmic growing Rad^+ cell; none is detected in stationary cells.

A DNA fragment of 4.5 kb containing the yNucR gene has been identified with the antiserum from a λgt11 yeast genome expression library. Tn10 insertion mutagensis into the fragment eliminates the CRM. When the yNucR gene is cloned into YEp213 plasmid and is transformed into Rad^+ cells, we observed an increase in expression of 3-10 times more of CRM and 1.5-2.0 times more of immuno-precitable nuclease activity. Furthermore, the transformants exhibits higher resistance to MMS than corresponding Rad^+ cells, but there is no detectable difference in ionizing sensitive for haploid. Disruption of the yNucR gene with leu2 insertion is lethal to the cell, suggesting the yNucR is essential. Preliminary genetic results with ts mutant suggest a growth phase dependent processing/regulation of the nuclease.

E 209 THE *RAD4* GENE OF *S. cerevisiae:* SEQUENCING OF WILD-TYPE AND MUTANT ALLELES AND PHENOTYPIC COMPLEMENTATION BY TRUNCATED GENES, Linda B. Couto, Wolfram Siede, Reinhard Fleer, and Errol C. Friedberg, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305. The *RAD4* gene is one of at least 5 genes required for the incision of DNA during nucleotide excision repair in *S. cerevisiae*. We have previously shown that propagation of this gene in *E. coli* leads to mutational inactivation, presumably because Rad4 protein is toxic to this organism (1). We have devisied a strategy for propagating the wild-type allele in *E. coli* and have completed the sequencing of this gene. The *RAD4* gene has an open reading frame of 2262 base pairs and is predicted to encode a polypeptide of 754 amino acids with a calculated molecular weight of 82.1 kDa. The sequences of 2 mutant alleles generated by propogation of the wild-type gene in *E coli* will also be presented. In addition we propose to rescue the mutant chromosomal alleles *rad4*-2, *rad4*-3 and *rad4*-4 on plasmids and determine their sequence, since all three are leaky alleles relative to *rad4* disruption mutants.

One of the mutant *RAD4* alleles recovered from *E.coli* (designated *RAD4*-EC1) contains a frame-shift mutation at codon 390, and is expected to encode a truncated polypeptide only 404 amino acids long. Nonetheless, when expressed from a multicopy plasmid, this mutant protein complements the UV sensitivity of a *rad4* disruption mutant (*rad4*-10) to near wild-type levels. Similarly, a *RAD4* allele containing an insertion mutation that interrupts the reading frame at codon 248, partially complements *rad4*-10 cells. These results suggest that only the amino terminal region of the Rad4 polypeptide is required for functional activity. A comprehensive deletion analysis is in progress to examine this hypothesis.

1. Fleer, R, Nicolet, C. M., Pure, G. A., and Friedberg, E. C. (1987) Mol. Cell. Biol. 7: 1180-1192.

E 210 INACTIVATION BY DNA-INTERSTRAND CROSSLINKS OF PLASMIDS INTRODUCED INTO NORMAL AND FANCONI ANAEMIA CELLS, Stephen W. Dean, Hazel R. Sykes and Alan R. Lehmann, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK.

An SV40-transformed Fanconi anaemia (FA) cell line, GM6914, exhibits approximately 2.4-fold sensitivity to the cytotoxic effects of nitrogen mustard (NM) when compared with the normal line, MRC5-V1. Host cell reactivation of NM-treated plasmid has been investigated using transient expression vectors which contain the chloramphenicol acetyltransferase (CAT) gene. In both cell types there is a similar, dose-dependent reduction in CAT expression which correlates with an increase in NM-induced DNA-interstrand crosslinking. Inactivation of plasmid expression results from either a single DNA-interstrand crosslink anywhere within the plasmid molecule preventing transcription of the CAT gene, or from a more frequent lesion.

E 211 MOLECULAR ANALYSIS OF REPAIR OF ALKYL ADDUCTS IN EXCISION REPAIR-DEFICIENT CELLS, Ruth L. Dusenbery and S-F Lee Chen, Southern Methodist University, Dallas, TX 75275.

Excision repair-deficient mutants of Drosophila melanogaster provide a useful model for understanding the biochemistry of DNA repair and for monitoring changes in the number and nature of mutations generated by alkyl adducts in the absence of the major excision repair pathway. Establidhed cell lines, derived from the excision repair-deficient mutants, mei-9 and mus201, exhibit no UDS activity in response to MMS, EMS, UV or X-ray damage, as previously determined for primary cells obtained from the same mutants. These established lines are used in HPLC analysis of the kinetics of removal of specific alkyl adducts from the genomic DNA of excision deficient cells compared to excision-proficient controls. Cells treated with N-[^{3}H]methyl-N-nitrosourea for 1-3 hrs are washed and harvested for DNA isolation and analysis at several time periods between 0 and 48 hrs post-treatment. Alkylated bases of interest are then obtained from two hydrolysis reactions: 1) neutral thermal hydrolysis for 3-MeA, 7-MeA, O^2-MeC, 3-MeC, 3-MeG and 7-MeG; 2) mild acid hydrolysis for 1-MeA, 6-MeA, O -MeG, residual 7-MeG, and unmethylated adenine and guanine. Residual methylation products remained in the DNA of both excision-proficient and excision-deficient cells after 48 hr recovery. 3-MeA, 7-MeG and O^6-MeG were removed with similar kinetics from control cells. Several-fold higher levels of 7-MeG and O^6-MeG adducts remained in the DNA of excision-deficient cells compared to control cells after 48 hr, while 3-MeA adducts were removed with kinetics similar to those observed for the excision-proficient cells. The relationship of this observation to the hypermutability of excision-deficient strains in response to alkylating agents will be discussed.

E 212 INHIBITORS OF TOPOISOMERASE AND THEIR ACTION IN REPAIR-COMPETENT AND REPAIR-DEFICIENT CHINESE HAMSTER CELLS, M. M. Elkind, H. Utsumi, T. Kosaka, W. Buddenbaum, M. Shibuya, and D. Suciu, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO 80523.

The lethal and DNA interactive properties of inhibitors of topoisomerase II have been examined using radiation repair-competent and repair-deficient V79 and CHO-K1 Chinese hamster cells. Of particular interest has been the differential effects produced by the simultaneous treatments with the DNA intercalator m-AMSA [4'-(9-acridinylamino)methanesulfon-m-anisidide] and the nonintercalator novobiocin (novo) as well as the effects of posttreatments with other enhancers of radiation killing. Compared to the responses to ionizing radiation, there are both similarities and differences. Mutants of CHO-K1 cells developed by Jeggo and Kemp [Mutat. Res., 122, 133-327 (1983)] are sensitive to m-AMSA as they are to X-rays but in contrast the radiation-sensitive V79-AL162/S-10 cells of Utsumi and Elkind [Radiat. Res., 96, 348-358 (1983)] have about the same response to m-AMSA as the parental V79 cells from which they were derived. Treatment with m-AMSA in the presence of nontoxic concentrations of novo considerably reduces the killing effectiveness in parental CHO-K1 or V79 cells as well as V79-AL162/S-10 cells but to a much lesser extent in CHO-K1 mutants. Posttreatment with caffeine does not alter the lethal effectiveness of m-AMSA on repair-competent V79 cells, but rescues repair-deficient V79 cells after m-AMSA or radiation. Protein-associated DNA strand breaks are produced by m-AMSA, but their frequency is not altered by concomitant treatment with novo. Because novo can inhibit mitochondrial function, the possibility is being explored that survival rescue by novo results from a reduction in the ATP available.

E 213 A DOMAIN MODEL FOR NUCLEOTIDE EXCISION REPAIR. PREFERENTIAL REPAIR OF PYRIMIDINE DIMERS IN "OPEN" AND TRANSCRIPTIONALLY ACTIVE CHROMATIN DOMAINS, Klaus Erixon, Karolinska Institute, Stockholm, Sweden.

UV-exposed human fibroblasts initially recognize and repair lesions in about 50% of their genome by an efficient, compartmentalized, DNA-polymerase alpha dependent (araC-sensitive) pathway. This repair is preferentially localized in a part of the genome that is in an "open" conformation. The fraction of pyrimidine dimer endonuclease sensitive sites accessible in native chromatin disappear more rapidly than in the genome overall. These accessible lesions are removed with the same kinetics as are "araC-sensitive sites" and lesions that give rise to repair replication.

From the identical UV-dose responses it is concluded that the target units for inactivation of transcription and for initiation of repair are identical. It is suggested that transcription and repair are initiated by a common event in potentially active chromatin domains and that both processes are blocked by the precense of a repair induced strand-break. The results are consistent with a requirement for tortional strain in DNA and/or an association with the nuclear matrix for the initiation of both transcription and repair.

E 214 **INDUCTION AND REPAIR OF DOUBLE STRAND DNA BREAKS WITHIN DEFINED GENOMIC REGIONS IN MAMMALIAN CELLS,** Maryann B. Flick and Robert E. Krisch, University of Pennsylvania, Philadelphia, PA 19104.

Double strand DNA breaks (DSB) are widely believed to be among the major biologically important lesions induced in cells by ionizing radiation. We are developing a technique to measure the induction and repair of DSB in specific subregions of the mammalian genome. Our experimental approach is to obtain long DNA segments by treatment of extracted cellular DNA with selected restriction endonucleases, to separate specific segments by neutral gel electrophoresis; and to identify and quantify them by DNA hybridization with probes for known internal sequences. In preliminary experiments, we have found that SV40 DNA appears to be integrated only within a single XbaI restriction fragment of size 30 kbp in the genome of an SV40-transformed WI38 human cell line. Using this approach, we have measured the induction of intracellular DSB by ionizing radiation within this restriction fragment. Our data are consistent with random single event induction of DSB, with an efficiency of 1-3 x 10^{-13} DSB/rad/dalton. This is closely comparable to values in the literature for similar experimental conditions, all based on methods which measure average numbers of DSB over the entire cellular genome. The radiation doses required to induce measurable numbers of DSB in this restriction fragment are too large to permit meaningful studies of DNA repair. We are currently attempting to rectify this problem by using pulsed field electrophoresis to separate substantially longer restriction fragments. (Supported in part by US DOE Contract No. AC02-83ER60163)

E 215 PYRIMIDINE DIMER FORMATION IS MODULATED IN NUCLEOSOME CORE DNA, James M. Gale and Michael J. Smerdon, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4660.

We have mapped the distribution of pyrimidine dimers (PD) at the single nucleotide level in nucleosome core DNA from UV-irradiated mononucleosomes, chromatin fibers and human cells in culture using the 3' → 5' exonuclease activity of T4 DNA polymerase. The results show that the frequency of PD formation in nucleosome core DNA is strongly modulated with a 10.3 (± 0.1) base periodicity. This periodicity is identical to the helical periodicity of DNA in nucleosomes. The positions of maximal PD formation map to positions where the DNA strands face "out" of the nucleosome core (i.e., at positions farthest from the core histone surface). Furthermore, the individual intensities of the distribution are modulated in a unique and characteristic manner which reflects core histone-DNA interactions, giving rise to a "photo-footprint" of core histone binding. Quantitation of the levels of PD in each 10.3 base ensemble indicates that this distribution is uneffected by folding of the 10 nm chromatin filament ("beads-on-a-string")to the 30 nm chromatin fiber (solenoid). These results suggest that the individual core histone-DNA interactions which direct this nonrandom distribution of PD formation remain unaltered by changes in the structural state of chromatin.

E 216 MNNG-INDUCED PARTIAL PHENOTYPIC REVERSION OF HeLa MER$^-$ CELLS, Regine Goth-Goldstein, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

When HeLa Mer$^-$ cells are treated with a highly toxic dose of N-methyl-N'-nitro-N-nitroso-guanidine (MNNG), the majority of the surviving cells revert to the MNNG resistance of Mer$^+$ cells, whereas MNNG treatment of HeLa Mer$^+$ cells does not alter the response of the surviving cells to a second exposure of MNNG. The Mer$^-$ revertants have a similar sensitivity as HeLa Mer$^+$ cells to all monofunctional alkylating agents, but are as sensitive as the Mer$^-$ parent-line to the crosslinking agent chloroethylnitrosourea. The revertant clones, like the Mer$^-$ cells, do not repair O^6-methylguanine (O^6MeG), as measured by the persistence of this base in DNA of ^{3}H-MNNG treated cells and by the inability of cell extracts to repair O^6MeG of in vitro methylated DNA. The results suggest that the two characteristics which are usually coupled with the Mer$^-$ phenotype - lack of O^6MeG repair and hypersensitivity to MNNG - can be separated. The findings will be discussed in relationship to other cellular responses to MNNG.

E 217 ANALYSIS OF THE SALMONELLA TYPHIMURIUM MUTS AND MUTL GENES REQUIRED FOR MISMATCH REPAIR, Lynne T. Haber, John A. Mankovich, and Graham C. Walker, MIT, Cambridge, MA 02139.

We have cloned the *mutL* and *mutS* genes of *Salmonella typhimurium* LT2. The *mutS* gene has been sequenced, and approximately 36% of the amino acids are identical to the *Streptococcus pneumoniae* repair protein *hexA* when the sequences are optimally aligned. Three regions of stronger similarity are worthy of note, including one region close to the amino terminus, and one located closer to the carboxy terminus, which includes similarity to a consensus sequence thought to be diagnostic of nucleotide binding sites. A third region, adjacent to the second, is similar to the consensus sequence for the helix-turn-helix motif found in many DNA binding proteins. The nucleotide sequence of *mutL* is being determined, and a similar comparison with the *hexB* gene will be made.

Several mutants of the cloned *mutS* and *mutL* genes have been isolated and characterized. Several mutants have been isolated which complement *mutL*$^-$ for mismatch repair, but do not result in conditional lethality when present in a *dam*$^-$ *recA*$^-$ *mutL*$^-$ strain. In addition, *mutL* mutants which are temperature sensitive for the mutator phenotype are viable at both temperatures in a strain that is *dam*$^-$ *recA*$^-$ *mutL*$^-$. This suggests that either a smaller amount of repair activity may be necessary for *in vivo* mismatch repair than for the conditional lethality of the *dam*$^-$ *recA*$^-$ combination, or mismatch repair may itself not be responsible for the conditional lethality.

E 218 CHINESE HAMSTER OVARY (CHO) MUTANTS HYPERSENSITIVE TO TOPOISOMERASE II INHIBITORS, A.L. Harris, S.M. Davies, C.N. Robson, S.L. Davies and I.D. Hickson, Cancer Research Unit, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

DNA topoisomerases are involved in DNA replication, transcription and recombination, and are a constituent of the chromosome scaffold and the nuclear matrix. To investigate the regulation of topoisomerase II (topoII) and its role as a mediator of drug-induced DNA damage, we have isolated CHO mutants hypersensitive to topoII inhibitors. One of these mutants, ADR-1, is hypersensitive to a variety of intercalating agents, including mAMSA and adriamycin, and to the non-intercalating agents VP16 and VM26. Higher levels of protein-associated single- and double-stranded DNA strand breaks are produced in the mutant than in parental cells by mAMSA. Strand break levels correlate well with cytotoxicity in both lines, suggesting that they are the underlying mechanism of cell death. Assays of topoII enzyme activity by filter binding of covalent topoII-DNA complexes and by decatenation of kinetoplast DNA showed a 2 - 3-fold increase in ADR-1 cells. Western blotting showed a 3-fold increase in the amount of topoII protein in the mutant cells. There was no change in the sequence specificity of DNA cleavage. Cell cycle analysis showed no increase in S-phase proportion. Thus, the novel phenotype of this mutant appears to be due to an elevation in topoII activity as a result of overproduction of the enzyme. The basis for this is under investigation. ADR-1 represents a model for studying the effects of topoII overproduction on cell proliferation and enzyme-mediated DNA damage.

E 219 TARGETED INTEGRATION USING SITE-SPECIFIC DOUBLE-STRAND CUT AND SINGLE-STRAND NICKED PLASMID SUBSTRATES, David R. Higgins and Jeffrey N. Strathern, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701.

It has been demonstrated that recombination of plasmids into the yeast genome at a specific site can be greatly enhanced by introducing a double-strand cut in the plasmid region homologous with the genome. This observation is one of several that support the notion that cuts are recombinagenic and has led to the Double-strand break model for recombination proposed by Szostak, Orr-Weaver, Rothstein, and Stahl. It is not known, however, whether nicks can provide a similar enhancement of targeted plasmid integration as might be predicted by the Meselson-Radding model. We are addressing the question of enhanced plasmid integration using a plasmid that can be introduced into the yeast cell as uncut (supercoiled), and site-specific nicked, or cut DNA. The plasmid contains the *LEU2* gene for selection of yeast transformants and internal fragments of the *URA3* and *TRP1* genes. The *URA3* and *TRP1* fragments contain unique restriction sites located near the middle of the fragments. A third unique restriction site is in pBR322 vector sequences. The plasmid has no yeast origin of replication and therefore stable *LEU2*$^+$ transformants result from homologous recombination with the genome. The unique restriction sites allow us to produce double-strand cut and single-strand nicked substrates at those specific sites which lie within the *URA3* and *TRP1* coding region. The cut and nicked plasmids are made *in vitro* and used to transform *TRP1*$^+$ *URA3*$^+$ *leu2-Δ* yeast cells to *LEU2*$^+$. Because internal fragments of *TRP1* and *URA3* are present on the plasmid, integration of the plasmid at the *TRP1* or URA3 locus creates a gene disruption of those genes and makes the cells *trp1*$^-$ or *ura3*$^-$ auxotrophs. Data on the relative frequency of Trp$^-$ and Ura$^-$ as a function of transformants (Leu$^+$) using uncut, cut, and nicked plasmid, which is an indication of the relative recombinagenic effect of each, will be presented. Physical evidence for the site of integration will also be presented. By this analysis we confirm our expectation that the double-strand cut plasmids integrate at the site corresponding to the site of the cut on the plasmid and we can test the hypothesis that single-strand nicked plasmids will promote targeting.

Research sponsored by the National Cancer Institute, DHHS, under Contract No. N0-CO-23909 with Bionetics Research, Inc.

E 220 DIFFERENTIAL RESPONSES OF A MOUSE MELANOMA CELL LINE AND A MAMMARY CARCINOMA LINE TO DNA-PROTEIN CROSSLINKING BY 254 AND 405 NM RADIATIONS, Helene Z. Hill, Jennifer G. Peak and Meyrick J. Peak, Argonne National Laboratory, Argonne, IL 60439

The much lower frequency of skin cancers in black skinned individuals is attributed to the protective effects of melanin. Melanin is reactive in O_2 radical species reactions, as well as being a potential screen for solar radiation. Hill and Setlow (Photochem. Photobiol. 35: 681, 1982) found fewer UV-endonuclease sensitive sites in Cloudman S91 melanotic melanoma cells than in EMT6 mammary carcinoma cells at wave lengths shorter but not longer than 290 nm. This suggested that melanin may not act by a screening mechanism in solar protection against near UV. The aim of this study was to determine the role of melanin in the production of DNA-protein crosslinks (DPC) by 405 nm light which produces few, if any, pyrimidine dimers, but does cause DPC with high efficiency, probably via O_2-radical species mechanisms. Melanotic Cloudman S91 and non-melanotic EMT6 cells were differentially prelabelled with ^{3}HdThd or ^{14}CdThd, harvested, mixed and exposed to increasing doses of monochromatic 254 or 405 nm UV light. The extent of DPC formation was evaluated by the alkaline elution technique after exposing UV-irradiated cells to 30 Gy of gamma rays. At 254 nm, the rates of increase of DPC were similar in the two cell lines, while at 405 nm, the rate for EMT6 was 2.5 times greater than that for S91 cells. Furthermore, the crosslinked DNA from the melanoma line eluted more slowly than that from the carcinoma line. It is concluded that the melanin in the S91 cells is protective at 405 nm most probably by a radical scavenging mechanism. In addition, it appears that the crosslinked DNAs in the two cell lines have different conformations.

E 221 EFFECTS OF DEMETHYLATION ON DNA REPAIR IN UV-IRRADIATED CHO CELLS
L. Ho, V. A. Bohr, and P. C. Hanawalt, Dept. of Biol. Sciences, Stanford Univ., Stanford, CA 94305.
We have previously reported an inverse correlation between DNA methylation and DNA repair within the dihydrofolate reductase (*DHFR*) amplicon in Chinese hamster ovary B_{11} cells (*J. Biol. Chem. 261*: 16666-16672 (1986)). We have now tested the hypothesis that hypomethylation is a positive determinant of DNA repair by quantitating and directly comparing, at both the genomic and specific-sequence levels, methylation and DNA repair levels in B_{11} cells and in a derivative cell line, B_{11}aza, generated by selection of B_{11} cells in increasing concentrations of 5-azacytidine, a cytidine analog and in vivo demethylating agent. Significant demethylation was demonstrated in the B_{11}aza cells at both levels of resolution by several independent techniques, and genomic repair replication was shown to increase concomitantly approximately two-fold. Defined sequences within the *DHFR* amplication unit were then examined for the efficiency of removal of T4 endonuclease-sensitive sites (ESS). Repair in several regions, representing both well repaired (*DHFR* 5' gene) and poorly repaired (3' flanking) sequences in B_{11} cells, remained constant, whereas repair in other sequences (e.g., the 3' end of the *DHFR* gene) exhibited reproducible increases. Thus, changes in DNA methylation appear to affect DNA repair to a significant extent, but the relationship between the two parameters is not a straightforward one, and methylation may be a secondary factor acting within the context of other, more immediately acting determinants such as transcription. (Supported by the National Cancer Institute and a Cancer Biology traineeship to L.H.)

E 222 GENETIC FUNCTIONS INTERACTING WITH A SITE-SPECIFIC DOUBLE-STRAND BREAK, Merl F. Hoekstra and Fred Heffron, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.
Studies on double-strand break repair in Saccharomyces cerevisiae have been informative for elucidating the overlapping roles that repair functions play in cellular metabolism. For example, some of the functions involved in repairing ionizing radiation damage are required to repair the double-strand break that initiates mating type switching. A single, un-repaired double-strand break is lethal. In addition, some of these same X-ray repair functions are involved in mitotic recombination and meiotic DNA metabolism. To gain further information on the complexity of double-strand break repair, we have isolated mutants sensitive to a single scission by the HO site-specific endonuclease. A yeast strain containing a chromsomal copy of a galactose-regulated HO gene has been constructed, mutagenized and isolates unable to grow on galactose-containing media identified. Of 60 galactose-inviable isolates, approximately one-third are sensitive to Methyl Methanesulfonate while none are sensitive to UV. From complementation studies, none of these mutations are rad52. At least one isolate may be a mutation in the recently discovered 140 kDa endonuclease NUC1. We are confident that the elucidation of the potential role(s) that these functions play in repairing the single, HO-induced double-strand break will be helpful in determining the specific functions involved in DNA damage repair.

E 223 MODE OF HYDROLYSIS OF POLY(ADP-RIBOSE) GLYCOHYDROLASE. Miyoko Ikejima and D. Michael Gill, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Mass. 02111.

After DNA damage poly(ADP-ribose) is synthesized in the cell with a constitutive enzyme, poly(ADP-ribose) polymerase. We have shown that the polymer is elongated by the proximal addition of new residues on the enzyme molecule so that the major event in the damaged cell is the construction of enzyme-bound poly(ADP-ribose) and that any poly(ADP-ribosyl)ation of other cellular proteins occurs secondarily. The major hydrolyzing enzyme of this polymer is poly(ADP-ribose) glycohydrolase, which accounts for the very rapid turnover of cellular polymer ($t_{1/2} < 1$ min).

We have developed a method of rapidly preparing enzyme-bound polymer which is labeled only in the distal position. The fate of such polymer can be followed using an SDS-gel system which clearly resolves oligomers of up to 30 residues. Therefore, we have been able to re-examine the mode of hydrolysis of the polymer by soluble poly(ADP-ribose) glycohydrolase. We find that : a) the initial attack by glycohydrolase is endonucleolytic and releases an oligomer from the distal terminus. The endonucleolytic attack clealy gives the glycohydrolase a greater target than if it had to locate the most distal residue. b) After the initial endo cut, hydrolysis proceeds rapidly in the 2'OH→ 1" direction, that is from the free end towards the enzyme. The combination of these properties helps account for the very rapid turnover observed.

E 224 MNNG- and ACNU-resistant mutants from HeLa S3 mer$^-$ cells, Ryoji Ishida and Taijo Takahashi, Aichi Cancer Center Res. Inst., Nagoya 464, Japan.
We isolated N-methyl-N'-nitro-N-nitrosoguanidine(MNNG)-resistant cells, MR10-1 from HeLa S3 mer$^-$ cells. The MR10-1 cells were also significantly more resistant to N-methyl-N-nitrosourea and slightly more resistant to methyl methanesulfonate and dimethyl sulfate than the parental cells. We found that the resistant cells had still little O^6-methylguanine-DNA methyltransferase activity and were sensitive to 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride(ACNU), like HeLa mer$^-$ cells. When induced 6-thioguanine (6TG)-resistant colonies were plotted as a function of the corresponding percentage survival, the resistant colonies of MR10-1 cells were induced much more frequently than in the case of HeLa mer$^-$ cells. However, induction of 6TG-resistant cells in the both cell lines did not differ significantly in the terms of mutant cells per μM MNNG. On the contrary, MR10-1 cells and HeLa S3 mer$^+$ cells differed in the induction of mutation as a function of MNNG concentration. We isolated ACNU resistant cells from MR10-1 cells, and found that MR10-1 AC^R cells were less mutable by MNNG than the MR10-1 cells. The above results show that HeLa mer$^-$ cell has at least two defects in the repair of alkylated adducts, O^6-methylguanine-DNA methyltransferase and an unidentified one, and also indicate that O^6-methylguanine is involved in the somatic mutation.

E 225 PROCESSING OF PSORALEN ADDUCTS IN DEFINED HUMAN SEQUENCES
A.L. Islas, J.-M. Vos, and P.C. Hanawalt, Stanford University, Stanford, CA. 94305-5020.
In order to study the efficiency of repair of bulky chemical adducts at the level of individual genes in human cells, we developed a procedure to determine the interstrand crosslinking levels produced by psoralens in defined genomic sequences. Our procedure involves the denaturation of DNA followed by rapid renaturation and isopycnic centrifugation to resolve readily double-stranded (crosslinked) and single-stranded (uncrosslinked) populations. By slot blotting fractions from the gradient one can probe defined genomic sequences for initial crosslinking levels as well as relative repair rates. Using human fibroblast cell lines, we have looked at adducts induced by 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT) in the housekeeping dihydrofolate reductase gene, the expressed c-*abl* and non-expressed c-*fms* proto-oncogenes and have compared these to the bulk DNA by probing with the highly repetitive interspersed human Alu sequence. Both the differential addition of HMT and the efficiency of its removal were characteristic features of the genomic regions examined. To understand further the relationship between transcriptional activity and the efficiency of repair of bulky DNA adducts we are studying the human promyelocytic HL60 cell line. When induced to differentiate into macrophages these cells repress certain genes such as c-*myc* and activate other genes such as c-*fms*. Altogether, results demonstrate that both the introduction of HMT and its repair are non-randomly distributed in the genome with particularly high accesibility of some actively transcribed sequences.

E 226 EPISOMAL EXPRESSION VECTORS FOR DNA REPAIR GENES IN HUMAN CELLS. Michael JAMES and Alain SARASIN, Institut de Recherches Scientifiques sur le Cancer, Villejuif,France.
Shuttle vectors which contain the Epstein-Barr virus origin of replication persist as stable multicopy episomes in the nucleus of human cells. These vectors overcome the obligatory chromosomal integration, probably the limiting step for production of stable DNA-transformants. Thus we have found up to 500 fold higher efficiency of DNA-transformation using EBV vectors relative to standard plasmids. Furthermore, integrated plasmids show highly variable expressionof co-transferred genes due to chromosomal position effects and physical interruption caused by the integration event. Worse still, it appears that human cells have a strong tendency to eliminate incorporated DNA. In contrast, EBV vectors are stably maintained and the genes carried by the episomal vector remain intact and in the original DNA (vector) context. This provides a system whereby expression of cloned genes can be designed and predictably obtained in the final DNA-transformants. For example, using the reporter gene CAT, our vectors reproduce the expected characteristics of the contitutively weak promoters mouse metallothein (mMT), mouse mammary tumour virus long terminal repeat (MMTV-LTR), the moderately strong promoters SV40-early, Adenovirus major late promoter (MLP) and the strongest known pol-II promoter, human cytomegalovirus immediate-early (hCMV-IE). Of special interest in the context of potentially-toxic or conditionallly-toxic DNA repair genes we have found that the mMT promoter retains metal regulation in the multicopy episomal state, giving up to 50 fold induction of CAT activity. Up to 5 fold induction occurs within 6 hours of addition of metal. Data will be shown on the application of this new genetic system, including in XP cells, for the controlled expression of several genes involved in DNA metabolism and DNA repair.

E 227 A β-ACTIN INTRON PROBE HYBRIDIZES PREFERENTIALLY TO THE REPAIRED DNA IN XP-C CELLS. G. J. Kantor and L. S. Barsalou, Wright State University, Dayton, Ohio 45435.
Ultraviolet (UV, 254 nm)-irradiated nondividing xeroderma pigmentosum complementation group C (XP-C) cells repair DNA to a very limited extent compared to normal human cells. The limited DNA excision repair is about as rapid as normal excision repair and is localized in undefined genomic domains (1). The selectively repaired DNA (sr-DNA) has been separated from the remainder of the DNA (bulk DNA) and probed with cloned DNA fragments that hybridize to the human β-actin gene. A 385 bp fragment of the 3rd intron that detects only the β-actin gene and a 14 kb Eco R1 human genomic fragment that encompasses the entire 3.5 kb β-actin gene and detects an additional 19 pseudogenes (2) were used as probes. We detect significantly more hybridization (3-10 times more) of the intron probe to blots of sr-DNA than to those of bulk and unfractionated DNA when equal amounts of the respective DNAs are used. The 14 kb Eco R1 fragment hybridizes equally well to both sr and bulk DNA. We interpret these results to mean that the transcriptionally active β-actin gene is preferentially repaired in XP-C cells. Domain-oriented repair in XP-C is evidently a non-random process, specific for selected DNA sequences that include some but not all (3) transcriptionally active genes. (Research supported by Research Challenge Award #66072, State of Ohio).
1. Mansbridge, J. N., and P. C. Hanawalt (1983) In: Cellular Responses to DNA Damage (Eds., E. C. Friedberg and B. A. Bridges), A. R. Liss, Inc., NY, pp. 195-207.
2. Ng, S-Y., et al. (1985) Molec. Cell. Biol. 5: 2720-2732.
3. Bohr, V. A., et al. (1986) Proc. Natl. Acad. Sci. USA 83: 3830-3833.

E 228 LACK OF PREFERENTIAL DNA REPAIR OF A MUSCLE SPECIFIC GENE DURING MYOGENESIS, Ofra Kessler and Ruth Ben-Ishai, Technion, Israel Institute of Technology, Haifa, Israel.
Bohr et al (1) have demonstrated that essential genes in rodent cells are preferentially repaired after UV damage. We have addressed ourselves to the question whether genes for specialized functions, such as muscle genes, are repaired when they become active during myogenesis. A rat muscle cell line that differentiates in vitro was tested for repair of UV light damage in the creatine kinase (CK) gene, a metabolically critical enzyme in muscle. Repair was tested as described (1), by quantitation of thymine dimers and Southern blot hybridization; in parallel CK mRNA levels and CK activity were determined. Dimer excision was not observed in either myoblasts, when the CK gene is not expressed, nor during differentiation when the gene becomes transcriptionally active. These results indicate that in the rat muscle cell line transcription of the CK gene is not sufficient for its preferential repair. To test whether postmitotic cells have repair capacity we tested for transient expression of an irradiated CAT plasmid transfected into myoblasts and differentiated cells. It was found that under both conditions UV damage to the plasmid could be overcome.

1) Bohr, V.A.; Smith, C.A.; Okumoto, D.S.; Hanawalt, P.C. : Cell, 40, 359-369, 1985.

E 229 ANALYSIS OF THE ROLE OF DNA HELICASE II (UVRD) IN DNA REPAIR AND DNA REPLICATION EMPLOYING IN VITRO MUTAGENESIS, Sidney R. Kushner, Valerie F. Maples, Brian Washburn and Carol Hamilton, Department of Genetics, University of Georgia, Athens, Georgia 30601.

The uvrD gene product (DNA helicase II) of Escherichia coli is involved in the repair of DNA mismatches, UV-induced photoproducts and the process of spontaneous mutagenesis. Indirect genetic evidence also suggests a role for the protein in DNA replication. In vitro the purified protein acts as a DNA dependent ATPase and in stoichiometric amounts as a DNA helicase. Although the uvrD has been cloned and sequenced, little progress has been made in correlating the in vitro enzymatic activities with the observed phenotypes of various uvrD mutants. Accordingly, a series of new uvrD mutations have been generated in vitro employing directed mutagenesis with linkers or random mutagenesis with a variety of chemicals. After phenotypically characterizing the mutations, attempts have been made to purify and characterize the altered DNA helicase II proteins. Another approach has been to generate chromosomal deletions of the gene. So far these efforts have not been successful. Finally, alterations in the promoter and transcription attenuator regions have been constructed to better understand the control of the gene. (This work was supported in part by NIH grant GM27997 to S.R.K.)

E 230 SERUM FACTORS CAN MODULATE DNA REPAIR IN HUMAN LYMPHOCYTES, Lyndon L. Larcom, Molly E. Smith and Calvin C. Wilhide, Clemson University, Clemson, SC 29634-1911.
We have developed an assay for determining relative capacities to perform unscheduled DNA synthesis repair in response to genetic damage. This assay removes artifacts present in previous assays used for such comparisons. The results are independent of: 1) varying levels of thymidine present in sera used to supplement the culture media, and (2) varying thymidine phosphorylase activities of the cells from different donors. Using this assay, we have studied UDS of freshly isolated peripheral blood lymphocytes damaged by 254 nm UV. The results indicate that: 1) the repair of UVC damage is suppressed by exposure to UVB radiation; 2) the repair capacity of lymphocytes from mononucleosis patients is significantly lower than for lymphocytes from healthy individuals; and 3) there are factors present in human sera which strongly affect the repair capacities of damaged lymphocytes.

E 231 REPAIR OF ALKYLATION DAMAGE IN SPECIFIC DNA SEQUENCES, Susan P. LeDoux, Vilhelm A. Bohr and Glenn L. Wilson, University of South Alabama, Mobile, AL 36688 and The National Cancer Institute, Bethesda, MD 20892
Recent evidence has demonstrated that within the genome there is a heterogeneity of DNA repair of UV dimers which correlates with transcriptional activity. To determine if a similar situation exists with repair of alkylation damage, we have modified the techniques used for detecting repair of UV dimers in order to investigate MNU-induced alkylation damage and its subsequent repair within the insulin gene. A uniquely suited model which consists of cell lines derived from two clonal isolates of a radiation-induced rat insulinoma (RINr 38 and RINr B2) is being utilized. In RINr 38 cells the insulin gene is actively transcribed, while in RINr B2 cells the insulin gene is silent. Furthermore, the transcription of the insulin gene in RINr 38 cells can be enhanced by elevating cellular cyclic AMP levels. For these studies, monolayer cell cultures are exposed to varying doses of MNU (1-10 mM) for 1 hour. The drug is removed and the cells are allowed to repair for designated intervals of time. The cells are then removed. The DNA is extracted, digested with BAM HI, and treated in standard conditions to break alkali-labile sites. The restriction fragments are then separated using alkaline gel electrophoresis. Following Southern transfer, the membranes are probed with a ^{32}P labeled probe for the preproinsulin I gene. Initial studies have demonstrated damage within the insulin gene in both cell lines. Currently, studies are in progress in which repair in the transcriptionally active RINr 38 cells is being correlated with that in RINr B2 cells in which the insulin gene is not expressed. (This work is supported by NIH Grant ES03456).

E 232 DIFFERENTIAL REPAIR OF UV DAMAGE IN THE HUMAN METALLOTHIONEIN GENE FAMILY, Steven A. Leadon and Margaret Snowden, Lawrence Berkeley Laboratory, Berkeley, CA 94720.
We have studied the repair of UV damage in the human metallothionein gene family using an immunological method that isolates DNA fragments containing bromouracil in repair patches by means of a monoclonal antibody that recognizes bromouracil. The presence of the metallothionein genes in the repaired and unrepaired DNA fragments is detected by using a nick-translated cDNA probe for the genes. We have measured the repair of damage in four members of this gene family: the expressed MT-IA and MT-IIA genes, the MT-IB gene whose expression is tissue-specific, and the non-expressed MT-IIB pseudogene. Following a dose of 10 J/m^2 UV, DNA damage is repaired faster in the transcribed MT-IA and MT-IIA genes than in the genome overall. By 6 hr post-UV, there is at least twice as much repair in these transcribed genes as in the rest of the genome. Repair in the MT-IB gene, which is not expressed in the cell line used in these experiments, and in MT-IIB, a processed pseudogene that is also not expressed, is about the same as in the rest of the genome. When the expression of the MT-IA and MT-IIA genes is increased 15-20 fold by incubation of the cell cultures with cadmium, the rate of repair in these genes is 4-5 fold faster than in the genome overall. The rate of repair in the MT-IB and MT-IIB genes, whose expression cannot be induced by cadmium, remained the same as in the rest of the genome. We have also examined the effect of UV irradiation on expression of the MT genes and found an early decrease in MT mRNA levels, followed by a return to control levels by 5 hr post-UV. These results indicate that the efficiency of repair of DNA damage in a particular sequence is influenced by the transcriptional activity of that sequence.

E 233 ISOLATION AND PRELIMINARY CHARACTERIZATION OF A MUTANT OF SCHIZOSACCHAROMYCES POMBE SHOWING ENHANCE SENSITIVITY TO UV LIGHT AT ELEVATED TEMPERATURE, Howard B. Lieberman and Richard Riley. Department of Radiation Oncology, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

During the screening of a population of S. pombe cells mutagenized with ethylmethane sulfonate, we have identified a mutant somewhat sensitive to UV light at 30°C and dramatically sensitive when grown at 37°C, compared to the parental strain. Further studies also indicated that the mutant was not more sensitive than the parental strain to low doses of 18 MeV electrons, yet statistically significant sensitivity, albeit slight, was observed at doses approximately 60 krads or higher, especially when mutant cells were grown at 37°C. Analyses of random spores generated by genetic crosses between the temperature-dependent mutant and known S. pombe DNA repair mutants sensitive to UV light indicate that the defect is not within the previously identified rad1, rad2, rad3, rad4, rad7 or rad17 locus. Work is in progress to characterize the genetic and biochemical basis for the defects expressed in the mutant.

E 234 SPECIFICITY OF THE DAM-DIRECTED MISMATCH REPAIR SYSTEM OF ESCHERICHIA COLI K-12. T-H. Wu and M.G. Marinus.* Univeristy of Massachusetts Medical School, Worcester, MA 01655.

The mismatch repair system of Escherichia coli removes mispaired bases from DNA and the products of the dam, mutH and mutL genes have been implicated in this process. We have used a forward mutation assay to identify independent mutations arising in dam, mutH, mutL and mutD mutant strains. The mutD gene product is not implicated in mismatch repair but specifies the epsilon subunit of DNA polymerase III holoenzyme. The mutation spectra in dam, mutH and mutL strains are very similar to each other but differ sharply from that of mutD. In dam, mutH and mutL strains, AT to GC transitions were favored and the majority of these occurred in three hotspots which are within six base pairs of the only two 5'-GATC-3' sequences in the target gene. In contrast no such hotspots were detected in mutD or wild type bacteria. Transversion and transition mutations were characteristic of the mutD strain. IS-element insertions were the predominant class of mutation recovered from the wild type strain. The above results show that Dam-directed post-replicative mismatch repair plays a significant role in the rectification of potential transition mutations in vivo and suggest that certain sequences are particularly prone to replication or repair errors.

E 235 ANALYSIS OF REMOVAL OF DNA INTERSTRAND CROSSLINKS FROM SPECIFIC GENES IN FANCONI'S ANEMIA CELLS OF DIFFERENT COMPLEMENTATION GROUPS J.-M. Vos, A. Matsumoto and P. C. Hanawalt, Dept. of Biol. Sciences, Stanford Univ. Stanford, CA 94305-5020.

Fanconi's anemia (FA), an inherited autosomal recessive human disorder, belongs to the class of diseases characterized by chromosomal instability and predisposition to cancer. An increased sensitivity of cells from FA individuals to DNA crosslinking agents implies a specific deficiency in the processing of these unique lesions. However, different laboratories have reported conflicting results on possible defects in repair. Such studies measured overall repair in cellular genomes and could not evaluate the processing of DNA interstrand crosslinks

The existence of genetic heterogeneity among FA individuals allows for the possibility that a deficiency in crosslink processing might be expressed at different levels. We have considered the possibility that one or more FA complementation groups may exhibit a defect in repair in specific DNA sequences. Using a novel procedure to measure interstrand crosslinking of DNA in specific genomic regions (Vos and Hanawalt (1987) Cell 50:789-799), we have analyzed the removal of such lesions from the pol I-transcribed endogenous ribosomal RNA genes and the pol II-transcribed established EBV genome in cultured FA lymphoblastoid cells. We report results of a comparative study on the psoralen derivative HMT and the anti-cancer drug Mitomycin C, inducing DNA crosslinking between pyrimidines and purines, respectively.

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E 236 ISOLATION OF A UV-RESISTANT CS CELL LINE AFTER DNA-MEDIATED GENE TRANSFER
L.V. Mayne, Sussex Centre for Medical Research, University of Sussex, England.

A uv-resistant derivative of CS3BE was isolated after transfection of normal human DNA into uv-sensitive CS3BE cells. Normal human DNA was partially digested and ligated to the dominant selectable marker pSV2neo. The DNA was added to CS3BE.S3.G1 (immortal derivative of CS3BE) as a calcium phosphate precipitate. A total of 3 x 10^4 G418 resistant colonies were isolated and screened for uv sensitivity. One G418 resistant cell line had a normal level of survival and showed a normal recovery of RNA synthesis after uv-irradiation. The uv-resistant phenotype of this line is unstable and is lost when the cells are subcultured in the absence of uv-irradiation. Preliminary Southern analysis data indicate that the loss of uv-resistance is associated with changes in the neo containing bands. Secondary transfers are currently in progress.

DNA-mediated gene transfer and selection for uv-resistance in a second complementation group of Cockayne's syndrome (CS1AN) has been initiated.

E 237 STRAND TARGETING SIGNALS FOR *IN VIVO* MUTATION AVOIDANCE BY POSTREPLICATION MISMATCH REPAIR IN *ESCHERICHIA COLI*, Jean-Pierre Claverys and Vincent Méjean, CRBGC - CNRS, 118 route de Narbonne, 31062 Toulouse Cedex France.

The involvement of GATC sites in directing mismatch correction for the elimination of replication errors in *Escherichia coli* was investigated in vivo by analyzing mutation rates for a gene carried on a series of related plasmids that contain 0, 1 and 2 such sites. This gene encoding chloramphenicol acetyl transferase (Cat protein) was inactivated by a point mutation. In vivo mutations restoring resistance to chloramphenicol were scored in mismatch repair proficient (mut^+) and deficient ($mutHLS^-$) strains. In mut^+ cells, reduction of GATC sites from 2 to 0 increased mutation rates by a factor close to ten. Removal of one GATC site (the one distal to the cat^- mutation) did not significantly change the rate of mutation, indicating that mismatch repair can proceed normally with a single site. The mutation rate increased three fold in the case of a single GATC site located farther from the mutation. In the absence of a GATC site, $mutL^-$ and $mutS^-$ cells exhibited a two to three fold increased mutation rate as compared to $mutH^-$ and mut^+ cells. This indicates that 50 to 70% of replication errors can be corrected in a mutLS-dependent way in the absence of any GATC site to target mismatch correction to newly synthesized DNA strands. Other strand targeting signals, possibly single strand discontinuities, might be used in mutLS-dependent repair. This result also argues in favour of the involvement of the MutH protein in recognition of the "GATC signal".

E 238 SELECTIVE REPAIR IN THE TRANSCRIBED STRAND OF MAMMALIAN DHFR GENES
I. Mellon, G. Spivak, and P. C. Hanawalt, Dept. Biol. Sciences, Stanford Univ., Stanford CA 94305-5020.

We have measured DNA repair independently in the transcribed and nontranscribed strands of genes using strand-specific RNA probes derived from specially constructed plasmids. We find a dramatic difference in the efficiency of removal of pyrimidine dimers from the respective strands of the DHFR gene in UV-irradiated human and hamster cells. In hamster cells, 80% of the dimers are removed from the transcribed strand in 4 hours while little repair occurs in the nontranscribed strand in 24 hours. In human cells, repair is significantly faster in the transcribed strand of the DHFR gene. Furthermore, in the 5' flanking region of the human DHFR gene, selective rapid repair is found in the opposite DNA strand relative to the transcribed strand of the DHFR gene. This strand is thought to serve as a template for transcription of a divergent transcript. Thus there is a switch in selective strand repair within a contiguous stretch of DNA where there is a switch in the strand that is used as a template for transcription (*Cell 51: in press*). Consequently, it seems likely that selective strand repair is directly related to transcription rather than to the polarity of replication forks.

UV-irradiated hamster and human cells exhibit similar levels of survival but they differ dramatically in removal of dimers from total DNA. However, most dimers are removed from the transcribed strand within 4 hours after UV in both cell types, so there is a direct correlation between levels of cell survival and rapid repair in the transcribed strand of an active gene in mammalian cells. To determine if there is a correlation between the timing or extent of selective strand repair and UV sensitivity, we are examining selective strand repair of active genes in cells from patients with Cockayne's syndrome or xeroderma pigmentosum. Preliminary evidence suggests a deficiency in the preferential repair of an active gene in Cockayne's syndrome cells. Supported by a grant from the National Cancer Institute and an American Cancer Society postdoctoral fellowship to I.M.

E 239 CONTROL OF *uvrC* EXPRESSION, Thomas F. Stark, Terry L. Timme and Robb E. Moses, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
We have shown that the 2.5 kb DNA region 5' to the *uvrC* structural gene contains five promoters and three putative LexA binding sites. In addition, there are two open reading frames (ORF) which encode expressed proteins of approximately 23 kd (5'-proximal) and 28 kd (5'-distal). We have now shown that: (1) the 28 kd gene is a positive element for *uvrC* gene transcription; (2) LexA binds specifically to a site inside the 23 kd open reading frame; (3) the 23 kd protein shows homology to omp^R, a conserved regulatory gene; and (4) transposon insertional mutagenesis in the *uvrC* gene is not lethal. Supported by USPHS GM24711.

E 240 PREFERENTIAL REPAIR OF NUCLEAR MATRIX ASSOCIATED DNA IN MAMMALIAN CELLS.
Leon H.F. Mullenders, A.T. Natarajan and Albert A. van Zeeland. State University of Leiden, Leiden, The Netherlands. The organization of eukaryotic DNA into a series of loops anchored to the nuclear matrix has been related to functional activities of the genome i.e. replication and transcription. In human fibroblasts we have investigated whether the matrix compartment is also involved in the regulation of DNA repair synthesis. Pulse labelling of UV(254 nm)-irradiated normal cells followed by enzymatic digestion of DNA-matrix complexes revealed that the repair label was preferentially located in matrix associated DNA (about 3 fold enriched) in cells exposed to low dose (5 J/m^2) but not to high dose(30 J/m^2). This non-random distribution was most abundant directly after UV-irradiation (5-10 min) and then gradually changed to a more random distribution within the first hour after treatment. Autoradiographic analysis of DNA-halo structures visualized the dose dependent preferential repair of matrix associated DNA. The results of chase experiments favour a preferential repair of DNA sequences permanently associated with the matrix. Xeroderma pigmentosum (group C) cells exhibited a preferential repair of matrix associated DNA, but unlike normal cells the distribution of repair events between matrix associated and loop DNA did not change at later stages after treatment. In Cockayne's syndrome cells the matrix associated DNA was repaired to a lesser extent then loop DNA. Southern analysis showed that the promotor region of the adenosine deaminase gene was significantly enriched in matrix associated DNA, consistent with the localization of transcribed DNA proximal to the matrix. The preferential repair of matrix associated DNA might represent the repair of active genes and be responsible for the rapid recovery of RNA synthesis (within 2 hours after irradiation) in UV-irradiated human cells. This idea is supported by the ability of XP-C cells and the inability of CS cells to recover UV-inhibited RNA synthesis.

E 241 EXCISION REPAIR IN DIFFERENT DOMAINS OF NUCLEOSOME CORE DNA IN HUMAN CELLS, **Karen A. Nissen and Michael J. Smerdon, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4660.**

Cyclobutane dipyrimidines (PD) are removed in at least two distinct phases in human cells following irradiation with UV light. These phases consist of an early, rapid phase, where some 50-80% of the PD are removed, and a prolonged, slow phase. Previously, we showed that during each of these phases, excision repair synthesis is followed by significant rearrangements in nucleosome structure which appear to involve the reformation of nucleosomes in newly repaired regions (*Biochemistry* 19:2992, 1980). More recently, we reported an exonuclease III mapping procedure to measure the distribution of repair synthesis in nucleosome core DNA following this reformation of nucleosome structure (*Biochemistry* 24:7771, 1985). We have used this method to compare the distribution within nucleosomes of repair synthesis occurring during the two repair phases in human cells. Whereas repair synthesis occurring during the early, rapid phase is located primarily in the 5' and 3' end domains of nucleosome cores, repair synthesis occurring during the slow phase is much more randomly distributed in nucleosomes. These results can be explained by (1) a nonuniform distribution of PD in nucleosomes, (2) preferential repair of nucleosome subdomains, or (3) insertion of different sized patches during the two repair phases. To shed light on these possibilities, we have measured the distribution and removal of PD in nucleosome core DNA at the single nucleotide level.

E 242 *HIGH PROPORTIONS OF 4,5',8-TRIMETHYLPSORALEN (TMP) PHOTOINDUCED FURAN-SIDE MONOADDUCTS CAN BLOCK CROSSLINK INCISION IN NORMAL HUMAN FIBROBLASTS,* *Dora Papadopoulo, Dietrich Averbeck and Ethel Moustacchi, Institut Curie, Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.*

Bifunctional psoralens photoinduce specific DNA lesions in well defined proportions, i.e. pyrone-side monoadducts (MA_p), furan-side monoadducts (MA_f) and interstrand crosslinks (CL). The rate and extent of CL removal are related to important biological endpoints, such as cell death, mutagenicity, carcinogenicity. Although the repair of psoralen induced DNA CL has been studied in a wide range of organisms, little is known about the influence of the presence of different types and proportions of monoadducts on the repair of CL.
We attempted to analyse the possible role of furan-side monoadducts on the incision repair step of DNA CL in normal human fibroblasts. For this, we used the highly photoreactive furocoumarin TMP in combination with either single exposure to 365 nm radiation or with two re-irradiation regimens, i.e. 405-365 nm or 365-365 nm radiations. At a given constant level of total adducts induced either by TMP plus 405 nm or TMP plus 365 nm radiation (as a first exposure), alkaline elution analysis allowed us to determine CL induction and incision.
Our results show that furan-side monoadducts induced in large proportions by TMP plus 365 nm radiation can block CL incision. When the amount of furan-side monoadducts is reduced by their conversion into even more CL, the incision step can take place.

E 243 RELATION OF TOPOISOMERASE II ACTIVITY TO DNA SINGLE-STRAND BREAKS INDUCED BY 5-BROMODEOXYURIDINE, TOPOISOMERASE II INHIBITORS, OR 313 nm ULTRAVIOLET LIGHT IN BLOOM SYNDROME FIBROBLASTS. Yves Pommier, Thomas M. Rünger, Donna Kerrigan, and Kenneth H. Kraemer. NCI, NIH, Bethesda, MD 20892.

Bloom syndrome cells form numerous sister chromatid exchanges upon exposure to 5-bromodeoxyuridine (BrdU). This effect has been proposed to result from abnormal topoisomerase II (topo II) activity (Heartlein et al., Exp. Cell Res.,169:245,1987). The possibility that BrdU could induce topo II-mediated DNA breaks was tested by alkaline elution in normal (GM0637) and Bloom syndrome (GM8505) fibroblasts. Three day exposure to BrdU induced DNA single-strand breaks (SSB) in both cell lines. Alkaline elutions performed with and without proteinase K showed that these breaks were not protein-associated and were alkali labile, indicating that they did not result from topo II action. Topo II inhibition by the anticancer drugs, amsacrine (m-AMSA) and etoposide (VP-16) was tested by alkaline elution in normal and Bloom syndrome cells. Both drugs produced similar SSB frequencies in the two cell lines, implying that they had similar topo II activities. The formation of SSB by ultraviolet light (313 nm for 10 min) was also tested in both cell lines by alkaline elution. Bloom syndrome cells formed more SSB than normal cells. Alkaline elution without proteinase K indicated that the breaks were protein-associated and alkaline elution following X-rays detected approximately one DNA-protein crosslink per DNA break in both cell lines. The nature of the crosslinking protein, its relationship to DNA cleavage and topoisomerases is under study.

E 244 EFFECT OF CARCINOGEN TREATMENT ON THE DNA REPAIR CAPACITY OF MAMMALIAN CELLS, Miroslava Protić-Sabljić, Emmanuel Roilides, Arthur S. Levine and Kathleen Dixon, Section on Viruses and Cellular Biology, NICHD, NIH, Bethesda, MD 20892.

We wished to determine whether DNA excision repair is enhanced in mammalian cells in response to carcinogen treatment. To address this question we used an expression vector-host cell reactivation assay to measure cellular DNA repair capacity. When UV-damaged pSV2catSVgpt vector DNA was introduced into monkey CV1 cells, the level of CAT activity was inversely related to the UV fluence due to inhibition of *cat* gene expression by UV photoproducts (Protić-Sabljić and Kraemer, PNAS USA 82:6622-6626, 1985). When CV1 cells were treated with either UV radiation or mitomycin C 24-48 hours before transfection, CAT expression from the UV-irradiated plasmid was increased. A similar increase occured in SV40 transformed normal human and XP group D cells but not in XP group A cells. Preliminary experiments suggested that the inducing signal was transferable by secreted cellular factor(s), since CV1 cells treated with conditioned media from UV-irradiated cells showed a similar (although smaller) response. We confirmed that this increase in CAT expression was likely due to repair, and not to production of damage-free templates by recombination; the frequency of generation of SupF+ recombinants after transfection with pairs of pZ189 vector mutants did not significantly increase in carcinogen-treated CV1 cells.

E 245 ENHANCED REPAIR SYNTHESIS IN HYPERACETYLATED NUCLEOSOMES, Brinda Ramanathan and Michael J. Smerdon, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4660.

Treatment of human fibroblasts in culture with millimolar concentrations of sodium butyrate induces histone hyperacetylation and stimulates DNA repair during early times after UV irradiation (*J. Biol. Chem.* 257, 13441, 1982). Since only a fraction of the total chromatin becomes hyperacetylated during sodium butyrate treatment and only a fraction of the DNA lesions are repaired more efficiently, it was not known if the enhanced repair of these lesions occurred in the hyperacetylated chromatin fraction. To this end, we have fractionated chromatin from sodium butyrate-treated human fibroblasts into different acetylated forms and monitored the level of repair synthesis occurring in each of these forms following UV irradiation. Our results indicate that most (or all) of the enhanced DNA repair synthesis occurring in these cells at early times after irradiation is located in the hyperacetylated chromatin fraction. Nucleosomes from moderately acetylated regions of chromatin contained only about half the level of repair synthesis found in hyperacetylated nucleosomes. These results could be explained by (1) changes in the initial level of UV damage in hyperacetylated chromatin or (2) increased DNA repair efficiency in the hyperacetylated regions of chromatin. To shed light on these possibilities, our current investigations have focused on (a) comparison of the distribution of repair synthesis in the different acetylated forms of nucleosomes and (b) determination of the levels of pyrimidine dimers in these regions both immediately after UV damage and following different extents of repair.

E 246 TOWARD ELUCIDATION OF THE BIOCHEMICAL LESION IN THE UVS-3 MUTANT OF NEUROSPORA. Dindial Ramotar and Murray Fraser, McGill University, Montreal, Canada H3G 1Y6. Neurospora endo-exonuclease, an enzyme implicated in DNA-repair, is present in both active (EE) and precursor (PRE) forms in nuclei. PRE is activated in vitro by treatment with proteases. Previously, it was found that nuclei of the mutagen-sensitive uvs-3 mutant contained only 10-12% EE and 32% PRE as wild-type nuclei. Furthermore, when cells were pre-treated with low doses of a mutagen, a dose-dependent activation of PRE was observed in wild-type, but not in uvs-3 nuclei. It was postulated that uvs-3 might be deficient in a protease which activates PRE in response to DNA damage. Others have shown that UVS-3 also exhibits constituitively high levels of mutagenesis and excision repair of UV-damge. The biochemistry of uvs-3 now appears much more complex. In searching for chromatin-bound protease activities with ^{3}H-casein as substrate, it has been found that nuclear extracts of uvs-3 contain at least 3 times as much as wild-type of a macropain-like protease. This high molecular weight protease is inhibited by p-hydroxymercuribenzoate and iodoacetamide, but not by phenylmethylsulfonyl fluoride (No ATP-dependent hydrolysis of ^{3}H-casein was detected). Furthermore, when immunoblots of the nuclear extract were probed with specific antibodies raised either to EE or to a specific inhibitor of EE now shown to be derived from PRE, high molecular weight immunoreactive polypeptides, especially one of 125 kDa (PRE?), were seen to be depleted in nuclear extracts of uvs-3. It is now suggested that the low level of EE in uvs-3 nuclei is due to a more rapid turnover of EE than in wild-type nuclei. The postulated PRE-activating protease remains to be identified. (Supported by the Med. Res. Council, Can.)

E 247 THE CENTROMERE AS A TARGET FOR DNA DAMAGE, Michael A. Resnick[1], James Westmoreland[1], Kerry Bloom[2], 1 National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 2 Department of Biology, University of North Carolina, Chapel Hill, NC 27514.
The centromere is required for proper chromosome segregation in mitotically growing and meiotically developing cells of the yeast Saccharomyces cerevisiae. Mutations in the DNA sequence of this organelle (approximately 200 bp) can result in chromosome malsegregation. We are investigating the sensitivity of the centromere DNA to damaging agents, the capability for repair and the induction of mutations. Because of the organization, we are able to sequence the positions of DNA damage and relate the sites of induction to protein-DNA interactions. UV induces considerable damage in the centromere due to the high AT content. Based on a comparison between irradiated purified DNA vs in vivo centromere DNA, the presence of centromere protein(s) results in a "cold region" for UV sensitivity. The centromere damage can be efficiently repaired. In order to more fully characterize damage in the centromere, we have developed a centromere plasmid system that allows us to develop lines of cells with as many as 10 centromere plasmids per cell corresponding to a "centromere barrier". The centromeres are conditional in that their function can be turned off. This allows us to sequence damage as well as examine repair at low doses in relation to the functional state of the centromere.

E 248 DNA POLYMORPHISM AND REACTIVITY TOWARDS CHEMICAL CARCINOGENS IN PLASMIDS CONTAINING ALTERNED PURINE/PYRIMIDINE INSERTS, Pascale Rio and Marc Leng, Centre de Biophysique Moléculaire, C.N.R.S., 1A avenue de la Recherche Scientifique, 45071 ORLEANS Cédex 2, France.
The reactivity of N-hydroxyaminofluorene (N-OH-AF) and N-acetoxyacetylaminofluorene (N-AcO-AAF) towards guanines has been compared by sequencing analysis in four plasmids : a) pBR322, b) pBR322 with a $(dC\text{-}dG)_{16}$ insert (pLP32), c) pCM4 which is a pBR322 containing a 300 base pairs segment of a repeated DNA from Cebus appella at the PstI site, d) pUC 19 with a $(dC\text{-}dA)_{30}$ insert. The binding spectrum of N-OH-AF adducts shows that the insert guanines, in highly supercoiled pLP32 are not reactive, while the insert is in the Z-form (according to immunochemical titration). A conformational change of the B DNA-Z DNA junctions occurs as a function of the superhelical density (as in the reaction towards N-AcO-AAF : Marrot et al., 1987, Nucl. Acids Res. 15, 5626). However, at high superhelical density of pBR322, guanine residues are still reactive while the segment 1448-1462 is in the Z-form (as shown by osmium tetroxide and diethylpyrocarbonate reactions, and Herr, 1985, Proc. Nat. Acad. Sci. USA 82, 8009). The reactivity of the insert guanines decreases slightly in the pUC and in the pCM4 $(dG\text{-}dT)_{15}$ sequences. The study of the reactivity of pCM4 and pUC with N-AcO-AAF shows that the reactivity of the guanine residues decreases with increasing superhelical density. No reactivity can be detected for highly supercoiled plasmids. At the opposite, N-AcO-AAF reacts equally with highly supercoiled and relaxed pBR322. We conclude that the reactivity of the guanine residues of the inserts towards carcinogens depends on DNA conformation : some specificity of reaction with chemical carcinogens may be induced by DNA polymorphism.

E 249 *PSORALEN-PHOTOINDUCED DNA CROSSLINKS IN NORMAL AND FANCONI ANEMIA FIBROBLASTS : AN ELECTRON MICROSCOPY ANALYSIS OF THEIR REMOVAL,* *Solange Rousset, Silvano Nocentini and Ethel Moustacchi, Institut Curie, Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.*
Fanconi anemia (FA) cells are characterized by a high cellular sensitivity to bifunctional genotoxic agents. The capability of normal (N) and FA cells to repair DNA interstrand crosslinks was evaluated by a quantitative electron microscopy analysis. Cells were treated by 8-methoxypsoralen (8-MOP) plus UV-A (365 nm) and allowed or not to repair for different periods of time. DNA was extracted, restricted to reduce fragment length and totally denatured before spreading. DNA crosslinks were visualized by a transmission electron microscope. Additional informations were obtained from DNA fragment length measurements performed with a computer assisted digitizer.
A direct relationship was found between the UV-A dose and the crosslinks induced in the DNA. For a same UV-A dose, a similar number of crosslinks was induced in N and FA cells. As a function of the repair period, the number of crosslinks decreased in all the cell lines studied. However, this removal was slightly reduced in some FA cell lines. Similar results were obtained using either confluent or rapidly growing cell cultures. FA cells appear thus to be able to accomplish at least the first steps of the repair process, i.e. recognition and incision of interstrand crosslinks, even if they seem somewhat affected in the efficiency of this process.

E 250 PHR1 PHOTOLYASE STIMULATES EXCISION REPAIR IN *SACCHAROMYCES CEREVISAE* BUT INHIBITS *E. COLI* EXCISION REPAIR, Gwendolyn B. Sancar and Frances W. Smith, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7260.

The *PHR1* gene of yeast encodes a 66 kd DNA photolyase containing two intrinsic chromophores. While repair of pyrimidine dimers by Phr1 requires light, formation of the photolyase-pyrimidine dimer complex does not; thus, in the absence of photoreactivating light, pyrimidine dimers are bound by an enzyme unable to repair these lesions. We have investigated whether the presence of such a complex affects dark repair of dimers in yeast. Yeast strains defective in either excision repair (GBS76; *rad2 phr1*) or "mutagenic repair" (GBS57; *rad18 phr1*) of pyrimidine dimers were transformed with the CEN plasmid pGBS107 in which transcription of *PHR1* is controlled by the galactose-inducible *GAL10* promoter, and the effect of Phr1 on the dark survival of the transformed strains was assessed by comparing survivals of plasmid-containing strains with those of isogenic segregants which had lost the plasmid. We find that induction of *PHR1* prior to irradiation has no effect on the dark survival of pGBS107/GBS76; in contrast survival is increased 10-fold in strain pGBS107/GBS57. Similar results were obtained when the survivals of GBS57 and GBS58 (*rad18 PHR1*) were compared. Thus we conclude that binding of dimers by photolyase enhances their removal by the excision repair pathway. In contrast, expression of Phr1 in *E. coli* inhibits excision repair; *in vitro* studies indicate that this is due to inhibition of the incision step catalyzed by ABC excision nuclease.

E 251 VARIATIONS IN dNTP POOLS IN MUTAGEN SENSITIVE AND WILD TYPE *NEUROSPORA CRASSA* STRAINS, Alice L. Schroeder and Vinod Srivastava, Washington State University, Pullman, WA 99164.

A precise assay for deoxyribonucleoside triphosphate [dNTP] pools in the fungus *Neurospora crassa* has been developed. After extraction in cold TCA-TOA in freon, ribonucleotides are eliminated by chromatography on boronate containing Biorad Affi-gel 601. Remaining ribonucleotides are destroyed by periodate-oxidation and extracts with +/- 50 pmols of each dNTP are subjected to HPLC on a Sax 10 anion exchange column in an ammonium phosphate-KCl gradient. In exponentially growing cells picograms dNTPs/μg DNA are: 10.6±0.36 dTTP; 8.90±0.80 dCTP; 7.39±0.68 dATP; and 5.16±0.33 dGTP. Stationary phase cells show about one-half these concentrations and hydroxyurea, a ribonucleoside reductase inhibitor, reduces the dC, G, and A pools by 40 to 60%, as in mammalian cells. Conidia (vegetative spores) have levels of dNTPs at least 20 fold lower. Pools are normal during log phase in *uvs-6*, a mutagen-sensitive mutant, but in stationary phase dT, C and A levels are slightly high while the dGTP level is low. Since dNTP ratios control *de novo* dNTP synthesis and perturbations lead to chromosome damage, we are measuring dNTP pools both in mutagen-sensitive mutants and after histidine treatment, which induces chromosomal damage in *Neurospora*.

E 252 SEQUENCE SPECIFIC REPAIR OF DNA DAMAGED BY METHYLATING AGENTS

D. A. Scicchitano, I. Mellon, C. A. Smith and P. C. Hanawalt, Stanford University, Stanford, CA 94305.

Methylating agents produce numerous types of deleterious lesions in DNA. The aberrant bases 3-methyladenine and 7-methylguanine, which constitute the major amount of damage, are removed by 3-methyladenine-DNA glycosylase, leaving an apurinic (AP) site in the DNA. Endonucleolytic incision at the AP site followed by repair replication and ligation completes the repair process. There is increasing evidence that AP sites in DNA may be promutagenic, indicating the importance of their repair to ensure fidelity of replication. It is, therefore, of some interest to learn whether repair of these sites differs throughout the genome. To investigate the differential repair of methylated bases, we have developed a quantitative method for examining their removal from specific genes analogous to that devised by Bohr et al. for pyrimidine dimers (*Cell*, 40: 359 (1985)). The procedure involves isolating parental DNA from cells damaged with dimethyl sulfate, treating it with an appropriate restriction enzyme, and heating it to release all remaining N-methylpurines. During the heating process, one portion in each set is heated with methoxyamine, an agent which reduces AP sites and protects them from subsequent alkaline degradation. Following alkaline hydrolysis, electrophoresis, transfer to a nylon membrane, and probing for the fragment of interest, the ratios of the band intensities of the DNA sample not treated with methoxyamine to its methoxyamine-treated counterpart are calculated to give the percentage of the restriction fragments containing no alkaline labile sites. From this information and the Poisson distribution, the frequency of N-methylpurines is determined for assessment of their removal. Currently, we are investigating the removal of N-methylpurines from specific genes in order to ascertain whether differential rates of repair occur in active genes relative to those in silent domains of the genome. (Supported by a grant from the N.I.G.M.S. to P.C.H. and an N.I.H. postdoctoral traineeship to D.A.S.)

E 253 PROTEOLYTIC CLEAVAGE OF ADA REGULATORY PROTEIN OF ESCHERICHIA COLI. T. Yoshikai, Y. Nakabeppu and M. Sekiguchi, Department of Biochemistry, Faculty of Medicine, Kyushu University 60, Fukuoka 812, Japan

Ada protein plays a central role in adaptive response of Escherichia coli in response to alkylating agents. It possesses two distinct methyltransferase activities and a transcriptional regulator activity. The protein is cleaved to smaller polypeptides by a cellular protease(s), and the process might be related to regulation of the adaptive response. The enzyme to cleave Ada protein was purified to a considerable extent. When various proteins were incubated with the enzyme, only Ada protein was cleaved. The 39-kDa Ada protein was cleaved to the 20-kDa and the 19-kDa polypeptides, each carrying methylphosphotriester methyltransferase and O^6-methylguanine methyltransferase activities, respectively. The cleavage products promoted transcription of the alkA but not of the ada gene. Based on these observations, biological significance of the proteolytic cleavage of Ada protein will be discussed.

E 254 A ROLE FOR DNA TOPOISOMERASES IN THE ACTIVE DISSOCIATION OF LIGANDS FROM THE MINOR-GROOVE OF CELLULAR DNA, Paul J. Smith, Paul G. Debenham and James V. Watson, MRC Clinical Oncology Unit, MRC Centre, Cambridge UK.

The bis-benzimidazole dyes (eg Hoechst 33258 and 33342) are DNA-specific agents which demonstrate considerable fluorescence enhancement upon binding to the minor-groove of DNA, such that dye-DNA binding and dissociation can be followed in single cells by flow cytometry. Minor-groove ligands can distort the inter-relationship of DNA with nucleosomal core particles and we have hypothesized that in an intact cell that resolution of such torsional stress could involve the action of cellular topoisomerases. We have used flow cytometry to study the effects of various inhibitors (including topoisomerase interactive drugs) on the responses of a mutant murine cell line to Hoechst 33342. The mutant shows Hoechst 33342-cytoxicity resistance due to an enhanced (10-fold) capacity to dissociate nuclear DNA-dye complexes. Dye-DNA dissociation was found to be energy dependent but not affected by the multi-drug resistance modifier verapamil or inhibitors of DNA synthesis. The type II topoisomerase inhibitors novobiocin, VP16, nalidixic acid and the type I inhibitor camptothecin blocked ligand-DNA dissociation to various extents. Ho33342 itself was found to inhibit DNA topoisomerase I activity *in vitro*. Enzyme activity measurements on cell extracts and the sensitivity of the mutant to topoisomerase-related drug induced DNA damage suggest that the mutant is not altered in cellular topoisomerase activities *per se*. We conclude that efficient topoisomerase activity is required for the operation of an active cellular process for the ejection of non-covalently bound ligands from the minor groove of DNA.

E 255 TWELVE GENES OF THE ALKYLATION EXCISION REPAIR PATHWAY OF DROSOPHILA MELANOGASTER, P. Dennis Smith and Ruth Dusenbery, Southern Methodist University, Dallas TX 75275.

Exposure of DNA to alkylating agents *in vitro* or *in vivo* can cause alkyl substitution reactions at 15 different nucleophilic base sites. Depending upon the nature of the substitution, these DNA lesions may alter base coding properties or interrupt DNA transactions, leading to mutagenic or cytotoxic consequences. Removal and replacement of the damaged base appears to constitute the major cellular pathway which acts to protect the genomes of both eukaryotes and prokaryotes. Genetic and molecular genetic characterization of these "base excision repair pathways" is an important aspect of DNA repair mechanisms.

29 genes of *Drosophila* have been identified by mutational analysis which confer increased cytotoxicity upon *in vivo* exposure to alkylating agents. These *mus* mutants are being analyzed for defects in alkylation excision repair. The inability to remove base alkylation lesions by the excision repair pathway has been shown with a number of organisms to result in enhanced mutation rates induced by alkylating agents. This "hypermutable phenotype" appears to be a reliable indicator of excision deficiency. From genetic studies, 12 of the 29 *mus* mutants have exhibited the "hypermutable phenotype" in response to monofunctional alkylating agents, from a sampling of 16 of the *mus* mutants. To date, the *mei-9*, *mus101*, *mus103*, *mus106*, *mus201*, *mus204*, *mus205*, *mus206*, *mus207*, *mus306*, *mus308* and *mus310* genes have been shown to confer the "hypermutable phenotype." We hypothesize that these 12 genes are defective in the excision repair of alkylation damage in genomic DNA. UDS analysis of 6 of these *mus* mutants demonstrates that primary embryonic cells cannot perform some repair step, up to or including,the resynthesis step in the excision repair process. These 12 genes are essential for excision repair in Dros.

E 256 SELECTIVE METHYLATION OF THE A:C MISPAIR BY THE HUMAN DNA (CYTOSINE-5) METHYLTRANSFERASE Steven S. Smith, David J. Baker, Lori Jardines, and Thomas A. Hardy Division of Surgery, City of Hope, Duarte, CA 91010

We have used the dA:dC mispair to study the response of the DNA methyltransferase to anomalies in the secondary structure of duplex DNA. When this mispair is the second base pair in what would otherwise be a recognition sequence for the *MspI* or *HpaII* restriction endonucleases, it is not recognized by these enzymes. In contrast, the mispair actually enhances the rate of methylation by the human DNA methyltransferase by about ten fold over the rate observed with the complementary sequence. The cytosine in the mispair is specifically methylated regardless of orientation. A $5^{m}C$ residue in place of the the central cytosine moiety on either strand in the complementary sequence enhanced the reaction rate by almost fifty fold. A $5^{m}C$ residue in place of the central cytosine opposite the cytosine in the mispair enhanced the reaction rate by more than 100 fold. A $5^{m}C$ residue in the mispair itself blocks methylation. Restriction analysis of the ^{3}H reaction products was used to assign preferred sites of methylation in these structures. Computer generated models of the structures based on published x-ray diffraction and NMR studies of the dA:dC mispair define structural features in the mispair that may predispose it to attack by the enzyme. The information that can be applied by the enzyme to structurally anomalous regions of DNA could be used by mammalian cells for several purposes.

E 257 **CHROMATIN STRUCTURE AND DNA REPAIR IN POLYAMINE-DEPLETED HUMAN CELLS**, Ronald D. Snyder, Merrell Dow Research Institute, Cincinnati, Ohio 45215

Mammalian cells depleted of polyamines by treatment with α-difluoromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG) or a combinaton of the two, display altered sensitivity to micrococcal nuclease, DNAse I and DNAse II. Released chromatin digestion products differ from those in non-depleted controls not only in extent of digestion, but also in proportion of DNA in mononucleosomes and in the Mg^{++}-soluble compartment. These chromatin alterations can not be accounted for by cell cycle changes and can be abolished by brief treatment of cells with polyamines. In addition, polyamine-depleted cells display a marked deficiency in their ability to seal X-ray-induced DNA strand breaks and those breaks resulting from the repair of ultraviolet irradiation and alkylating agent treatment. This inhibition of repair is also circumvented by polyamine addition. The mechanism of this inhibition of repair will be discussed. Depressed clonogenicity relative to controls is observed in polyamine-depleted cells following X-ray- or hyperthermia plus X-ray-treatment but to a much lesser degree following alkylation or UV-irradiation. These studies demonstrate a role for cellular polyamines in maintenance of chromatin structure and in the DNA repair process in human cells.

E 258 SELECTIVE STRAND REPAIR IN A CHO CELL LINE EXPRESSING THE HUMAN *ERCC-1* GENE G. Spivak, I. Mellon, and P.C. Hanawalt, Dept. of Bio. Sci., Stanford Univ. Stanford, CA 94305-5020

Rodent cells in culture exhibit high UV resistance but remove only a small fraction of the cyclobutane pyrimidine dimers from their total DNA. It has been suggested that their UV resistance is due to preferential repair of active genes (Bohr et al, *Cell 40*: 359-369, 1985). We have found that only the transcribed strand of the DHFR gene is efficiently repaired in CHO cells (*Cell 51*, in press). Little repair is detected in the nontranscribed strand even 24 hours after UV irradiation. Both strands of the DHFR gene are repaired in human cells but the transcribed strand is repaired more rapidly than the nontranscribed strand. Thus, differential strand repair is manifested in CHO cells as a difference in extent and in human cells as a difference in rate of dimer removal.

CHO lines of five complementation groups have been isolated that are hypersensitive to UV light. The UV sensitivity of a mutant belonging to complementation group II has been partially corrected by transfection with a human DNA sequence that contains a human excision-repair gene, *ERCC-1* (van Duin et al, *Cell 44*: 913-923, 1986). We examined this transformant CHO line to see if selective strand repair occurs, and if so, whether it resembles that in UV resistant CHO cells or that in human cells. We find that the transcribed strand of the DHFR gene is efficiently repaired in the *ERCC-1* containing CHO cells while little repair is detected in the nontranscribed strand. Little repair is found in either strand of the DHFR gene in the UV sensitive mutant CHO cell line. We conclude that the presence of the human *ERCC-1* gene in the UV sensitive cell line restores selective strand repair characteristic of the parental UV resistant CHO cell line. We are extending this type of analysis to other sets of UV resistant CHO cells, their UV sensitive derivatives that belong to different complementation groups, and the corresponding transformants containing DNA sequences that restore their UV resistance. This work is supported by a grant from N.C.I. and an A.C.S. postdoctoral fellowship to I.M.

E 259 A GENERAL METHOD FOR QUANTITATING ADDUCTS IN SPECIFIC MAMMALIAN GENOMIC SEQUENCES USING ABC EXCISION NUCLEASE, David C. Thomas, Andrew Morton, Vilhelm Bohr and Aziz Sancar, University of North Carolina School of Medicine at Chapel Hill, Chapel Hill, NC 27599 and National Cancer Institute, NIH, Bethesda, MD 20892.

We have recently developed a method to quantitate adducts at the level of specific single-copy mammalian genes. Damaged DNA is first digested with a restriction enzyme and then ABC excision nuclease, separated on an alkaline agarose gel and probed by the method of Southern for a specific gene sequence. Because ABC excision nuclease removes DNA adducts from the gene of interest and thereby fragments it, the result is a decrease in the intensity of the full length signal, from which the number of adducts in the gene is calculated. We have demonstrated that this assay can be used to quantitate adducts induced by UV, psoralen, *cis*-platin, and 4-nitroquinoline-1-oxide in the human *ras* gene. Adducts in fragments as small as 2 kilobases and as large as 20 kilobases can be successfully quantitated, and as little as 1 μg of genomic DNA can be used for measuring damage and repair in single-copy genes. Because ABC excision nuclease recognizes a wide range of DNA lesions, this system has the potential to measure virtually any type of damage in any sequence of interest from various tissues, and thus screen putative carcinogens and chemotherapeutic compounds. As one example, we are currently using this assay to study the mechanism of drug resistance in mouse L1210 cell lines.

E 260 EFFECTS OF CELLULAR DIFFERENTIATION ON DNA REPAIR, P.J. Tofilon and R.E. Meyn, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
It has been suggested that terminal differentiation results in a decrease in the ability to repair DNA damage. To investigate this hypothesis, we have examined the repair of the DNA damage induced by ionizing and UV radiation in the murine proadipocyte stem cell line 3T3-T, which forms terminally differentiated (TD) adipocytes upon exposure to medium containing 25% human plasma. γ-ray-induced DNA single strand breaks (SSBs) and their repair were evaluated in stem and TD cells using the alkaline elution assay. No difference was detected between cell types in the initial level of SSBs induced by γ rays. At each repair time point examined after 4 Gy, however, the percentage of SSBs remaining unrejoined was 4-6 times greater in TD cells than in stem cells. These data indicate that the ability to repair DNA damage induced by ionizing radiation is compromised in TD 3T3-T cells. In response to UV irradiation, the ability to perform unscheduled DNA synthesis was significantly reduced in TD cells relative to the stem cells. The induction of incision events as a function of time after UV irradiation was determined using the alkaline elution assay. In stem cells, a slight degree of incision was detected at 4 min after UV with essentially no further increase at longer times. TD cells, however, exhibited a significantly larger degree of incision at 4 min; incision events continued to accumulate in a linear manner out to at least 16 min after UV irradiation. These results indicate that TD cells are also deficient in their ability to repair the DNA damage induced by UV radiation and suggest that this deficiency is in a postincision step of the excision-repair process.

E 261 REPAIR OF SPECIFIC GENES IN HUMAN CELLS. Albert A. van Zeeland*, Lynne Mayne+ and Leon H.F. Mullenders*. *State University of Leiden, Leiden, The Netherlands and +Sussex Medical Research Center, Sussex, UK. A likely explanation for the preferential repair of nuclear matrix associated DNA (Mullenders et al, 1987) and the rapid resumption of UV-inhibited RNA synthesis in human cells (Mayne et al, 1982) is the existence of a repair pathway which selectively removes damage from transcribing DNA. To study the repair of active and inactive DNA sequences in more detail, we measured the removal of pyrimidine dimers in defined DNA fragments as described by Bohr et. al. (1985). The induction and removal of UV-induced dimers (sites sensitive to T4 endonuclease V) was analysed in a restriction fragment of the active adenosine deaminase gene (ADA, 18.5 kb) and the inactive 754 locus (14 kb) which is located at the X-chromosome. Induction and removal of pyrimidine dimers from the genome overall was determined by alkaline sucrose gradient centrifugation. Quantification of pyrimidine dimers at various doses showed no differences in the number of these lesions induced in the ADA fragment when compared to the genome overall. In primairy human fibroblasts exposed to 10 J/m^2 the removal of pyrimidine dimers from the ADA fragment was more efficient then from the genome overall or the 754 fragment. After 8 hours of post-UV incubation about 60% of the pyrimidine dimers were removed from the ADA gene compared to about 30% from the genome overall or the 754 fragment. The removal of pyrimidine dimers from the 754 locus was similar to the genome overall measured over a period of 4, 8 and 24 hours. In immortalized Cockayne's syndrome cells exposed to 15 J/m^2 about 20% of the pyrimidine dimers in the ADA fragment was removed after 8 hours compared to 50% in normal cells. The results of a single experiment with confluent xeroderma pigmentosum (group C) cells exposed to 10 J/m^2 indicated that these cells can remove pyrimidine dimers from the ADA gene to a considerable extent. The efficient repair of active DNA in normal cells and its absence in CS cells correlates with ability (normal cells) and inability (CS cells) to recover UV-inhibited RNA synthesis.

E 262 SISTER CHROMATID EXCHANGE FORMATION DOES NOT CORRELATE WITH CLEAVABLE COMPLEX FORMATION BY TOPOISOMERASE II, Raymond L. Warters[1] and William F. Morgan[2], [1]University of Utah,Salt Lake City,UT 84132 and[2]University of California, San Francisco, CA 94143.
A relationship has been proposed between the formation of a "cleavable complex" between topoisomerase II and DNA, and the induction of sister chromatid exchanges (SCE). Cleavable complex formation in the presence of the intercalator m-AMSA can be estimated by determining the frequency of protein-associated, DNA strand breaks detectable at pH 12.2 in the presence of proteinase K. The formation of cleavable complexes was reduced by a factor of 0.5 in Chinese hamster ovary cells exposed to 15 mM caffeine prior to m-AMSA. In m-AMSA exposed cells the frequency of existing cleavable complexes was reduced by a factor of 0.5 (i.e., complex reversal) by addition of 15 mM caffeine. The frequency of SCE per chromosome in control cells or cells exposed to 15 mM caffeine, to 0.1 ug/ml m-AMSA or to 15 mM caffeine 60 min prior to 0.1 ug/ml m-AMSA was 0.43, 0.61, 1.11 and 1.25, respectively, indicating that the two drugs interact independently and additively to produce SCE. Thus SCE induction in response to m-AMSA exposure does not appear to be a function solely of the frequency with which cleavable complexes are formed between DNA and topoisomerase enzymes. (Supported by NIH grant CA 25957 and the US Department of Energy).

E 263 Molecular cloning of part of the human excision repair gene ERCC-3.

*G. Weeda, *R.C.A. van Ham, *R. Masurel, J.H.J. Hoeijmakers, A. Westerveld, D. Bootsma and *A.J. van der Eb.

*Dept. of Medical Biochemistry, State University of Leiden, The Netherlands, Dept. of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

The UV-sensitive Chinese hamster (CHO) mutant 27-1 (isolated and generously provided by R. Wood) is defective in excision repair and belongs to complementation group 3 of UV-sensitive CHO mutants (Thompson et al. PNAS 78, 3734). In order to clone the human gene correcting the defect of this mutant, cultures were co-transfected with HeLa DNA and plasmid pSV3GPT, encoding the bacterial xanthine-guanine-phosphoribosyl transferase. Repair-proficient transformants were found to exhibit UV-survival and UDS levels close to wild type and had integrated considerable amounts of human DNA. DNAs extracted from each transfectant were used for secondary transfection into 27-1 cells. One secondary transformant (B2-56) was obtained by linked transfer of two marker copies and the presumed repair gene, 7 others by addition of free gpt copies. All secondary transformants displayed UV-survival and UDS levels in the wild-type range. Southern blot analysis revealed that they had several human repeat-containing fragments in common. Analysis of gpt marker-containing cosmids of B2-56 indicated that the human fragments that also occurred in other transformants were not within 30 kb of the gpt copies. Therefore, a lambda library was constructed from which a 4.3-kb common human EcoRI fragment was isolated. Hybridization with unique probes from this fragment confirmed its human origin and revealed that it was present in all of the 6 independently isolated primary and 4 of the 8 secondary transformants, indicating that it is situated close to, but not within the ERCC-3 repair gene. Hybridization to a rodent/human hybrid panel showed that this fragment mapped on chromosome 2, in agreement with the previous assignment of ERRC-3 (Siciliano et al. HGM 9, in press). A cosmid clone of B2-56 DNA containing the 4.3-kb EcoRI fragment and flanking sequences, presently under analysis, should contain at least part of the ERCC-3 gene.

E 264 VECTOR CONSTRUCTIONS FOR THE ANANLYSIS OF DNA MISMATCH REPAIR IN MAMMALIAN CELLS. Gerhild Wildner and Hans-Joachim Fritz, Max-Planck-Institut für Biochemie, Abteilung Zellbiologie, D-8033 Martinsried bei München, F.R.G.

In recent years, we have studied DNA mismatch repair in E.coli by means of a genetic assay which rests on transformation by heteroduplex DNA molecules constructed *in vitro* such that structurally predetermined base/ base mismatches are formed (Kramer et al., 1984, Cell 38; Zell and Fritz, 1987, The EMBO Journal 6). A prerequisite for similar studies on the repair properties of higher cells is the availability of host/vector combinations which have to meet the following criteria:

1. Efficient physical DNA tranfer into the recipient cell followed by genetic transformation;
2. Easily detectable phenotypes to follow the intracellular fate of the transforming heteroduplex DNA by genetic methods;
3. Optional rescue of the vector from the transformed cell for DNA sequence analysis;
4. Proliferation of the vector in E.coli and facile construction of heteroduplex DNA molecules.

Presently we construct and test various such cloning vectors the starting point being the pOPF-cosmid of Grosveld et al.(1982, Nucl.Acids Res. 10) and the *neo*-gene from transposon Tn 5.

E 265 EVIDENCE FOR A ROLE FOR DNA METHYLATION IN MISMATCH CORRECTION IN GENOMIC DNA IN MAMMALIAN CELLS, William P. Diver, P.J. Crowther and David M. Woodcock, Peter MacCallum Cancer Institute, Melbourne, Victoria 3000, Australia.

Hare and Taylor have produced evidence for hemimethylated sites directing mismatch repair in extrachromosomal SV-40 DNA in mammalian cells. We have used a series of cell subclones derived from Chinese hamster ovary CHO-K1 cells with stably altered genomic methylation levels (generated by 5-aza-2'-deoxycytidine treatment) to examine the role of cytosine methylation in mismatch correction in the genomic DNA. We have two lines of evidence which implicate DNA methylation in correction of mismatches. The first is the relationship between total genomic methylation levels and the toxicity of 2-aminopurine (2AP). It is well documented the 2AP incorporated into DNA results in base pair mismatching. We have found that cell killing by 2AP is inversely proportional to the overall genomic level of cytosine methylation in this series of mammalian cell subclones. This toxicity is unrelated to any secondary effects or nucleotide pool sizes in that, while coadministration of deoxycytidine reverses the 2AP depression of DNA synthesis, it has no effect on 2AP toxicity as measured by colony formation. Secondly, we have examined the spontaneous mutation rate in a specific cellular gene, HPRT. Some of these clones (which have lost at most 1/3 to 1/4 of their total genomic methylation) show frequencies of spontaneous mutations elevated more than 5 to 6 fold over that of the cell clone from which they were derived. Current work involves a correlation between sites of methylation in the HPRT gene region in the different cell subclones and the spontaneous mutation rate.

E 266 THE REPAIR OF DNA ADDUCTS AT THE C8 OR N2 OF GUANINE IN MAMMALIAN CELLS, [1]R. Waters, [1]C. Jones, [1]A-L Yang, [2]J. W. I. M. Simons and [2]M. Z. Zdienicka, [1]University College of Swansea, Swansea, UK and [2]Leiden University, Leiden, The Netherlands. The repair of 4NQO induced adducts at the C8 or N2 of guanine and the repair of 3me4NQO adducts which occur uniquely at the C8 of guanine has been investigated in a range of transformed XP fibroblasts from different complementation groups, and in Chinese hamster mutants sensitive to 4NQO but not UV. The data indicate some gene products are required for UV and 4NQO repair, whereas other gene products are required for 4NQO repair alone. Furthermore some different gene products are required for the repair of C8 guanine versus N2 guanine 4NQO adducts. XPA cells cannot remove 4NQO adducts at N2 guanine, but can remove 4NQO and 3me4NQO adducts at C8 guanine. Contrarily XPG and XPD appear to be defective in the repair of both types of adducts (i.e. N2G and C8G). The 4NQO and 3me4NQO sensitive Chinese hamster cell line VH4 cannot repair C8G adducts, but can repair UV damage as normal. Some repair of N2 adducts occurs in this line, but this appears to be slower than normal. These results therefore clearly indicate that some mammalian gene products are required for the repair of adducts at both the N2 and C8 of guanine, whereas others are required for the repair of N2 guanine adducts alone. Finally some gene products involved in large adduct repair are clearly not required for the repair of UV induced DNA damage.

Repair Genes, Genetic Control of Repair, Intragenomic and Structural Effects - II

E 300 STIMULATION OF p53 CELLULAR TUMOR ANTIGEN AFTER UV-TREATMENT OF NORMAL HUMAN AND CERTAIN XERODERMA PIGMENTOSUM CELLS.

Peter J. Abrahams, Henk J.M. Rosdorff, Ron Schouten, Arno A.M. van der Kleij and Alex J. van der Eb. Department of Biochemistry, State University of Leiden, The Netherlands.

We have investigated the occurrence of SOS-phenomena such as Enhanced Reactivation (ER) and Enhanced Mutagenesis (EM) of Herpes Simplex Virus type 1 (HSV-1) after UV-treatment of normal human and Xeroderma Pigmentosum (XP) skin fibroblasts. ER and EM followed similar kinetics in normal and in XP cells from complementation groups A,C and D ($XPER^+$). Maximum activities occured when infection with HSV-1 was delayed 1-2 days after UV-treatment. However, certain XP strains did not express an ER phenomenon, whereas the EM response was normal. Interestingly, these latter XP cells ($XPER^-$) originated from patients that were reportedly (still) free from cancer in sunlight-exposed skin areas.
These results suggest that the ER response possitively correlates with cancer induction.

In order,to characterize the $XPER^+$ and $XPER^-$ cells, we studied the stimulation of the p53 cellular tumor antigen after UV-exposure of normal human and XP cells. In UV-treated normal ER^+ and $XPER^+$ cells we observed a considerable stabilisation of p53 protein compared to untreated controls. In contrast, no stabilisation of p53 cellular antigen was detected in UV-exposed $XPER^-$ cells, indicating that the p53 cellular oncogen could be involved in expression of some of the SOS-phenomena in human cells.

E 301 EXPRESSION OF THE ada AND alkA GENES OF ESCHERICHIA COLI IN RESPONSE TO ALKYLATING AGENTS: INDENTIFICATION OF TRANSCRIPTIONAL REGULATORY ELEMENTS,

Motoaki Anai, Takanori Nakamura, Yu Chen-Guo, Yusaku Nakabeppu and Mutsuo Sekiguchi, Kyushu University, Fukuoka 812, Japan.

Ada protein plays a central role in the regulatory synthesis of DNA repair enzymes on exposure of *E. coli* to alkylating agents. Methyl groups of alkylated DNA are transferred to Ada protein by its own methyltransferase activity and the methylated Ada protein then acts on the ada and the alkA promoter regions to overproduce the respective gene products. To elucidate the regulatory mechanisms for expression of the ada and the alkA genes, we analyzed the two promoter regions by site-directed mutagenesis. Series of deletion analysis revealed that sequences up to 53 and 48 nucleotides upstream from the transcription initiation sites are required for the controlled expression of the ada and the alkA genes, respectively. Libraries of point substitution mutations in the two regulatory elements were constructed with the use of synthetic oligonucleotides. By using these mutants the -10 and the -35 boxes of the two promoters as well as the ada and the alkA regulatory sequences were identified. There are some differences between the ada and the alkA regulatory elements, probably reflecting the fact that expression of the two genes is controlled by Ada protein in slightly different manner.

E 302 HOST-CELL REACTIVATION OF A UV-DAMAGED PLASMID EXPRESSION VECTOR IN XERODERMA PIGMENTOSUM AND NORMAL HUMAN LYMPHOCYTES

William F. Atnas and Lawrence Grossman, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205.

Cells from patients with the cancer-prone genetic disorder xeroderma pigmentosum (XP) are defective in the repair of UV-damaged DNA. Recently, this DNA repair defect has been measured by demonstrating a reduced capacity of XP fibroblasts to reactivate the expression of a transfected UV-treated gene coding for chloramphenicol acetyltransferase (CAT) [Protic-Sabljic, M. and Kraemer, K.H., Proc. Natl. Acad. Sci., **82**, 6622-6626, 1985.] With the intent of applying this methodology in population-based studies of DNA repair proficiency and cancer susceptibility, we have developed a similar plasmid host-cell reactivation assay of DNA repair in human lymphocytes. The assay utilizes the plasmid vector pCMVcat, which permits quantitative expression of CAT enzyme activity in transfected lymphocytes. Gene expression is inhibited by inducing inactivating photoproducts with 254 nm UV-light, and the irradiated plasmid DNA subsequently introduced into lymphocytes by DEAE-dextran mediated transfection. Following a 40-hour post-transfection expression/repair period, cells are harvested and cell-free extracts assayed for CAT activity. DNA repair capacity is then expressed in terms of the percentage CAT activity present in cultures receiving damaged plasmid relative to parallel cultures receiving non-damaged plasmid.

Studies with Epstein-Barr virus-transformed lymphoblastoid cell lines indicate the following: (i) an inverse dose-response relationship between input UV fluence and percent CAT reactivated; (ii) a markedly reduced capacity for XP cell lines to reactivate UV-inhibited CAT expression; (iii) a differential capacity between various XP complementation groups to reactivate CAT expression (i.e. A=D<B<C).

E 303 **COMPLEMENTATION OF DNA REPAIR DEFICIENCY IN CHINESE HAMSTER AND HUMAN CELLS BY CHROMOSOME TRANSFER** R.S. Athwal, G.P. Kaur and P. Shashidharan, Department of Microbiology and Molecular Genetics, New Jersey Medical School, Newark, New Jersey 07103

Cells from xeroderma pigmentosum (XP) patients of complementation group A and Chinese hamster repair deficient mutant UV24 were used as recipients to transfer individual human chromosomes. The protocol used in these experiments involved the integration of a dominant selectable marker, Ecogpt, into human chromosomes by gene transfer methods. The marked chromsomes were then transferred to mouse cells by microcell fusion to produce mouse/human hybrids each containing a single human chromosome (Athwal et al., 1985, Somatic Cell and Mol. Genet. 11:177). Monochromosomal hybrids were then used as donors to transfer, preidentified human chromosomes to XP and UV24 cells. Microcells prepared from the monochromosomal hybrids were fused with the recipient cells and the resultant hybrids were isolated by selection in medium containing mycophenolic acid and xanthine. The microcell hybrids with UV24 and XP cells were analysed for complementation of the repair defect by sensitivity to UV-irradiation and unscheduled DNA synthesis. The result of these experiments show that the gene complementing the repair defect in UV24 cells is present on the long arm of human chromosome 2. We have transferred six different chromosomes to XP cells but no definitive human chromosome complementing the repair defect has yet been identified. These experiments show the feasibility of our approach of chromosome transfer to identify the gene(s) responsible for DNA repair in mammalian cells. The data on DNA transfection and isolation of repair gene(s) will also be presented.

E 304 INTERNATIONAL FANCONI ANEMIA REGISTRY (IFAR): FIVE YEAR REPORT, Arleen D. Auerbach, André Rogatko and Traute M. Schroeder, The Rockefeller Univ. and Memorial Sloan-Kettering Cancer Center, New York, NY and Univ. of Heidelberg, FRG.

In 1982, the IFAR was established at The Rockefeller University as a central repository for clinical, hematologic and genetic information on patients with Fanconi anemia (FA). Our current database consists of 310 patients who have been tested for sensitivity of peripheral blood lymphocytes to the clastogenic effect of DNA crosslinking agents such as diepoxybutane (DEB). To test the clinical basis of the classification of patients by the DEB sensitivity test, a stepwise multivariate logistic regression was applied. The dependent variable was group (DEB^+) or (DEB^-), and the predictors were the hematologic manifestations and congenital abnormalities scored. Results indicate that the DEB^+ and DEB^- patients belong to discrete groups and should be considered as distinct clinical entities. For purposes of the registry, these groups are designated as FA (202 patients) and non-FA (108 patients). Our data indicate that the clinical diversity in FA is much more widespread than previously recognized. An analysis of the multiplex sibships in the registry shows a high heritability for the age of onset of hematologic manifestations but not for the number of congenital abnormalities present. We are currently attempting to map the FA gene by genetic linkage to anonymous DNA markers.

E 305 EFFECTS OF UV IRRADIATION ON CULTURED HUMAN KERATINOCYTES. Claude Backendorf, Susan Gibbs, Wilma Teubel, Peter Belt and Pieter van de Putte, University of Leiden, 2333 AL Leiden, NL

Epidermal keratinocytes constitute the outermost layer of human skin and play an important role in the protection of the organism against external agents such as carcinogens and radiation. In vitro human keratinocytes can be cultured on 3T3 feeder layers where they actively divide,undergo terminal differentiation and form cellular multilayers reminescent of the in vivo situation. Hence primary cultures of human keratinocytes constitute an attractive model to study the effects of UV irradiation on human cells. During the last years differential screening of cDNA libraries has been used to identify genes whose expression is modulated following the exposure of epidermal keratinocytes to short wave UV. A special emphasis has been put into the study of those genes where the relative amount of polyA RNA increases after UV irradiation. The contribution in this process of transcriptional activation,differential repair of UV induced lesions or mRNA stability will be discussed.

E 306 CHARACTERIZATION OF THE FAR-5' REARRANGEMENT ASSOCIATED WITH CARCINOGEN-INDUCED ACTIVATION OF THE HAMSTER THYMIDINE KINASE GENE, Frederic G. Barr*, Sridharan Rajagopalan, and Michael W. Lieberman, Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111.

We have previously shown that the chemical carcinogen N-methyl-N'-nitro-N-nitroso-guanidine can activate a quiescent thymidine kinase (TK) gene and that 20% of the TK^+ variants have a rearrangement in the far-5' region of the TK gene (Barr et al., Mol. Cell. Biol. 6:3023-3033, 1986). To obtain detailed mapping data and hybridization probes for further characterization of these sequence alterations, we cloned the 16 kb Hind III fragment containing the far-5' region of the wild-type hamster TK gene. The 3' boundary of the rearrangement was then mapped in 3 cell lines to the region 6 kb 5' to the origin of transcription and in 1 cell line to the region 8 kb 5' to the origin. Southern blot analysis revealed that, in each cell line, the rearrangement consisted at least partly of a deletion of wild-type sequences 5' to this boundary. By synchronization of cells containing an active, rearranged TK gene, we demonstrated that the rearrangement does not abolish the normal growth-related regulation of TK activity. (Supported by NIH Grant CA39392).

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E 307 ALZHEIMER'S DISEASE PATIENT'S MONOCYTES AND T-LYMPHOCYTES DISPLAY DECREASED DNA REPAIR EFFICIENCIES AFTER EXPOSURE TO ALKYLATING AGENTS. John D. Bartlett, Jeanette N. Hartshorn and Steven H. Robison, University of Vermont, Burlington, VT 05401.

Our laboratory and others have previously shown that various cells from Alzheimer's disease (AD) patients have increased sensitivity to the alkylating agents methyl methane sulfonate (MMS) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Putative AD patient's leukocytes were subjected to three different DNA repair assays in an effort to further elucidate this generalized DNA alkylation damage repair defficiency. Adherent cells (monocytes) displayed decreased MMS and MNNG induced unscheduled DNA synthesis (UDS) as compared to normals. Nonadherant cells were cultured in the presence of T-cell growth factor (11-14 days) and, after MMS and MNNG exposure, were analyzed for DNA repair by alkaline elution (AE). T-cells were also assayed for O^6-methyl guanine acceptor protein (O^6-MGAP) levels. Analysis of DNA repair by AE after alkylation damage in T-cells from putative AD patients revealed reduced recovery when compared to normals. However, no significant difference between putative AD patients and normals was found for O^6-MGAP levels. These results suggest an ineffeciency in an enzyme common to both UDS and excision repair. Since AD patient's neurons have been shown to contain an altered chromatin structure, perhaps the altered chromatin masks certain DNA lesions which remain undetected or makes them inaccessible to a specific repair enzyme or enzymes. This work was supported by grants from the NIA and the American Federation for Aging Research.

E 308 INCREASED FREQUENCY OF SPONTANEOUS AND BLEOMYCIN-INDUCED MICRONUCLEI IN FIBROBLAST STRAINS FROM TUBEROUS SCLEROSIS PATIENTS, N.T. Bech-Hansen and M. Dodds, University of Calgary, Calgary, Alberta, Canada T2T 5C7.

Tuberous sclerosis (TS) is an autosomal dominant disorder which manifests variably with seizures, mental retardation, of skin lesions and hamartomas of the retina, heart and kidney. Skin fibroblasts from tuberous sclerosis patients show impaired colony-forming ability following gamma irradiation and bleomycin treatment, suggesting that DNA metabolism is defective in TS. We have investigated the in vitro manifestations of the TS mutation(s) by measuring the frequency of both the spontaneous and induced frequency of micronuclei in TS fibroblast strains. Micronuclei formation can be an indication of impaired ability to repair DNA strand breaks, of aberrant centromere division, or of other defects in cell division.

The spontaneous frequency of micronuclei in four TS fibroblast strains, all previously found to be ϑ-ray sensitive, was 1.8 to 4.7 times greater than in the normal control fibroblast line (GM38). Micronuclei frequencies in bleomycin treated (0.5 μg bleomycin/mL for 3 hr and 50.5 hr post-treatment recovery) fibroblasts were 2.1 to 3.7 times greater in TS strains than the control strain GM38. The ratio of the spontaneous frequency of micronuclei and the induced frequency ranged from 3.2 to 5.8 times: the mean ratio was 3.96 and the normal strain showed a ratio of 3.8. The mitotic index in each of the untreated cell strains was within a normal range, 9.5 to 16.3 mitosis per 1250 cell. In bleomycin treated experiments, the mitotic index was reduced in 4 of 5 strains; in the fifth, a TS strain, there was an increase of 63%.

These results indicate that TS fibroblasts are defective in the maintenance of the integrity of the genome both under stressed and unstressed conditions.

E 309 SYNERGISTIC TUMOR CELL KILL AND INHIBITION OF DNA REPAIR BY 2'-DEOXYCOFORMYCIN (dCF) AND DEOXYADENONSINE (dAdo) IN IRRADIATED L5178Y LYMPHOBLASTS IN VITRO, Asher Begleiter, Linda Pugh and James B. Johnston, University of Manitoba, Winnipeg, Canada.
The adenosine deaminase innibitor, dCF, is effective in the treatment of lymphoid malignancies. The lymphocytolysis produced by dCF has been attributed to inhibition of repair of naturally occurring DNA single strand breaks (SSB), as a result of accumulation of dATP. We have studied the effect of dCF on radiation-induced cytotoxicity and on repair of the resulting SSB in log- and plateau-phase murine L5178Y lymphoblasts in vitro. Treatment of plateau-phase cells with 5 μM dCF and 50 μM dAdo for 1 h before irradiation, and 4 h after irradiation, resulted in synergistic (35% > than additive) tumor cell kill. The enhanced cytotoxicity produced by dCF/dAdo was dependent on the concentration of dAdo, but was uneffected by prolonging pre- or post-irradiation drug treatment. Treatment of plateau-phase cells with dCF/dAdo did not effect the formation of SSB by irradiation but produced a 25% inhibition in the extent of repair of this DNA damage and this was associated with an increase in cellular dATP. dATP returned to the pretreatment level 1 h after removal of dCF/dAdo and this was accompanied by additional repair of SSB. Neither synergistic tumor cell kill nor inhibition of repair of DNA SSB were seen in log-phase cells, despite a greater accumulation of dATP compared to plateau-phase cells. These studies indicate that dCF/dAdo can enhance the cytotoxic activity of radiation in plateau-phase L5178Y lymphoblasts in vitro and this effect is associated with inhibition of repair of SSB, perhaps as a result of an increase in cellular dATP. The lack of effect of dCF/dAdo on log-phase cells may be due to higher DNA repair capacity in these cells. (Supported by MRC Canada).

E 310 AN INDUCIBLE RESPONSE TO DNA DAMAGE AND HEAT SHOCK IN HUMAN CELLS, Ruth Ben- Ishai, Ilana Kepten and Rivka Sharon, Technion, Israel Inst. Technology, Haifa, Israel.
DNA damage responsive genes that are also induced by heat shock have been detected in Saccharomyces cerevisiae (1) and Drosophila melangaster (2). We have isolated such a dually regulated human clone by differential hybridization of a cDNA library from UV irradiated transformed Xeroderma pigmentosum group C cells. This cDNA clone hybridized to 1.2kb transcripts which were elevated after UV irradiation, 4-nitroquilonine-1-oxide treatment and heat shock of repair proficient and repair defective human fibroblasts. Enhanced levels of this transcript were, however, not observed after mitomycin C treatment or arrest of DNA synthesis by aphidicolin. Whereas the yeast and drosophila gene are represented in the genome by unique sequences, southern blot analysis showed that the human clone contains a sequence repeated many times in the genome. Partial sequencing of the human clone did not reveal any homology to known sequences including those of the heat shock genes.
A clue as to the possible relation of the induced response to DNA damage and heat shock is indicated by our data that heat shock pretreatment promotes repair capacity.

1) McClanaham, T. and McEntee, K. : Mol. Cell. Biol. 6, 90-96, 1986.
2) Vivino, A.A.; Smith, M.D.; Minton, K.W. : Mol. Cell. Biol. 6, 4767-4769, 1986.

E 311 ISOLATION OF CHINESE HAMSTER OVARY CELL LINES WITH AN INCREASED STABLE RESISTANCE TO ALKYLATING AGENTS. M. Bignami, G.Aquilina, G. Frosina, A.Abbondandolo and E. Dogliotti, Istituto Superiore Sanita', Roma, and Ist. Ricerca sul Cancro, Genova, Italy.

An O^6-methylguanine-DNA methyltransferase (MT)-proficient CHO cell line was obtained by transfection of the original MT-deficient cells with human liver DNA (Ding et al., Mol. Cell. Biol., 1985, 5: 3293-3296). Sequential treatment wth N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) of MT-proficient cells led to the isolation of several cell lines with increased levels of resistance to this agent. MNNG-resistance ($MNNG^r$) was stable upon cultivation in the absence of the selective agent over a period of several months. When two cell lines (clone 13 and clone B) with different levels of $MNNG^r$ (D_{37} of 0.8 and 3.4 μg/ml MNNG, respectively) were assayed for MT activity, a 2-fold increase was found in both cell lines, as compared to the parental cell line (D_{37} of 0.27 μg/ml MNNG).
These results suggest that resistance to alkylating agents is likely to involve O^6-methylguanine repair as well as other unknown cellular functions.

E 312 CLONING OF A DROSOPHILA GENE (mei-9) THAT IS ESSENTIAL FOR EXCISION REPAIR, Robert K. Brodberg, David M. Binninger, Paul V. Harris, Aki Yamamoto, James M. Mason, Department of Genetics, University of California, Davis, CA 95616, and National Insitute of Environmental Health Science, Research Triangle Park, NC 27709. A mutation was introduced in the mei-9 gene under conditions that mobilize transposable elements. In situ hybridization revealed that the mutant harbors a P element in chromosome region 4B, the cytogenetic location of the mei-9 gene. Revertants that have simultaneously lost the P element and recovered mutagen resistance reveal that the P element is responsible for mei9 mutation. A P element probe was employed to recover wild type genomic clones that surround the site of P insertion. Identification of additional restriction fragment length polymorphisms in other mei-9 mutants further localized the mei-9 gene within the cloned region. Northern blotting indicates that a single transcript is associated with the site of P insertion. This information is being employed to explore the structure and function of a gene that is essential for meiotic recombination and DNA repair.

E 313 CHARACTERIZATION OF LIVE AND DEAD SUBPOPULATIONS OF MNNG-TREATED T5-1 CELLS BY FLUORESCENCE ACTIVATED CELL SORTING, Kurt A. Black, Richard D. McFarland and Gary J. Smith, Dept. of Pathology, Univ. of North Carolina, Chapel Hill, NC 27599.
The mechanisms involved in cell death produced by spontaneously reactive methylating agents, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), are poorly understood. In the present study, we used flow cytometric techniques to study the relationship between MNNG-induced cell cycle perturbation and cell death. Logarithmically proliferating T5-1 human lymphoblastoid cells were treated with a dose of MNNG that killed about 50% of the population. Cell viability was quantified by measuring the conversion of fluorescein diacetate to fluorescein, and cell cycle analysis was performed on ethanol-fixed cells which were stained with Hoechst dye 33342. The reduction in cell viability caused by MNNG coincided with the accumulation of cells in the late S phase. Fluorescence activated cell sorting was used to isolate viable and dead subpopulations of MNNG-treated T5-1 cells, and the distribution of cells throughout the cell cycle was determined for each sorted fraction. The subpopulation of dead cells contained primarily cells that possessed a DNA content which was much less than that of cells in the G1 phase and was also enriched with cells that posssessed an S phase DNA content. The viable subpopulation contained relatively few cells that possessed a DNA content less than that of G1 phase cells, and cells from all phases of the cell cycle were well represented in this sorted fraction. These results suggest that the late S phase block produced by MNNG is associated with cell death and that degradation of cellular DNA appears to be related to this process.
(Supported by NIH grants CA24144, ES03813 and ES07017.)

E 314 ENHANCED REACTIVATION OF UV-IRRADIATED SV40 IS DUE TO THE RESTORATION OF VIRAL EARLY GENE EXPRESSION, Thomas C. Brown and Peter A. Cerutti, Swiss Institute for Experimental Cancer Research (ISREC), 1066 Epalinges, Switzerland.
Mammalian cells respond to UV radiation by inducing an increased ability to reactivate UV-damaged virus. We identified the genetic function altered to produce enhanced reactivation by transfecting irradiated and unirradiated CV-1 cells with SV40 DNA containing UV-induced damage in different regions of the viral genome. Lesions in the SV40 regulatory region, early genes region and late genes region inactivate viral DNA by disrupting different sets of genetic functions. We reasoned that enhanced survival would reduce the lethal effect of damage in one of these three functionally distinct regions. We observed enhanced reactivation corresponding to a dose reduction factor of 43% for damage in the early genes region. No enhanced reactivation was observed for damage in either the regulatory or late genes regions. Thus, UV-enhanced reactivation reverses the lethal disruption of an essential function peculiar to the early genes region. This function is almost certainly transcription.

E 315 COMPLEMENTATION OF THE UV SENSITIVE PHENOTYPE OF A XERODERMA PIGMENTOSUM HUMAN CELL LINE BY TRANSFECTION WITH A cDNA CLONE LIBRARY, Dan Canaani, Tal Teitz and Tova Naiman, Department of Biochemistry, Tel Aviv University, Tel Aviv 69978, Israel.

We report the complementation by gene transfer of the UV sensitivity of an XP-C established cell line. A human cDNA clone library constructed in a mammalian expression vector, and itself incorporated in a lambda phage vector, was introduced into the cells as a calcium phosphate precipitate. Following selection to G418 resistance, transformants were selected for UV resistance. Twenty-one cell clones were obtained with UV resistance levels typical of normal human fibroblasts. Upon further propagation in the absence of selection for G418 resistance, about half of the primary transformants remained UV resistant. Secondary transformants were generated by transfection with a partial digest of total chromosomal DNA from one of these stable transformants. This resulted in G418 resistant clones, several of which exhibited UV resistant phenotype. The other primary clones lost UV resistance rapidly when subcultured in the absence of G418. Importantly, several retained UV resistance under G418 selection pressure. The acquisition of UV resistance by secondary transformants derived by transduction of DNA from a former clone, and the linkage between G418 and UV resistances in the second group, strongly suggests that the XP-C transformants acquired UV resistance through DNA-mediated gene transfer and not by reversion.

E 316 OXYGEN EFFECTS ON MAMMALIAN CELLS IN CULTURE, Adriana Cantiello and William G. Thilly, Massachusetts Institute of Technology, Cambridge, MA 02139.

William G. Thilly, Massachusetts Institute of Technology, Cambridge, MA 02139.

Spontaneous mutations at the hypoxanthine, guanine phosphoribosyl-transferase (HPRT) locus in cultured mammalian cells belong to two major categories, large structural alterations and point mutations. However, the underlying causes of these mutations are not well understood.

One contribution to such mutations could come from the DNA damage caused by increased levels of oxygen radicals like superoxide, hydrogen peroxide and hydroxyl radicals. To test this hypothesis we studied the effects of continuous high concentrations of molecular oxygen on survival and mutation in TK6 human lymphoblasts grown in culture.

Lymphoblasts grew with a 16 h doubling time at concentrations between 60-250 uM oxygen. Below and above this range cell growth slows. At 500 and 600 uM oxygen, there is an initial toxic response that disappears almost completely after 6 days of treatment. At 880 uM oxygen (95% oxygen in gas mixture) cells die in a log-linear fashion with time of exposure.

Mutation at the HPRT locus was observed only when toxic effects were evident. After an initial increase in the mutant fraction (1-2 days), the mutation rate returned to control values. This adaptative response is similar to the recovery from toxic effects seen at 500 and 600 uM oxygen but is also evident at 880 uM when no recovery from toxicity is observed.

E 317 GENE TRANSFER AND REVERSION IN XERODERMA PIGMENTOSUM (XP), J.E. Cleaver[1], D. Mitchell[2], L. Lutze[1], A.M. Player[1], L. Vuksanovic[1], and D. Karentz[1], [1]Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA, and [2]University of Texas System Cancer Center, Smithville, TX.

An XP group A cell line has been restored to wild type resistance to ultraviolet (UV) light, by transfer of a hamster chromosomal fragment or by chemically-induced reversion. The XP-hamster hybrids can repair both cyclobutane and (6-4) photoproducts, indicating they carry a hamster homolog of the wild type XP gene. The hamster chromosome fragment is being mapped by field inversion gel electrophoresis using hamster alu probes and a hamster cDNA library. The revertant has the unique property of only repairing (6-4) photoproducts and overproduces several novel proteins. Despite its limited excision capacity, the revertant exhibits approximately normal UV responses to chromosomal damage (survival, SCEs, mutations, DNA and RNA synthesis, and repair replication), but remains sensitive to monofunctional psoralens. The revertant, however, remains as host cell reactivation negative as its parent XP cell line and does not repair damage in either a mutational (pZ189) or a transcriptional (pRSVcat) shuttle vector system, indicating that the shuttle vectors are poor indicators of chromosomal events in this cell line. These studies demonstrate (a) the biological importance of (6-4) photoproducts, (b) that we have successfully corrected the XP defect by chromosome mediated gene transfer and (c) that we may be able to distinguish transfectants from revertants as a necessary step to cloning XP genes. Work supported by the U.S. Department of Energy.

E 318 DNA REPAIR AND DNA PRECURSOR POOLS, Andrew Collins* and David Oates†, *Department of Biochemistry, University of Aberdeen, AB9 1AS, and †Department of Zoology, University of Cambridge, CB2 3EJ, UK.
Hydroxyurea has paradoxical effects on DNA repair. It causes incomplete repair sites to accumulate as DNA breaks, suggesting an interference with DNA resynthesis through the known inhibition by hydroxyurea of ribonucleotide reductase, the enzyme that provides dNTPs. Yet DNA resynthesis is apparently not reduced by hydroxyurea. Hydroxyurea also potentiates the effects of DNA polymerase inhibitors cytosine arabinoside and aphidicolin. By measuring the changes in dNTP concentrations in various cell lines (normal human, transformed human (HeLa) and the permanent hamster line CHO K1) incubated with hydroxyurea, we have attempted to explain the effects of this drug on repair. (i) Hydroxyurea does not significantly alter the concentration of dTTP, so measurements of resynthesis in terms of [^{3}H]dThd incorporation are valid, not being distorted by changes in specific activity of the precursor pool. (ii) Hydroxyurea rapidly depletes the other pools, until the dNTP originally present in smallest amount is exhausted. The reduced availability of precursors easily accounts for the blocking of repair, with resynthesis continuing at a reduced rate and repair sites remaining open for longer than normal. (iii) In human cells, depletion of the dCTP pool reduces competition at the DNA polymerase binding site between dCTP and araCTP or aphidicolin, and this can account for the enhancement of the effects of these inhibitors. In hamster cells, however, the dCTP pool remains at a very high level with hydroxyurea present, and yet hydroxyurea still enhances the effects of the other inhibitors. Alternative explanations for this potentiation must therefore be sought.

E 319 THE NUCLEOTIDE SEQUENCE OF THE GENE FOR EXONUCLEASE III, Richard P. Cunningham*, Susan M. Saporito*, and Brian J. Smith-White†, *SUNY Albany, NY 12222 and †The Upjohn Company, Kalamazoo, MI 49001. The xth gene of Escherichia coli K-12 which encodes exonuclease III has been sequenced. Exonuclease III from a cloned copy of the E. coli K-12 gene has been purified and characterized. The molecular weight, the amino terminal amino acid sequence and the amino acid composition of the polypeptide predicted from the nucleotide sequence are in excellent agreement with the properties determined for the purified enzyme. Inspection of the nucleotide sequence reveals two possible promoters for the gene and a region of dyad symmetry capable of forming a hairpin stem and loop structure characteristic of a rho-independent terminator immediately downstream from the xth gene. The xth promoter was mapped by primer extension of in vivo transcripts. Only one of the two promoters identified by inspection of the nucleotide sequence is utilized under normal growth conditions. A possible role for the use of dual promoters for the regulation of exonuclease III expression will be discussed.

E 320 *TRANSFECTION OF MOUSE DNA CORRECTS THE DEFECT OF FANCONI'S ANEMIA (FA) FIBROBLASTS. STRATEGY FOR CLONING FA CORRECTING DNA SEQUENCES,* *Catherine Diatloff-Zito and Ethel Moustacchi, Institut Curie, Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.*
Fanconi's anemia is a genetic disorder characterized by a specific chromosomal and cellular hypersensitivity to DNA crosslinking agents. Complementation analysis involving somatic hybridization have provided evidence for the presence of 2 genetic complementation groups (group A and group B) (Duckworth-Rysiecki et al., 1985) corresponding to 2 phenotypic classes of FA cells (Moustacchi et al., 1987).
We have shown that FA cells sensitivity to killing by Mitomycin C (MMC) as well as DNA semiconservative synthesis after 8-methoxypsoralen (8-MOP) plus UVA can be corrected by transfection with high molecular weight DNA from normal human cells (Diatloff-Zito et al., 1986).
A strategy aiming at the cloning of DNA sequences complementing the FA defect was devised. It consists of : 1. transfecting FA fibroblasts with calcium phosphate precipitated DNA from mouse cells. 2. selecting the MMC resistant transfectants. 3. preparing a genomic DNA library from FA transfectants in bacteriophage lambda. 4. screening the library with mouse repetitive B1 sequences. 5. selecting hybridizing phages and testing by transfection assay their capacity to correct the FA defect.
The data demonstrated that introduction of heterologous mouse DNA can rescue the cellular FA phenotype on FA cell lines belonging to complementation groups A and B. DNA hybridization studies show that these transformants are transgenic, i.e. they contain foreign mouse DNA integrated in their genome. A genomic library from FA group B transfectants has been constructed in lambda phage. B1 positive recombinants phage are analyzed.

E 321 COMPLEMENTATION OF THE INCISION REPAIR DEFECT IN A GROUP 4 CHO MUTANT WITH HUMAN DNA THAT LACKS ABUNDANT REPETITIVE SEQUENCES, Ann M. Dulhanty and Gordon F. Whitmore, Ontario Cancer Institute, Toronto, Canada

We transfected DNA from the repair proficient human cell line, HeLa, along with the dominant selectable marker, pSV_2gpt, into the repair deficient mutant, UV-41. UV-41, which belongs to complementation group 4 of the incision deficient CHO mutants, is sensitive to UV light and mitomycin C. Eight transfectants, each with the repair proficient phenotype (wild type levels of UV and mitomycin C resistance, and incision proficiency) have been isolated. It was expected that the human repair gene would be identifiable in the hamster genome by detection of human repetitive sequences; however, four of the transfectants contain no detectable human repetitive sequences, while the other four do. We conclude that this human gene contains no highly abundant repetitive sequences. This has forced us to explore alternative strategies for cloning the gene. One possibility is that the gpt gene is closely linked to the repair gene in the transfectant genome. If this is the case, loss of one gene could be accompanied by loss of the other. One transfectant line, S2-25, which lacks repetitive sequences, gave 21 clones which are resistant to 6-thioguanine, demonstrating loss of the gpt gene. Twenty of these 21 clones also lost the repair gene function, when they became thioguanine resistant. The coincident loss of the functional gpt and repair genes argues against S2-25 being a revertant. We are now in the process of cloning the gpt containing fragments from S2-25 that are lost when the cells become thioguanine resistant. All or part of the human repair gene that complements the defect in UV-41 should be contained in some of these fragments.

E 322 TRANSFER OF HUMAN O^6-METHYLGUANINE-DNA METHYLTRANSFERASE GENE TO CHINESE HAMSTER OVARY CELLS. W. C. Dunn, K. Tano+, R. S. Foote+, G. J. Horesovsky+, R. J. Preston, and S. Mitra. Biology Division and Univ. Tenn. Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, TN 37831

Chinese hamster ovary (CHO) cells with no detectable O^6-methylguanine-DNA methyltransferase (MGMT) (< 100 molecules/cell) were transfected by electroporation with 10-20 Kb fragments of human (HeLa) DNA together with pSV2neo DNA plasmid (that confers G418 resistance). Two stable drug-resistant clones, GC-1 and GC-2, were isolated that were also resistant to 2-chloroethyl-N-nitrosourea (CNU). GC-1 is completely resistant to 200 μM CNU and contains ~40,000 MGMT molecules/cell while GC-2 contains ~2000 MGMT molecules/cell and has an LD_{50} of 100 μM CNU. The MGMT levels are stably expressed in these cell lines in the absence of CNU for at least 50 doublings. Both lines have an undetectable amount of chromosomally integrated repetitive human DNA, as checked by Southern blotting with nick-translated total human DNA or AluI repetitive sequence, and only a few copies of neo gene. Current efforts include (a) comparing sensitivities of CHO, GC-1 and GC-2 lines to cell killing and mutation induction by different alkylating agents and (b) cloning of the human MGMT gene into a cosmid vector from GC-1 and GC-2. (Research supported by NCI grant CA 31721 and by U. S. Dept. of Energy contract DE-AC05-840R21400 with Martin Marietta Energy Systems.)

E 323 DNA DAMAGE INDUCIBLE TRANSCRIPTS IN MAMMALIAN CELLS, Albert J. Fornace Jr., Isaac Alamo Jr., and M. Christine Hollander, Radiation Oncology Branch, N.I.H., Bethesda, MD 20892.

While there is good evidence in bacteria and increasing evidence in yeast that DNA damage can induce genes whose products are involved in the cellular responses to such damage, the evidence for a specific DNA damage inducible response in mammalian cells has been unconvincing. Unlike previous investigations, our strategy was based on the assumption that DNA damage inducible transcripts in mammalian cells would have some of the characteristics of the SOS response in bacteria: low abundance, rapid induction of 2-10 fold, and induction specific for DNA damage. Hybridization subtraction at low ratios of RNA:cDNA was used to enrich for the cDNA of nonabundant transcripts induced in Chinese hamster cells by UV radiation. 49 different cDNA clones were isolated which coded for transcripts which were rapidly induced 2-28 fold by UV. Sequence analysis of many of these cDNA clones did not match any known sequence. However, the predicted amino acid sequence of one cDNA, *DDIA18*, did have 2 localized areas of homology with the rat helix destabilizing protein. These areas of homology were at the 2 major binding sites of this nucleic acid single strand binding protein with DNA which indicates that *DDIA18* codes for a DNA damage inducible nucleic acid single strand binding protein. With a variety of different damaging agents, there was a complex pattern of induction. In many cases, induction was *specific* for DNA damaging agents; the highest induction was often seen with agents at doses where high levels of base damage occurred. Many of these cDNA hybridized to specific transcripts in human fibroblasts, and in several cases these transcripts were found to be UV-inducible. In addition, examples of altered expression in both Chinese hamster and human DNA repair mutants were also found. These results support the conclusion that multiple transcripts in mammalian cells are specifically induced by DNA damaging agents, and that their protein products may be involved in the cellular response to such damage.

E 324 INTER-INDIVIDUAL VARIATION IN EXCISION REPAIR OF UVB-INDUCED PYRIMIDINE DIMERS IN DNA OF HUMAN SKIN *IN SITU*. S.E. Freeman, Lovelace Medical Foundation, Albuquerque, NM 87108

The high incidence of skin cancer in individuals with xeroderma pigmentosum is thought to be related to their inability to repair ultraviolet radiation (UV)-induced pyrimidine dimers in their DNA. However, it is not clear whether those individuals in the "normal" population who have an increased tendency to develop skin cancer also have a reduced ability to repair pyrimidine dimers. In order to establish whether or not there is a correlation, a baseline for DNA repair must first be established. Therefore, we have begun to establish a baseline for DNA repair of UV-induced pyrimidine dimers in DNA of human skin *in situ*.

The untanned skin of seventeen healthy volunteers was exposed to UVB (280-320 nm) from an FS-40 sunlamp and shave biopsies obtained either immediately or 6 h after irradiation. The epidermis was separated from the biopsies and the DNA isolated by phenol and sec-butanol extraction and ethanol precipitation. DNA was incubated with *Micrococcus luteus* UV-endonuclease, which makes a single-strand scission at pyrimidine dimers in DNA. An alkaline agarose gel electrophoresis method was used to quantitate the number of UV-endonuclease sensitive-sites per 1000 bases in the extracted DNA. In each individual a dose response curve was determined at 0 and 6 h after irradiation. Removal of pyrimidine dimers from human skin could be detected within 6 h after irradiation and the average half-life for removal of pyrimidine was calculated to be 11.0 +/- 4.3 h. However, there was significant inter-individual variability as determined by a coefficient of variation of the half-life of 38%. (This study was funded by the Lovelace Medical Foundation).

E 325 THE REGULATION OF THE BACILLUS SUBTILIS BACTERIOPHAGE ϕ105 IMMUNITY REGION. Bradford M. Friedman and Ronald E. Yasbin, Department of Microbiology & Immunology, University of Rochester, Rochester, NY 14642

In E. coli, following insult to host cellular DNA by the addition of certain DNA damaging agents or abnormal cessation of DNA replication, a pleiotropic response is coordinately induced, termed the SOS repair system. A similar system has been characterized in the gram positive bacterium Bacillus subtilis denoted as the SOB response. Our laboratory has been interested in elucidating the molecular mechanisms of this inducible response and its relationship to the development of the competent state. A specific interest has been the identification of the fundamental physiological and molecular processes associated with the maintenance of lysogeny, and to even greater extent, the mechanism(s) of induction of bacteriophage ϕ105.

Overproduction of the repressor protein in a host lysogenic for ϕ105 abolishes spontaneous and UV-induced prophage. In addition, transformation frequencies are increased closer to non-lysogenic levels due to suppression of ϕ105 prophage induction during competence. The presence of only the ϕ105 repressor promoter/operator region on a multicopy plasmid in a host lysogenic for ϕ105 significantly reduced UV-induced prophage. This result suggests a titration of the cro-like protein due to the increased presence of its DNA-binding site and/or a possible increase in the production of repressor protein due to autoregulation. Presently, these promoter/operator regions are being fused to the structual gene for B-galactosidase in order to study further their regulation in vivo.

E 326 COMPLEMENTATION OF A DNA REPAIR DEFECT IN A MAMMALIAN CELL BY EXPRESSION OF A CLONED BACTERIAL GENE. Janet Hall, Hiroko Kataoka, Claire Stephenson and Peter Karran, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Potters Bar, Herts EN6 3LD

Mammalian expression vectors derived from pSV2gpt and encoding both or one of the two catalytic domains of the E.coli ada^+ gene have been constructed. Following stable integration into CHO cells, the whole ada^+ gene conferred resistance to both cell killing and mutagenesis by MNNG. An N-terminal fragment of the ada^+ gene which encodes only the methylphosphotriester DNA repair domain, conferred significant resistance to killing by MNNG only at high concentrations, indicating that methyl-phosphotriesters in DNA may contribute to the cytotoxic effects of MNNG but only at high levels of DNA damage. This apparent correlation between methylphosphotriester repair activity and increased cell survival has been further investigated by the isolation of MNNG resistant clones from a population expressing methylphosphotriester repair activity. These cloned MNNG resistant cell lines express higher levels of the methylphosphotriester DNA repair activity. A third plasmid has been constructed encoding only the C-terminal sequence of the Ada protein. Two independent clonal cell lines which express O^6-MeGua methyltransferase activity were isolated. The level of enzyme activity as measured in cell-free extracts was the same in both cell lines and both exhibited increased resistance to killing by MNNG. These data indicate a role for O^6-MeGua in the cytotoxic effects of agents such as MNNG. However the correlation between O^6-MeGua repair activity and cytotoxicity is not quantitative.

E 327 CLONING AND EXPRESSION OF A MICROCOCCUS LUTEUS UV-DEPENDENT ENDONUCLEASE IN E. COLI, Richard W. Hamilton, and R. Stephen Lloyd, Departments of Molecular Biology and Biochemistry, Vanderbilt University, Nashville, Tennessee 37232.
Two enzymes, T4 endonuclease V and Micrococcus luteus UV endonuclease, have been previously shown to incise UV-irradiated DNA at the site of pyrimidine dimers by an identical two-step mechanism. In addition, the denV gene, encoding endonuclease V, has been cloned, and sequenced. In order to clone the M. luteus UV endonuclease, an M. luteus genomic library was made by ligating sonicated M. luteus DNA into pEMBL-18. The library was transformed into E. coli UT481, amplified and then retransformed into AB2480, an E. coli uvrA$^-$ recA$^-$ strain. After screening $\sim$1.2 x 10^4 transformants for enhanced UV-resistance, two colonies were identified. These colonies contained two hybrid plasmids which confer UV resistance to AB2480, upon retransformation. These plasmids both contain $\sim$1-kb inserts as determined by restriction digestion. Both of these clones appear to be utilizing their own promoters rather than the lacZ promoter since induction with IPTG does not enhance the UV survival of the E. coli host. Each of these two clones have been shown to encode a UV-dependent endonucleolytic activity in vitro as well as enhancing UV-irradiated plasmid repair in vivo. The clones also enhance the UV resistance of an E. coli uvrA$^-$ recA$^+$ strain suggesting that the proteins encoded by these clones are not involved in recombinational repair. Based on these observations we feel that these clones encode the M. luteus UV endonuclease. Supported by BRSG 2507-RR05424-25, NIH ES 04091 and NIH ES 00267.

E 328 EXCISION REPAIR IN THE NEMATODE Caenorhabditis elegans.
Phil Hartman, Department of Biology, Texas Christian University, Fort Worth, TX 76129 and David Mitchell, University of Texas System Cancer Center, Smithville, TX 78957.
Cyclobutane dimer and (6-4)photoproduct excision were measured in wild-type and radiation-sensitive (rad) animals using a radioimmunoassay (Mitchell et al., 1985. Mutat. Res. 143, 109). In wild type, five developmental stages, ranging from embryos to adults, were assayed. Both photoproducts were removed at roughly equal rates, with repair proficiency declining slightly throughout development. C. elegans excised these photoproducts with kinetics more typical of mammalian cells in tissue culture than prokaryotes and lower eukaryotes. Cyclobutane dimer excision was also assayed using the UV specific-endonuclease from Micrococcus luteus. Rates were experimentally identical to those obtained with the RIA. Photoreactivation was not detectable. Of the four rad mutants assayed (rad-1, rad-2, rad-3 and rad-7), only rad-3 was significantly deficient in photoproduct removal. These data will be related to the epistatic interactions of these rad mutants, which place rad-1 and rad-2 in one epistatic group and rad-3 and rad-7 in another(Hartman. 1985. Genetics 109, 81). In addition, the hypersensitivity patterns of the rad mutants, which vary dramaticly throughout development (Hartman. 1984. Photochem. Photobiol. 39, 169), will be related to their DNA repair capacities.

E 329 REPAIR OF UV-IRRADIATED PLASMID DNA IN XENOPUS OOCYTES, John Hays, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331, and Eric Ackerman, Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD 20892.
Plasmids (derivatives of pBR322) were irradiated at 254 nm with fluences of 100 to 400 J/m^2, producing about 2 to 8 cyclobutane pyrimidine dimers (CBPD's) per plasmid, as assayed by treatment with *M. luteus* UV endonuclease and alkaline sucrose sedimentation. Irradiated plasmids were injected into *Xenopus laevis* oocytes (20 to 50 ng per oocyte), incubated 2 to 24 hours, then extracted and concentrated by an SDS-pronase-phenol-ethanol procedure. Repair, as judged by the ability of plasmids to transform recombination-deficient *E. coli* (*uvrB*$^-$*recA*$^-$*phr*$^-$) was highly efficient [more than 10^{10} lethal lesions (presumably CBPD's) removed per oocyte in 2 h]. Efficient repair was also observed using a modification of the procedure of Hanawalt and coworkers (treatment of UV-irradiated [^{32}P] plasmid preparations with *M. luteus* UV endonuclease, alkaline agarose electrophoresis, autoradiography). (Supported by American Cancer Society grant #NP-564 and an NIH IPA sabbatic year grant to JBH.)

E 330 A HAMSTER GENE WHICH COMPLEMENTS THE XERODERMA D DEFECT, Robert T. Johnson, Janet E.Arrand and Neil Bone, Cambridge University, Cambridge CB2 3EJ, England.
A transfection-competent derivative of the xeroderma D/HeLa hybrid cell HD2 (Johnson et al., 1985, J.Cell Science 76, 115) has been transfected with normal hamster DNA "tagged" with a neo gene borne on a cosmid vector. Using a UV selection protocol designed to enrich for partially corrected phenotypes (Arrand et al., 1987, EMBO J. in press), stable primary $G418^{R}UV^{R}$ transfectants have been isolated at a frequency of 2×10^{-5}.

A second round of transfection using cloned DNA from the primary transfectant with no further addition of dominant neo marker produced a stable secondary $G418^{R}UV^{R}$ colony (frequency 2.5×10^{-5}). The primary and secondary transfectants show identical phenotypes of reduced sensitivity to UV light and a partial restoration of UV-induced excision repair.

Southern blot analysis of transfectant DNA reveals the presence of 10 and 2 copies of an Alu-like hamster repeat sequence together with 10 and 5 copies of neo in the primary and secondary transfectants respectively. Attempts to clone the intact hamster gene from the secondary transfectant by cosmid rescue are in progress. Furthermore, hamster sequences from a λEMBL3 library made from secondary transfectant DNA are being used to probe an existing human cosmid library. Progress in the characterisation of the hamster and corresponding human clones will be reported.

E 331 **CHARACTERIZATION OF THE IONIZING RADIATION SENSITIVE MUTANTS *irs1* AND *irs2*,** N.J. Jones, L.H. Thompson, S.A. Stewart, J.D. Tucker, J.L. Minkler and A.V. Carrano, Biomedical Sciences Division, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, CA 94550.

Irs1 and *irs2* were isolated from the Chinese hamster cell line V79-4 on the basis of their enhanced sensitivity to ionizing radiation, and they belong to distinct complementation groups (1,2). Assay of strand-break rejoining by filter elution showed that both mutants have the capacity to repair both single and double strand breaks at normal rates following γ-irradiation. The baseline levels of sister chromatid exchange are also normal in both mutants. However, the level of spontaneous chromosomal aberrations is elevated to ~22% in *irs1*, compared to 1-4% in *irs2* and V79. *Irs1* shows an unusual phenotype of marked cross-sensitivity to both UV radiation (3-fold) and mitomycin C (~60-fold), suggesting there might be more than one genetic defect (1). The extreme hypersensitivity to MMC of *irs1* has been utilized to isolate a panel of interspecific hybrids by fusion with human lymphocytes. Analysis by isozymes and DNA probes will be used to identify the human chromosome complementing MMC sensitivity. These hybrids will be used to test whether the same chromosome also restores UV and/or γ-ray resistance. Concordant phenotypic correction would argue in favor of a single-gene defect in *irs1*. Complementation analysis showed that the mutation causing MMC-sensitivity is in a locus different from those of three CHO mutants (UV20, UV41, and UV-1) that also exhibit extreme MMC-sensitivity. 1. Jones et al. Mutat. Res. 183:279-286, 1987. 2. Jones et al. Mutat. Res. in press. This work was done under the auspices of the U.S. DOE by the LLNL under Contract W-7405-ENG-48.

E 332 DEFECTIVE REPAIR OF O^6-METHYL GUANINE AND DNA SINGLE-STRAND BREAKS/ALKALI LABILE SITES INDUCED BY N-METHYL-N'-NITRO-N-NITROSO GUANIDINE IN A HeLa CELL VARIANT. Ramaswami Kalamegham, Susan Rodenheiser, Helen MacDonald and Kaney Ebisuzaki, Cancer Research Laboratory, University of Western Ontario, London, Ontario, Canada N6A 5B7.
Sensitivity to alkylating agents may result from different mechanisms and the role of O^6-methyl guanine (O^6-MeG) as a cytotoxic lesion has been controversial. We have attempted a genetic approach to study this problem in human cells. Variants sensitive to the monofunctional alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) were selected from a mutagenized population of HeLa cells (CCL2, American Type Culture Collection) that are proficient in the removal of O^6-MeG from DNA. One such variant, designated A2-8, was found to be extremely sensitive (>10 fold difference in D_{37} values) to the S_N1 alkylating agents, MNNG and N-methyl-N-nitrosourea (MNU), and moderately sensitive (≈2 fold) to the S_N2 alkylating agent, dimethyl sulfate. O^6-MeG-DNA methyl transferase assays and HPLC analysis after ^{3}H-MNU treatment of A2-8 cells revealed a defective repair of the promutagenic lesion, O^6-methylguanine. The repair of 3-methyladenine, considered to be an important cytotoxic lesion, was unaffected in the variant. The repair of single-strand breaks (SSB) and/or alkali labile sites (ALS) in DNA after alkylation damage was studied by the sensitive alkaline elution technique. MNNG treatment resulted in a greater frequency of SSB/ALS and their delayed repair in A2-8 compared to HeLa CCL2. Studies to identify the lesion(s) responsible for the increase in SSB/ALS in A2-8 are in progress.
This study was supported by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada.

E 333 MECHANISMS OF TRANSCRIPTIONAL REGULATION OF A DNA DAMAGE RESPONSIVE (DDR) GENE IN SACCHAROMYCES CEREVISIAE, Naoko Kobayashi, Terrill McClanahan and Kevin McEntee, UCLA School of Medicine, Los Angeles, CA 90024.

The DDRA2 gene is a member of a group of genes in S. cerevisiae that shows increased transcript levels following exposure of cells to a variety of DNA damaging agents. In addition, the DDRA2 gene and at least one other member of this group are transcribed at elevated levels following heat shock treatments. Using a set of plasmids containing the cloned DDRA2 gene, we have identified two regions of the DDRA2 which are involved in its regulation by these stress treatments. Using Bal 31 exonuclease we have constructed deletions that delineate an upstream element necessary for both DNA damage and heat stress responses of DDRA2. A second region lying close to the transcription initiation sites contains one or more binding sites for negative regulatory factors which are present in limiting amounts in the cells. The structures of these regulatory sequences and a mechanism for DDR gene regulation by stress will be presented.

E 334 ISOLATION OF OPERON FUSIONS (soi::lacZ) INDUCIBLE BY OXIDATIVE STRESS IN Escherichia coli, Tokio Kogoma, Spencer B. Farr, Kelly Joyce and Donald O. Natvig, University of New Mexico, Albuquerque, NM 87131.

Aerobic life forms are under the constant threat of reactive oxygen species which arise primarily as a result of incomplete reduction of molecular oxygen during the respiration process. These reactive oxygen species include the superoxide radical, hydrogen peroxide and the hydroxyl radical. Earlier, we demonstrated that pretreatment of cells with non-lethal concentrations of plumbagin, a superoxide radical generator, invokes a cellular response that promotes cell survival upon subsequent challenge with lethal doses of plumbagin but not upon challenge with hydrogen peroxide (Farr, Natvig and Kogoma, J. Bacteriol. 164:1309-1316, 1985). In order to elucidate this novel oxidative stress response, we used Mu dX phage to isolate three random lacZ fusions (soi::lacZ for superoxide radical inducible) which were inducible by treatment with paraquat or plumbagin, superoxide radical generators. The induction of the fusions with superoxide radical generating agents required aerobic metabolism. Hyper-oxygenation (i.e., bubbling of cultures with oxygen gas) also induced the fusions. On the other hand, hydrogen peroxide did not induce the fusions at concetrations that are known to invoke an adaptive response. Introduction of oxyR, htpR or recA mutations did not affect the induction. Two of the fusion strains exhibited increased sensitivity to paraquat but not to hydrogen peroxide. All three fusions were located in the 45-61 minute region of the E. coli chromosome.

E 335 A SYSTEM FOR DETERMINATION OF NUMBERS OF CELLULAR AND MOLECULAR EVENTS IN HIGHER ORDER (SECOND ORDER-TWO HIT-AND ABOVE) KINETICS, W. Clark Lambert and Warren Tanz, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Most molecular and cellular events of significance in DNA damage and its cellular processing occur according to kinetics of first order or greater. One of us (WCL) has previously shown that such events obeying first order kinetics may be accurately determined using the expressions, $c=\ln P_o - \ln P$ and $c=NP_o^{-1/N}(P_o^{1/N}-P^{1/N})$, for stoichiometric and nonstoichiometric systems, respectively, where c is the number of events per unit (i.e., molecule, molecular unit (e.g., gene) or cell), P_o and P are the proportion of unaltered units under control and assay conditions, respectively, and N, in non-stoichiometric processes, is the possible number of such events per unit (J Cell Biochem 1983, Supplement 7B:189; Gene Anal Techn 1986; 3:75-77). Processes obeying higher order kinetics, however, such as the commonly occuring "two-hit" processes in radiation biology, give rise to functions of the general class $f(x)+\ln g(x)=k$, where k is a numberical constant, which require numerical methods for solution and are therefore awkward to use. We now introduce a readily applicable graphic method in which these equations are expressed, for stoichiometric systems, in the form $\ln P+c=\ln f(c)$. In our system, each side of this expression is plotted separately with the intercept(s) corresponding to (the) corresponding value(s) of c. Analagous plots have also been developed for higher order non-stoichiometric processes. This system provides an instantly applicable method for analysis of data from many higher order molecular and cellular processes of significance in DNA damage and its cellular processing. Supported by grants AM-35148 from the NIH, 86-490-CCR from the New Jersey State Commission on Cancer Research, and grants from the ALS Association and the Sandoz Research Institute.

E 336 INDUCIBLE REPAIR OF ALKYLAYED BASES IN MAMMALIAN CELLS, Patricia Lefebvre and Françoise Laval, Institut Gustave Roussy, 94805 Villejuif, France.

A single treatment with various agents (UV light, X-rays, Bleomycin, alkylating agents...) increases the O^6-methylguanine-DNA-methyltransferase and the 3-methyladenine glycosylase activities in H4 cells (rat hepatoma cells). The enhancement is maximum 48 hours after the treatment and needs protein synthesis. The transferase activity is increased by 2 to 5-fold and the glycosylase activity by 2 to 3-fold, according to the treatment delivered to the cell

The enhanced transferase activity is produced by a protein having the same characteristics than the constitutive one, as judged by gel electrophoresis. The enhanced activities are biologically active, as the treated cells are more resistant to the killing and the mutagenic effects of MNNG than control cultures. Furthermore, the removal of 3-methyladenine, 7-methyl guanine and 3-methylguanine was measured in cells incubated with ^{3}H MNU, with or without a pretreatment. Results show that the 3-methyladenine and the 3-methylguanine are removed more rapidly from the cellular DNA in pretreated, compared to control cells, whereas the 7-methyl guanine removal is not affected by the pretreatment.

This increased repair of alkylated bases was observed in other cell lines from rat or human origin. It is only observed after treatment with chemical or physical agents which produce damage in the cellular DNA. The enhancement is higher when the cells are treated both with these agents and inhibitors of the poly (ADP-ribose) polymerase.

E 337 PROGRESS ON CLONING THE GENE DEFECTIVE IN XERODERMA PIGMENTOSUM GROUP A CELLS, Randy Legerski, Carolyn Peterson, Paul Moore and David Brown, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Xeroderma pigmentosum (XP) group A and G cells can be transiently complemented, as determined by unscheduled DNA synthesis, by microinjection of poly(A)+RNA derived from HeLa cells. This assay has been utilized to obtain an approximately 10 fold enrichment of the XP-A-complementing mRNA by fractionation on non-denaturing sucrose gradients and a 50-100 fold enrichment by methylmercury agarose gel electrophoresis. These results indicate that this mRNA species is approximately 1 Kb in size. Active fractions have been used to prepare cDNA libraries in the expression vector pT7/T3-18. This vector contains the bacteriophage T3 and T7 promoters which can be utilized in vitro to produce either sense or anti-sense transcripts from cDNA clones. RNA prepared from pools of clones from these libraries are currently being assayed by microinjection for complementation of XP-A cells.

E 338 TRICHOTHIODYSTROPHY - A UV-SENSITIVE DISORDER, Alan R. Lehmann and Colin F. Arlett MRC Cell Mutation Unit, Sussex University, Falmer, Brighton BN1 9RR, UK.

Trichothiodystrophy (TTD) is an autosomal recessive disorder characterised by brittle hair with reduced sulphur content, ichthyosis, peculiar face and mental and physical retardation. Some patients are photosensitive. A previous study by Stefanini et al. (Human Genet., 74 (1986), 107-112) showed that cells from 4 patients with TTD had a molecular defect in DNA repair, which was in the same complementation group as xeroderma pigmentosum, group D. We have found a variety of different responses in a detailed molecular and cellular study of the effects of UV light on cells cultured from 4 further TTD patients. Cells from patient 1 were normal in cell survival, excision repair, DNA and RNA synthesis following UV irradidation, whereas in cells from patient 2 all these responses were similar to those of excision-defective XP cells. In cells from patient 3 cell survival was normal following UV-irradiation, even though excision repair was only 50% of normal, and RNA synthesis was severely reduced. In patient 4 excision repair was normal but RNA synthesis was reduced. Our results suggest that the abnormal UV response of most TTD cell strains may be used for confirmation of the clinical diagnosis and for prenatal diagnosis of TTD. They pose a number of questions about the relationship between the molecular defect in DNA repair and the clinical symptoms of XP and TTD.

E 339 SURVIVAL OF HUMAN COLON TUMOR CELLS AFTER TREATMENT WITH RESTRICTION ENDONCUCLEASES: EFFECTS OF DIFFERENTIATION-INDUCING AGENTS SODIUM BUTYRATE AND N-METHYLFORMAMIDE, John T. Leith, Rhode Island Hospital and Brown University Providence, RI. 02903.

Studies on the effects of the differentiation-inducing agents sodium butyrate (NAB) and N-methylformamide (NMF) have shown that chronic adaptation of human colon tumor cells in vitro to these agents changes the phenotype of the cells so that they become sensitized to the effects of x-irradiation. This change is particularly associated with a decrease in the "shoulder" region of the dose-survival curve indicating interference with cellular ability to recover from radiation injury. As part of our efforts to characterize these phenomena, we are using various restriction endonucleases which produce double strand breaks in permeabilized cells and are 1) measuring cell survival after exposure of cells to increasing concentrations of REs, 2) correlating cell survival with induction of double strand breaks as measured neutral filter elution, and 3) determining if this RE activity is modified by adaptation to NAB or NMF.

(Research supported by ACS Grant PDT 243B)

E 340 EXCISION REPAIR CHARACTERISTICS OF *den*V-TRANSFORMED XERODERMA PIGMENTOSUM CELLS, Ronald D. Ley, Lee A. Applegate, Jon K. de Riel† and Earl E. Henderson†, Lovelace Medical Foundation, Albuquerque, NM 87108 and †Temple University School of Medicine, Philadelphia, PA 19140.

Introduction of the *den*V gene of phage T4, encoding the pyrimdine dimer-specific endonuclease V, into xeroderma pigmentosum cells XP12RO(M1) was reported to result in partial restoration of colony forming ability and excision repair synthesis (Valerie, *et al.*, Cancer Res. 47:2967, 1987). We have further characterized three *den*V-transformed XP clones in terms of rates of excision of pyrimidine dimers and size of the resulting resynthesized regions. In the *den*V-transformed XP cells we observed 50% dimer removal within 3-6 h after UV exposure as compared to no measurable removal in the XP12RO(M1) line and 50% dimer excision after 18 h in the GM637A normal cells. Dimer removal was assayed with *Micrococcus luteus* UV-endonuclease in conjunction with sedimentation of treated DNA in alkaline sucrose gradients. The size of the resulting repaired regions was determined by the bromouracil photolysis technique. Based on the photolytic sensitivity of DNA repaired in the presence of bromodeoxyuridine, we calculated that the excision of a dimer in the GM637A cells appears to be accompanied by the resynthesis of a region ~100 nucleotides in length. Conversely, the resynthesized regions in the *den*V-transformed clones were considerably smaller and were estimated to be between 15-20 nucleotides in length. These results may indicate that either the endonuclease that initiated dimer repair dictated the size of the resynthesized region or, possibly, that the long-patch repair observed in the normal cells resulted from the repair of non-dimer DNA lesions. (This study was funded by the Lovelace Medical Foundation).

E 341 ISOLATION OF HERPES SIMPLEX VIRUS TYPE 1 TEMPERATURE SENSITIVE MUTANTS MAPPING TO THE VIRALLY ENCODED dUTPase LOCUS, Ronald Lirette and Sal Caradonna, UMDNJ-SOM, Piscataway, NJ 08854.

dUTPase functions in the maintenance of low intracellular concentrations of dUTP, and thus limits its incorporation into DNA. Compromising the cell's ability to exclude this base from its DNA can result in fragmentation of the genome and cell death. Herpes simplex viruses encode a species of this enzyme that is distinct from the cellular form. If selective inhibition of the virally encoded form of dUTPase is possible, then a target for cell specific anti-viral chemotherapy would be uncovered. Here we report the isolation and characterization of site specific mutants deficient in the production of dUTPase at the non-permissive temperature (39°). Sequence analysis and marker rescue have verified the location and the nature of the mutations, placing the defects within the coding region of the dUTPase gene. Permissive cells infected with these ts mutants at 39° yield 60% less virus specific dUTPase activity than at 34°. In addition, the yield of infectious virus at the non-permissive temperature is greater than ten thousand fold less than at 34°. Density gradient analysis of the viral DNA indicates a smaller overall size when isolated from mutants at 39°, consistent with the genomic fragmentation hypothesis. These results confirm the effects of elevated dUTP concentrations, and indicate the utility of the viral system as a model to study consequences of altered intracellular nucleotide concentrations as a result of physical or pharmacologic manipulations.

E 342 ISOLATION AND CHARACTERIZATION OF HeLa CELL VARIANTS SENSITIVE TO ALKYLATION INDUCED DAMAGE, Helen MacDonald, Ramaswami Kalamegham and Kaney Ebisuzaki, Cancer Research Laboratory, University of Western Ontario, London, Ontario N6A 5B7.
Monofunctional alkylating agents produce both mutagenic and carcinogenic lesions. We have directed research toward the study of the mechanisms by which these lesions are repaired in human cells. We have isolated a number of HeLa cell (CCL2:ATCC) variants by selection for hypersensitivity to the cytotoxic effects of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). These variants are divided into two broad classes based on their colony-forming ability after MNNG treatment. One class, represented by the variant A2-8, possesses the following properties: an extreme hypersensitivity to MNNG evidenced by a decreased colony-forming ability, a greater frequency of MNNG-induced single-strand breaks and/or alkali-labile sites and a defective repair capacity for O^6-methylguanine (O^6-MeG) lesions (Kalamegham et al., this meeting). Variants of the second class display an average 2-fold, intermediate sensitivity in D_{37} values to MNNG, as compared to wild-type cells. One member of this class, the variant 6a12, also shows sensitivity to dimethylsulfate, but possesses wild-type sensitivity to uv- and γ-irradiation. Alkaline elution studies revealed that no additional strand breaks or alkali-labile sites are produced in 6a12 after alkylation by MNNG at various doses. Preliminary HPLC analyses of alkylated bases following [^{3}H]-N-methyl-N-nitrosourea (400μM) treatment indicated that the 6a12 variant fails to repair O^6-MeG adducts while repairing 3-methyladenine lesions to wild-type levels. Further characterizations of the variants of this second class of MNNG cytotoxicity will be presented. (Supported by the NCI (Canada) and MRC (Canada).)

E 343 MOLECULAR-GENETIC EVIDENCE FOR IDENTIFICATION OF THE ERCC-5 HUMAN DNA EXCISION REPAIR GENE, Mark A. MacInnes, Richard T. Okinaka, David J. Chen, Joyce W. Nickols, Judy G. Tesmer, Linda S. McCoy and Gary F. Strniste, Genetics Group, Los Alamos National Laboratory, Los Alamos, NM 87545.
We seek to understand the important biochemical elements of human DNA excision repair for UV-induced DNA damages, and the role of genetic variation in this system for mechanisms of human carcinogenesis. In order to approach these questions, we are attempting to identify and isolate a putative human DNA repair gene (ERCC-5) for nucleotide excision repair of UV-induced DNA photoproducts. Chinese hamster ovary cell mutant UV-135 (complementation group 5) was used for $CaPO_4$-DNA cotransformation with ligated mixtures of high molecular weight human fibroblast DNA and plasmid pSV2gpt. These experiments produced rare, UV resistant (UV^r) and mycophenolic acid-adenine-xanthine resistant (MAX^r) cotransformant colonies. Primary, secondary and several tertiary cotransformants have been isolated from UV-135 cells. The frequency of tertiary UV^r, MAX^r cotransformants is 1 per 10 to 20 MAX^r colonies isolated. We are currently examining which human DNA fragments show concordant inheritance with the UV^r phenotype in our cotransformants and segregants. A cosmid library of one tertiary cotransformant has been screened for functional inserts located in proximity to the pSV2gpt. Our initial results did not reveal any single cosmid which conferred UV resistance. More library clone isolation and cosmid mixing experiments are in progress. Available evidence suggests that the genomic ERCC-5 DNA sequences are either fairly extensive in size and/or they are located at some distance from the nearest pSV2gpt copies. We will also report on attempts to detect DNA repair related mRNA expression for the purpose of cDNA library construction and screening. (Supported by the U. S. Department of Energy, under Contract No. W-7405-ENG-36.)

E 344 MECHANISM OF INHIBITION OF ALKYLATED T7 BACTERIOPHAGE DEVELOPMENT IN REPAIR-DEFICIENT HOST CELLS, M. D. Mamet-Bratley, G. Czaika, J.-F. Racine and B. Karska-Wysocki, Dép. de Biochimie, Univ. de Montréal, Montréal, Canada H3C 3J7.
Alkylated T7 phage displays greatly reduced host cell reactivation in *E. coli* cells defective for 3-methyladenine-DNA glycosylase I. To understand the molecular basis of this inhibition, we compared DNA metabolism in wild-type (AB1157) and mutant (BK2114) cells infected with methyl methanesulfonate-treated T7 phage. With mutant cells, there was a total absence of phage DNA synthesis and no detectable breakdown of host DNA. These results indicated that the inhibition in phage development, due to defective DNA repair, was not caused by direct interference with phage DNA replication but rather occurred prior to replication. Neither adsorption nor DNA injection was changed in mutant cells as compared with wild-type cells. Protein synthesis in infected mutant cells was delayed for Class I proteins and very reduced for Class II and III proteins. Thus expression of those genes which depend on T7 RNA polymerase for their expression was much more affected than expression of those which depend on host RNA polymerase. We propose that transcription by T7 RNA polymerase is the step in intracellular phage development which is the most hindered by unrepaired lesions in DNA. We can then account for all our experimental results within the framework of the transcription-assisted model for T7 DNA injection.

E 345 DESCRIPTION AND ANALYSIS OF A NEW METHYLATION REPAIR DEFICIENT MUTANT DERIVED FROM CHINESE HAMSTER V79 CELLS, Linda S. McCoy, Kelly L. Wirfel, and David J. Chen, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545. V79 cells, a Chinese hamster lung-derived cell line, were mutagenized by treatment with 300 μg/ml of EMS, and clones from mutant cells were initially selected on the basis of radiation sensitivity relative to the parental line. One of these clones, designated S2-8, was selected for further study. S2-8 shows marked sensitivity to the methylating agents MMS, MNNG, and DMS. These sensitivities are in the range of 3- to 10-fold (compared to V79 cells), as measured by cell survival. S2-8 is slightly sensitive to damage induced by EMS and by the cross-linking agent mitomycin C. Compared to its' sensitivity to methylating agents, it shows minimal sensitivity to X-irradiation, and no appreciable sensitivity to damage induced by UV radiation. Biochemical experiments are presently underway to determine the nature of the unrepaired DNA lesion which results in this sensitivity, and to determine the mutability of S2-8 relative to the V79 parental cell line. Preliminary evidence indicates that the S2-8 cell line shows a deficiency in DNA damage repair not yet described for Chinese hamster cell lines, and thus a description of this deficiency should add valuable information to present knowledge of DNA repair. (This work was supported by DOE and Los Alamos National Laboratory.)

E 346 CHO MUTANT UV61 REMOVES (6-4) PHOTOPRODUCTS BUT NOT CYCLOBUTANE DIMERS D.L. Mitchell*, L.H. Thompson#, J.D. Regan+, S.A. Stewart#, W.L. Carrier+, and R.S. Nairn*, *University of Texas Cancer Center System, Science Park/Research Div., Smithville, TX 78957, +Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831, #Biomedical Sciences Div., Lawrence Livermore Natl. Lab., Livermore CA 94550.
The CHO mutant UV61 was previously assigned to UV complementation group 6. UV61 is more resistant to killing by UV than mutants such as UV5, which were earlier found to be fully defective in the incision process after UV irradiation. The D_{37} for cell survival is ~4 J/m^2 for UV61, compared with 10 J/m^2 for the parental AA8 line and ~2 J/m^2 for UV5. Similarly, mutation induction at the *hprt* and *aprt* loci shows an intermediate response. Biochemical assays that measure different aspects of repair support the hypothesis that this mutant is defective specifically in removing pyrimidine dimers of the cyclobutane class, i.e. (5-6) photoproducts. Using alkaline elution of DNA from filters, assay of the rate of strand incision immediately (0 to 6 min) after UV exposure to 6 J/m^2 showed a normal response in UV61. This result correlates with our finding that during the first 2 hr after exposure, the kinetics of rapid removal of (6-4) photoproducts is normal as measured by a radioimmunoassay that is specific for this class of damage. After exposure to 15 J/m^2, no detectable removal of cyclobutane dimers from DNA was found in UV61 while AA8 cells removed 28% by 24 hr. We suggest that the mutation in UV61 specifically lowers the affinity of the repair complex for (5-6) photoproducts, which are inefficiently removed even in normal CHO cells. This work was done under NIH grants CA24540 and 36361, the auspices of the U.S. DOE by the LLNL under Contract W-7405-ENG-48 and, by ORNL under contract DE-AC05-840R21400 the Martin Marietta Energy Systems, Inc.

E 347 CHROMOSOMAL LESIONS CAUSED BY INHIBITING DNA POLYMERASES IN G_2 MAMMALIAN CELLS, Ruth C. Moore, Cell Biology Group, Peter MacCallum Cancer Inst., Melbourne, Victoria 3000, Australia.

It is known that the α-polymerase inhibitor aphidicolin (APC) and the α- and β-polymerase inhibitor 1-β-D-arabinofuranosylcytosine (ara-C) cause chromosomal lesions when mammalian cells are exposed to them continuously in G_2.

Data will be presented to show that lesions are of two types; chromatid lesions in cells exposed throughout G_2, and isochromatid lesions (involving both replicated sister chromatids at the same site) in cells exposed in early G_2. Exposures to APC or ara-C in G_2 act synergistically with damage induced in S by incorporation of ara-C, and in this case the damage which is repaired in G_2 cells appears to be single strand regions of DNA. Inhibition of G_2 repair also prevents completion of the misrepair which leads to chromosome exchanges, after cells are irradiated in G_2, or in G_1 or early S.

E 348 RELATIONSHIP OF SUPEROXIDE:DISMUTASE-I (SOD-I) GENE DOSAGE AND CYTOTOXICITY BY DNA DAMAGING AGENTS IN HUMAN FIBROBLASTS, Hatsumi Nagasawa and John B. Little, Laboratory of Radiobiology, Harvard School of Public Health, Boston, MA 02115.
We previously reported the hypersensitivity of mitomycin-C (MMC) induced cytotoxicity in Fanconi's anemia (FA) and dyskeratosis congenita (DC) cells and the enhanced survival witnessed in these cell strains after the addition of SOD-I. Human SOD-I genes are located in the long arm of chromosome-21. In this report we examined the cytotoxicity of trisomy-21 and monosomy-21 cells with MMC and X-rays. Confluent cells were treated with MMC for one hour at 37°C or X-irradiated at a dose rate of 80 rads per min. Immediately after treatment the cells were trypsinized and seeded onto 100 mm plastic petri dishes. Both GM1309 (FA) and GM230 (monosomy-21) cell strains showed extremely sensitive to MMC (D_{10} ~0.04 μg/ml and ~0.15 μg/ml, respectively). The D_{10}s of three normal cell strains were 0.35-0.43 μg/ml with shoulders in the survival curves. Three trisomy-21 cell strains were more resistant to MMC treatment (D_{10} ~0.57-0.74 μg/ml). FA and monosomy-21 showed intermediate X-ray sensitivities (D_{10} ~210-300 rads) between the extremely X-ray sensitive ataxia-telangiectasia (AT5BI) cell strain (D_{10} ~100 rads) and normal cell strains (D_{10} ~450 rads). Trisomy-21 cell strains, however, showed a wide range of X-ray sensitivities. However, these cell strains showed enhanced survival reaching the same level as normal cell strains when the cells were irradiated in presence of 3% dimethyl sulfoxide (DMSO) or under hypoxic conditions. The cytotoxic effects of MMC or X-irradiation may be mediated by active oxygen species and SOD-I may play an important factor in their detoxification. (This research is funded by an NIH Grant No. CA11751 and the U.S.D.O.E. under contract W-7405-ENG-36.)

E 349 EFFECTS OF UV DAMAGE ON HOMOLOGOUS AND NON-HOMOLOGOUS RECOMBINATION IN REPAIR-PROFICIENT AND -DEFICIENT CHO CELLS, Rodney S. Nairn, Gerald M. Adair, and Ronald M. Humphrey, University of Texas Science Park, Smithville TX 78957.
Both homologous and non-homologous recombination can occur in mammalian cells. We have exploited two different, selectable genetic sequences to investigate the effects of UV damage in transfected DNA molecules on their participation in both types of recombination events. By co-transfecting UV-irradiated hamster APRT sequences containing non-overlapping deletions, we have observed that moderate UV damage in covalently-closed circles stimulates homologous recombination in repair-proficient and class II (UV20 class) repair-deficient UV-hypersensitive mutant cell lines, but not in class I (UV5 class) repair-deficient UV-hypersensitive mutant cells. This effect is abolished if UV-irradiated linear molecules are used in transfections. In other experiments using the selectable genetic chimera pSV2gpt, it was observed that UV damage in transfected DNA neither stimulated nor inhibited non-homologous recombination between transfected DNA and the DNA of recipient cells, regardless of their repair proficiency. These results suggest that the processing of UV damage in mammalian cells may affect the participation of damaged DNA sequences as substrates in homologous, but perhaps not in non-homologous, recombination events. This work is supported by NCI grants CA36361 and CA04484.

E 350 EXCISION REPAIR IN *MICROCOCCUS LUTEUS*: EVIDENCE FOR A UVRABC HOMOLOG, Hiroaki Nakayama and Susumu Shiota, Department of Microbiology, Dental School, Kyushu University, Higashi-ku, Fukuoka 812, Japan.

Contrary to the widely held view, the evidence so far available is not compatible with a major role for the *M. luteus* UV endonuclease (pyrimidine dimer DNA glycosylase/AP endonuclease) in the UV survival of this bacterium, and rather indicates the presence of another enzyme acting as an excinuclease. To substantiate this prediction, we cloned and sequenced *M. luteus* DNA fragments capable of transforming two different excision-negative mutants (UV^SN1 and #7) to full resistance to UV and mitomycin C. Two ORFs, each containing the site of the corresponding mutation, were discovered in those fragments. The deduced protein products of the ORFs were shown to have extensive amino acid sequence homologies to the *E. coli* UvrA and UvrB proteins, respectively. These results strongly suggest that an enzyme homologous to the UvrABC excinuclease of *E. coli* is functioning in *M. luteus*. However, the actual role for the UV endonuclease remains to be clarified.

E 351 THE RESPONSE TO DNA DAMAGE IN THE CHLOROPLAST AND NUCLEAR COMPARTMENTS OF CHLAMYDOMONAS, Timothy Opperman and Stefan Surzycki, Department of Biology, Indiana University, Bloomington, IN 47405. Chlamydomonas reinhardtii appears to maintain genetically and biochemically distinct DNA repair mechanisms in the chloroplast and nuclear compartments. Inactivation and the frequency of induced mutations in the chloroplast and nuclear compartments were quantitated over a range of DNA damage produced by Ultraviolet light (UV) and the UV-mimetic agent 4-Nitroquinoline-N-oxide (NQO). The frequency of induced mutation increased 10 and 100-fold above the spontaneous mutation frequency after relatively low and high levels of DNA damage, respectively. Ultraviolet senstive mutant strains of Chlamydomonas exhibited 3 distinct mutagenic phenotypes with respect to the frequency of induced mutation: 1) Wild type frequency of induced mutations in the chloroplast and nucleus. 2) Wild type frequency of induced mutation in both compartments after a high level of DNA damage only. 3) No induced mutations in both compartments. Southern analyses showed that the chloroplast genome contains sequences homologous to the following DNA repair genes from E. coli: recA, lexA, uvrA, uvrB, uvrC, uvrD, umuDC, and ssb. Experiments are underway to determine whether these sequences are expressed and induced in response to DNA damage. We are currently sequencing the chloroplast genomic fragment containing the recA and uvrC homologies. Proteins produced after various doses of UV were labeled with $^{35}SO_4$ in vivo, separated by SDS polyacrylaminde gel electrophoresis, and visualized by flourography. The synthesis of all the cellular proteins was inhibited by low doses of UV. Three proteins appeared to be produced in response to high doses of UV.

E 352 POSSIBLE ROLE OF POLYPLOID CELLS IN STUDIES ON GENE AMPLIFICATION IN DRUG-TREATED MAMMALIAN CELLS. Robert B. Painter, Laboratory of Radiobiology, University of California, San Francisco, CA 94143.

Polyploid cells arise spontaneously in cultures of Chinese hamster and other aneuploid cell lines. In previous studies (Hahn, *et al.*; Cancer Research, 46,4607,1986) it was observed that the frequency of polyploid CHO B11 cells arriving at mitosis increases 2-3 fold after a 6 hr hydroxyurea (HU) treatment, even though DNA overreplication was not detectable. In this study, two polyploid cell lines were derived from CHO B11 cells; one of the polyploid lines but not the other has a very high spontaneous resistance to HU and methotrexate (MTX). The resistance to MTX is accompanied by markedly increased intracellular concentrations of mRNA specific for dihydrofolate reductase (dhfr). These resistant polyploid cells arrive at mitosis after a 6 hr HU treatment much sooner than do the parental B11 cells. These results suggest that: 1) Naturally occurring polyploid cells may be the forerunners of cells with amplified genes and, 2) Selection of polyploids may confuse the FACS analysis of DNA contents in cell populations at early times after drug treatment.

E 353 MOLECULAR ANALYSIS OF REARRANGEMENTS INVOLVING A NEO GENE CHROMOSOMALLY INTEGRATED IN A RAT2 CELL LINE, Ileana Quinto and Giuseppe Scala, Institute of Cellular and Molecular Biochemistry and Institute of Biochemical Sciences, 2nd Unversity Medical School, Naples, Italy.

A cellular system has been developed to study the frequency and the molecular analysis of rearrangements of a neo gene, chromosomally integrated in a Rat2 cell line, occurring either spontaneously or following the treatment with genotoxic compounds.
Rat2 cells were cotransfected with the pEMBLneo and pHMR272 vectors by calcium phosphate precipitation. The pHMR272 vector confers resistance to the hygromycin B antibiotic in eukaryotic cells; the pEMBLneo vector carries a neo gene without promoter and, thus, unable to confer resistance to the G418 antibiotic. About 300 hygromycin B resistant (Hg^R) clones were obtained (corresponding to 46 Hg^R clones/gamma DNA). 70 Hg^R clones were amplified and analysed for the genomic integration of neo by Southern blotting analysis. Hg^R Rat2 clones, which carried the neo gene integrated at one single locus and were still sensitive to the G418 antibiotic (Neo^S), were selected for further analysis.
We plan to use a few Hg^R Neo^S Rat2 clones to investigate the mechanisms of rearrangements of the neo gene conferring resistance to the G418 antibiotic.

E 354 TRANSFECTION ENHANCEMENT OF BACTERIOPHAGE DNA MAY REFLECT A NOVEL REPAIR PATHWAY FOR UV-IRRADIATED DNA IN B. SUBTILLIS, Eric H. Radany, Gregory Malanoski, Nicholas Ambulos, Errol C. Friedberg and Ronald E. Yasbin, Univ. Pennsylvania, Philadelphia, PA 19104 (E.H.R.), Stanford Univ., Stanford, CA. 94305 (E.H.R. and E.C.F.), Univ. Rochester, Rochester, N.Y. 14642 (G.M., N.A. and R.E.Y.)

Transfection of B. subtilis by isolated bacteriophage DNA previously has been shown (for some phages) to be greatly enhanced in efficiency by UV-irradiation of host cells, by cotransfection of UV-irradiated heterologous DNA, or by preinfection of host cells with phage under conditions permissive for protein synthesis; these observations have been attributed to nonspecific intracellular inactivation of transfecting phage DNA by a host enzyme(s) that is inhibited by damage in UV-irradiated DNA or by a phage-encoded factor.

We show that this transfection enhancement (TE) is restricted to those B. subtilis phages that contain the minor base 5-hydroxymethyl uracil (HMU) in place of thymine in their DNA. We also show that infecting DNA of HMU phages SP82G and SP01 is inactivated intracellularly in the presence of chloramphenicol, and that this inactivation is blocked by UV-irradiation of the host cell. We suggest that these results may reflect the action of a host DNA repair process on HMU phage DNA in a system analogous to the uracil-containing B. subtilis phage PBS2 and the inhibitor of host uracil DNA-glycosylase that it encodes. We failed to detect uracil in SP82G DNA. The photoproduct in cotransfecting heterologous UV-DNA that promotes TE is shown not to be pyrimidine dimer or pyrimidine hydrate. Our present approaches to elucidating TE and its relationship to metabolism of damaged DNA in B. subtilis include further characterization of the TE photoproduct and molecular cloning of the putative inhibitor gene.

E 355 DECREASED REPAIR OF ALKYLATING AGENT INDUCED DAMAGE IN FAMILIAL ALZHEIMER'S DISEASE Steven H. Robison, Susan K. Jones, Linda E. Nee*, Ronald J. Polinsky* and Walter G. Bradley, University of Vermont, Burlington, VT 05401 and * The National Institutes of Health, NINCDS, Bethesda, MD 20892.

We have previously shown that Alzheimer's disease skin fibroblasts have deficient repair of of methyl methane sulfonate (MMS) induced DNA damage. In order to further understand the origin of this DNA repair defect we have undertaken a study of MMS and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced DNA repair in familial Alzheimer's disease lymphoblasts. Analysis of DNA repair was done by using alkaline elution and cesium chloride equilibrium density gradient centrifugation. The mean recovery of 5 different control cell lines 3hrs after 200uM MMS treatment was 75.9% (±2.3,SEM), while 7 cell lines from Alzheimer's disease patients repair only 58.2% (±6.7) of the damage, with $p = .03$. When lymphoblasts from at risk individuals were studied 4 individuals were found to have normal repair and 1 person was found to have decreased repair. Similar studies using MNNG revealed that the same control cell lines repair 75.8% (±4.3) of the damage induced by 6uM MNNG, but Alzheimer's disease cells repaired only 41.6% (±2.1) of the lesions ($p = .001$). A study of the at risk group indicated that 3 individuals have normal levels of DNA repair (68-92%) and three individuals have decreased levels of repair (31-50%). Several cell lines from each category were analyzed by cesium chloride equilibrium density gradient centrifugation. The results from these studies in all cases confirmed the results obtained from alkaline elution analysis. This work was supported by grants from the NIA and the American Federation for Aging Research.

E 356 CHARACTERISATION OF MUTAGEN-HYPERSENSITIVE CHINESE HAMSTER OVARY (CHO) CELL MUTANTS C.N. Robson, P.R. Hoban, J. Reid, A.L. Harris and I.D. Hickson, Cancer Research Unit, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

We have isolated a bank of CHO cell mutants which exhibit hypersensitivity to DNA damaging agents. Of these, 5 were isolated as mitomycin C-sensitive (designated MMC-1 to -5), 2 as bleomycin-sensitive (BLM-1 and -2) and 6 as MMS-sensitive (MMS-1 to -6). Genetic analysis shows that the mitomycin C- and bleomycin-sensitive mutants are phenotypically recessive and represent 6 different complementation groups (with MMC-1 and MMC-5 being identical). BLM-1 and BLM-2 are also genetically distinct from the X-ray and bleomycin-sensitive xrs-6 mutant isolated by Jeggo and Kemp [Mutat. Res. 112, 313 (1983)].

MMC-1 is hypersensitive to a range of cross-linking agents and shows delayed repair kinetics for cross-links induced by mitomycin C. MMC-2, a representative of UV complementation group 3 (Thompson), is also defective in cross-link repair. Mutants MMC-3 and MMC-4 show no apparent repair defect. More cross-links are induced in MMC-4 and MMC-5 cells than in CHO-K1 cells following exposure to mitomycin C.

Mutants BLM-1 and -2 are hypersensitive to several free radical-generating agents, but show cross-resistance to neocarzinostatin (NCS). BLM-2 cells show a defect in the repair of both single- and double-strand breaks. DNA ligase activities are being assayed in this mutant. Both BLM-1 and BLM-2 accumulate less DNA strand breaks than CHO-K1 cells after NCS treatment, but BLM-2 still repairs these breaks with reduced efficiency.

A number of the mutants exhibit increased spontaneous mutation frequencies, and are being screened for alterations in AP endonuclease activity or mismatch repair proficiency.

E 357 DNA-PROTEIN CROSSLINKING IN HUMAN AND ICR 2A CELL LINES FOLLOWING UV-IRRADIATION, Barry S. Rosenstein, Department of Radiation Medicine, Brown University, Providence, RI 02912. The number of DNA-protein crosslinks (DPC) was measured using the alkaline elution assay in normal human skin fibroblasts exposed to 25, 50, 100, or 200 J/m^2 of 254 nm UV radiation and incubated 0-24 hours. The level of DPC decreased upon incubation of cells treated with either 25 or 50 J/m^2. However, the level of DPC increased about 3-fold in cells irradiated with either 100 or 200 J/m^2 following a 2 hour incubation and then decreased. In addition, when the cell lysate was treated with proteinase K to eliminate the DPC, a large number of strand breaks was revealed. A similar result was detected with ICR 2A cells exposed to sunlamp UV > 315 nm. But, two mutant cell lines isolated from ICR 2A that are hyper-sensitive to wavelengths in this region showed a much smaller enhancement in DPC following sunlamp UV-irradiation. One interpretation of these results is that there may be a protein that becomes covalently associated with DNA following the induction of DNA damage. This protein may be involved in a repair process or some other cellular response to UV-irradiation. Potential candidates for this protein could be topoisomerase I or II as these enzymes may recognize perturbations in DNA structure resulting from the presence of DNA damages. The identification of this protein is currently being followed. This work was supported by grants CA45078 and ES04355, DHHS.

E 358 CORRELATIONS BETWEEN ADDUCT FORMATION AND BIOLOGICAL ENDPOINTS IN MOUSE CELLS WHICH HAVE BEEN TREATED WITH ALKYLATING AGENTS. L. Rudenko and R. B. Setlow, Biology Department, Brookhaven National Laboratory, Upton, New York 11973

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and dimethyl sulfate (DMS) are simple alkylating agents which generate different spectra of adducts in DNA, with MNNG producing a higher ratio of O^6/N7 methyl guanine than DMS. To determine the absolute and relative concentration of adducts, NIH-3T3 cells were treated with either DMS or MNNG. Alkali labile sites, including apurinic sites and phosphotriesters, were determined by analysis of alkaline sucrose gradients and alkaline gel electrophoresis. Inactivation of the endogenous O^6 alkyltransferase was at least ten-fold more efficient by pretreatment of the cells with MNNG than DMS. In addition, on a molar basis, MNNG was several-fold more cytotoxic than DMS. Sister chromatid exchange (SCE) induction differed both in the total number of SCE's per cell, and in the kinetics of induction, with MNNG the more potent inducer. Modulation of these effects was studied by transforming the parental NIH-3T3 cell line with a plasmid containing the ras oncogene, which effectively abolished the activity of the endogenous alkyl transferase. ras transformed cells were more sensitive to the cytotoxic effects of both alkylating agents, as well as exhibiting an altered sensitivity to SCE induction. These results will be discussed with respect to mutation induction and correlation to actual adduct formation.

This work was supported by the Office of Health and Environmental Research of the United States Department of Energy.

E 359 ASSESSMENT OF IN VIVO DNA LIGASE ACTIVITY IN BLOOM'S SYNDROME CELLS USING PLASMID VECTORS. Thomas M. Rünger, Kenneth H. Kraemer, NIH, NCI, Bethesda MD 20892

Extracts of cell lines from patients with Bloom's syndrome, a rare disease with a high cancer risk and an elevated frequency of sister chromatid exchanges, were recently reported to contain reduced activity of DNA ligase I, an enzyme that ligates double stranded, blunt ended DNA. *In vitro* activity of DNA ligase II, which ligates DNA with overlapping ends, was normal (Willis and Lindahl, Nature 125, 1987,355-357).

In order to assess *in vivo* ligation ability of Bloom's syndrome cells, we prepared linearized plasmids with either blunt or overlapping ends by digestion with restriction endonucleases. Following transfection we looked for evidence of re-ligation during passage through the cells. We examined Bloom's syndrome transformed fibroblasts GM 8505 (BSF) and lymphoblasts GM 3403C (BSL), and normal transformed fibroblasts GM 0637 (NF) and lymphoblasts GM 606 (NL). Two different approaches were chosen:

First, we transfected pRSVCAT, a non-replicating plasmid containing the chloramphenicol acetyltransferase gene (CAT), linearized by cutting either inside or outside the CAT gene. After two days CAT activity was compared to controls transfected with the intact, circular plamid. The activity of the samples with the plasmid cut outside the gene was higher than in those cut inside the gene (averages of 1.7 - 14.4% versus 0.63 - 3.5%). No significant difference was found between Bloom's syndrome and normal cell lines, neither for plasmids with blunt, nor with overlapping ends.

In the second assay we transfected linearized pRSVCAT or pZ189, a replicating plasmid, and utilizing Southern blotting looked directly for relaxed or supercoiled circular plasmids produced *in vivo*. Plasmids with overlapping ends yielded circular forms in the NF and BSF lines, which indicates DNA ligase II activity in both cell lines. Blunt end ligation could be detected in NF; experiments to detect blunt end ligation in BF are in progress.

E 360 ONCOGENE ACTIVATION IN SKIN TUMORS FROM A REPAIR DEFICIENT SYNDROME, XERODERMA PIGMENTOSUM, Horacio G. Suarez, L. Daya-Grosjean, D. Schlaifer, P. Nardeux and A. Sarasin, Institut de Recherches Scientifiques sur le Cancer, B.P. n° 8, 94800-Villejuif (France).
The recessive autosomal hereditary disease, xeroderma pigmentosum, is characterized by a high incidence of tumors in sun-exposed skin. The defect in early steps of excision repair of xeroderma pigmentosum cells leads to hypermutability towards UV-mimicking agents. We report here activation of the N-ras oncogene in two skin tumors isolated from a xeroderma pigmentosum child. The N-ras activation, detected by the DNA transfer assay in 3T3 mouse cells, was accompanied by an increase in the level of specific mRNA and in one example, by the alteration of the p21 protein. In the same tumors, c-myc amplification and over transcription, and Ha-ras gene rearrangement were also detected. The untransformed fibroblasts from the same patient and from other XP patients show normal patterns and levels of N-ras, c-myc and Ha-ras sequences. The presence of several oncogene alterations in the same tumor is believed to be due to the large number of UV-induced DNA lesions found in the exposed skin cells, in the absence of efficient repair. The same oncogene amplifications have been found in other XP tumors that we are at present analysing.

E 361 *Escherichia coli* MUTANTS (*xmu*) RESISTANT TO GAMMA RADIATION MUTAGENESIS, Neil J. Sargentini and Kendric C. Smith, Department of Therapeutic Radiology, Stanford University School of Medicine, Stanford, CA 94305.
E. coli umuC shows about 30% of the gamma radiation mutagenesis (*argE3*oc –> Arg^+) seen in the wild-type strain (N.J. Sargentini and K.C. Smith, 1984, *Mutation Res.*, 128, 1-9). We have mutagenized the *umuC* strain with MNNG to obtain mutants deficient in this residual gamma radiation mutability. Several *xmu* (for *X*-ray *mu*tability) mutants have been obtained. One mutation, *xmu-1138*, is located at 40.8 min on the genetic linkage map for *E. coli* K-12. This mutation has been transduced into *E. coli* K-12 AB1157 and we have just begun characterizing its phenotype. The *xmu-1138* strain is quite sensitive to the lethal effects of radiation, showing dose-modification factors (at 1% survival) of 2.9 for gamma irradiation and 2.0 for UV irradiation. The *umuC xmu-1138* combination produces a synergistic sensitization of gamma- or UV-irradiated cells, suggesting that the two genes function in separate pathways for the repair of the same class of lethal DNA lesions. The *xmu-1138* ($umuC^+$) strain is nonmutable using either gamma or UV radiation, which suggests a more general role in radiation mutagenesis for this *xmu* gene than for the *umuC* gene. Since this *xmu* gene maps near the location of the *ruvAB* locus, we have used complementation tests to determine that this *xmu* gene is not *ruvA* or *ruvB*. Work supported by CA-33738, NCI, DHHS.

E 362 CHARACTERIZATION OF *E. coli* K12 *ada* MUTANTS, Diane E. Shevell, Peter K. LeMotte and Graham C. Walker, M.I.T., Cambridge, MA. 02139.
We have isolated and characterized a set of ordered deletions from the 3′ end of the *E. coli* K12 *ada* gene. These Ada deletion mutants are fusion proteins, which derive their amino-terminus from *ada* and their carboxy-terminus from the downstream vector (pBR322) sequence that occurs before an in-frame stop codon. Using a patch mutagenesis assay to screen different strains with plasmids carrying these *ada* derivatives, we separated the plasmids into three classes. (i) The first class of plasmids makes strains containing the ada^+ allele more resistant to the mutagenic and toxic effects of MNNG. These Ada derivatives constitutively activate *ada* transcription. (ii) The second class of plasmids makes strains carrying a ada^+ allele more sensitive to the mutagenic and killing effects of MNNG. These *ada* derivatives are dominant inhibitors. (iii) The last class of plasmids have no detectable phenotype in any of the strains tested. Our data suggests that the carboxy-terminus of the Ada fusion protein, which is derived from the vector, modulates the activity of the *ada* mutant. Two mutants composed of the same Ada sequences but fused to different vector derived tails are strikingly different. The gene product of the first mutant constitutively activates *ada* expression to levels 200 fold above that seen with the uninduced wild type Ada protein. In contrast, the gene product of the second mutant and the wild type Ada protein have similar effects on *ada* expression. Interestingly, while the products of these activator alleles constitutively activate *ada* expression, they are less effective than the wild type Ada protein at promoting *alkA* transcription.

E 363 REGULATION OF THE DAMAGE-INDUCIBLE *RAD2* GENE OF *S. cerevisiae*. Wolfram Siede, Gordon W. Robinson, David Kalainov and Errol C. Friedberg, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305. The *RAD1*, *RAD2*, *RAD3*, *RAD4* and *RAD10* genes of *S. cerevisiae* are required for the incision of damaged DNA during nucleotide excision repair. Of these 5 genes only *RAD2* is inducible following exposure of cells to DNA-damaging agents. We have generated a series of deletions in the 5' non-coding region of a *RAD2/lacZ* fusion gene carried on a centromeric plasmid. These plasmids were transformed into yeast cells and expression of ß-galactosidase was monitored as an indicator of *RAD2* induction. A number of deletions resulted in a reduction or loss of expression of ß-galactosidase. No deletions resulted in spontaneous induction of the fusion gene. This suggests that induction of *RAD2* is positively regulated. Several deletions identified a region located ~150-170 bp upstream of the ATG start codon that may function as an upstream activator sequence (UAS). Additionally, several poly(AT) tracts were identified in positions flanking the putative UAS that are required for normal levels of induction of the plasmid-borne *RAD2/lacZ* fusion gene. Induction of the plasmid-borne *RAD2/lacZ* fusion gene is observed in cultures which are held in stationary phase. This suggests that induction of *RAD2* is not the result of cell cycle regulation. Additionally, there is no indication of cell cycle-dependent regulation of ß-galactosidase activity in synchronized cultures. In an effort to evaluate the functional significance of *RAD2* induction we have examined the phenotype of *rad2* mutant cells transformed with centromeric plasmids carrying either the wild-type *RAD2* upstream sequence or the sequence deleted of the putative UAS. The only difference observed to date is a slight increase in UV sensitivity in late-log phase cells carrying the mutant plasmid. We are searching for mutants in genes which might regulate induction of *RAD2*. Several candidates have been isolated which show a complete defect in enhanced expression of ß-galactosidase after exposure of cells to UV radiation. These potential mutants are under detailed investigation.

E 364 RESTORATION OF HOST CELL REACTIVATION ACTIVITY OF XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP A CELLS, J. Christopher States, Children's Hospital Research Foundation, Cincinnati, Ohio 45229

Xeroderma pigmentosum is an autosomal recessive disorder caused by dimished capability to repair DNA. It is characterized by severe sensitivity to UV irradiation and high occurrence of epidermal neoplasms. Currently, there are nine complementation groups of defects in excision repair and the variant form which is defective in post-replication repair. The most severe defect in excision repair is seen in complementation group A (XPA) cells. Toward the end of isolating a cDNA for the XPA gene, an effort to complement XPA cells by gene transfer was undertaken. SV40 transformed XPA fibroblasts were cotransfected with a human fibroblast cDNA expression library and a dominant selectable marker. Transfected cells were selected for expression of the marker. Complemented XPA cells were enriched from the pools of transfectants by selecting for those cells which were able to reactivate an UV irradiated second dominat selectable marker. These pools were then screened for the ability to survive a UV dose of 6 J/m^2. Five pools which showed enhanced UV survival were then tested for the ability to reactivate a chloramphenicol acetyltransferase shuttle vector in transient assay. Two of the pools contained cells capable of host cell reactivation. Blot hybridization analysis of genomic DNA from these pools demostrates the presence of cDNA vector promoter sequences. This indicates that these pools have stably integrated cDNA expression vector sequences. Individual members of these pools are currently being cloned in order to isolate the complemented XPA cells. These should provide a means of obtaining a cDNA for the XPA gene.

E 365 SEQUENCE AND FURTHER CHARACTERIZATION OF THE IMP OPERON (I-GROUP MUTATION AND PROTECTION) OF THE PLASMID TP110. Peter Strike and David Lodwick, Department of Genetics, University of Liverpool. L69 3BX. U.K.

The imp operon of the IncI plasmid TP110 encodes the genes involved in the UV protection and mutation phenotype conferred by this plasmid. Previous studies have suggested that two genes are involved in this operon, impA and impB, and that they produce proteins of approximately 10kD and 50kD respectively. The presence of two genes has been confirmed by subcloning experiments in which the separated genes are shown to be able to complement in trans. Effective complementation is observed irrespective of the fact that the impB gene in this configuration is expressed constitutively from a vector promoter, and is no longer under rec/lex control.

Cloning of the impA gene, still under rec/lex control, into a high copy number vector results in marked sensitization of the host strain to UV irradiation. The sensitization is particularly dramatic in a uvrA background, but is not observed in recA or lexB strains. Full (enhanced) resistance can be conferred upon these sensitized strains by the introduction of high copy number clones in which impB is expressed constitutively. The sensitization effect of impA$^+$ clones may correlate with their ability to prevent induction of the SOS system, as measured by β-galactosidase production from a uvrA-lacZ fusion. Whether this is a direct repression effect or an indirect effect on the induction process is not yet clear. However, the supression of SOS induction is not overcome by the introduction of an impB$^+$ clone.

DNA sequence determination of impA$^+$ reveals an ORF for a protein of 19.5kD. As both in vitro transcription/translation of impA$^+$ DNA, and a T7-promoter-based in vivo system, indicate that impA$^+$ produces a protein of approx. 10kD, the data strongly suggest that the product of impA$^+$ is subject to rapid processing. A comparison of impA$^+$ sequences with the lexA$^+$, umuD$^+$ and mucA$^+$ gene products reveals striking amino acid homology at the C-terminal end of the protein, but very little at the N-terminal end. Taken together these data support the notion that the C-terminal end of the protein is essential for UV-induced mutagenesis, perhaps in a processed form.

E 366 REPAIR OF ALKYLATING AGENT-INDUCED DNA DAMAGE IN MOTOR NEURON DISEASES, Rup Tandan, Steven Robison and Walter Bradley, University of Vermont, Burlington, VT 05401.
The etiology of the widespread though predominantly motor system degeneration in amyotrophic lateral sclerosis (ALS) is unknown. Altered nuclear chromatin, and decreased cytoplasmic ribonucleic acid and protein content suggest that lack of normally functioning nuclear deoxyribonuclic acid (DNA), perhaps from poor repair of DNA damage, underlies this neuronal loss. We have studied repair of DNA_1 damage produced by the alkylating agents methyl methane sulfonate (MMS) and N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG) in peripheral T-lymphocytes (T-Lys) and monocytes (Mns) from normal subjects and patients with sporadic and dominantly-inherited ALS (SALS and FALS) and postpolio muscular atrophy (PPMA). We used alkaline elution (AE) to measure repair of strand breaks in T-Lys, and unscheduled DNA synthesis (UDS) to study base excision repair in Mns. Quantitative DNA damage produced by 50uM MMS and 1uM MNNG in T-Lys was similar in all groups. Mean (± SEM) repair of damage (in %) after 3 hours recovery following MMS and MNNG damage were 87 ± 3 and 85 ± 4, 60 ± 8* and 60 ± 6*, 55 ± 9* and 66 ± 6*, and 80 ± 4 and 78 ± 3 in normal (n=12), SALS (n=8), FALS (n=8) and PPMA (n=8) cells, respectively. The mean (± SEM) UDS after treatment with 300uM MMS and 10uM MNNG (measured as counts per minute /ug of DNA) was significantly lower in sporadic ALS (n=5) than normal (n=9) or PPMA (n=4) Mns (237 ± 93* and 160 ± 99*, 417 ± 72 and 797 ± 73, not done and 806 ± 298, respectively). These results indicate that SALS and FALS cells are deficient in repairing alkylated DNA, possibly of apurinic-apyrimidinic sites which result from such damage (Supported by grants from the ALS and Muscular Dystrophy Associations). *p<0.05

E 367 TRANSFER OF THE E. COLI O^6-METHYLGUANINE DNA-METHYLTRANSFERASE (MT) GENE OR A TRUNCATED FORM OF THE GENE AND THEIR EXPRESSION IN HUMAN METHYL-REPAIR-DEFICIENT CELLS RESTORES RESISTANCE TO MNNG, Kanji Ishizaki, Tohru Tsujimura, Chikau Fujio, Hideo Yawata, Zhang Yangpei,† Yusaku Nakabeppu,† Mutsuo Sekiguchi and Mituo Ikenaga, Radiation Biology Center, Kyoto University, Kyoto and† Dept. of Biochemistry, Kyushu University, Fukuoka, Japan.
A plasmid carrying the MT gene (ada gene) linked to an SV40 promoter and a poly (A) site was transferred into Mer HeLa MR cells by transfection. G418-resistant transfectants were selected for resistance to a derivative of chloroethylnitrosourea and eight independent clones tested showed MT activity. The resistance of two cell lines to killing and sister chromatid exchanges induced by MNNG was tested and found to correlate with the level of MT activity and gene copy number, suggesting that MNNG-induced damage repairable by E. coli MT is a major lethal lesion in these cells and that this same lesion is involved in SCE formation. When a second plasmid, containing a truncated MT gene (ada gene lacking the methylphoshotriester repair activity) was transferred into the HeLa MR cells, the transfectants expressing the truncated form showed the same degree of resistance to killing and SCE formation by MNNG and the same amount of host cell reactivation as those expressing the complete MT gene. This suggests that the phosphotriester does not contribute to these effects of MNNG. (Supported by Grants for Cancer Research from the Ministry of Education, Science and Culture, Japan.)

E 368 *E. coli recF* mutations reduce expression of the adaptive response to alkylating agents. Michael Volkert, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01605.
E. coli recF143 mutant strains are hypermutable by methylating agents such as methyl methanesulfonate (MMS) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). In wild type cells, the induction of the *ada* regulated adaptive response genes (*ada*, *alkA* and *alkB*) protects cells against the mutagenic and lethal effects of methylating agents. When expression of *alkA*::Mud1(Ap^r *lac*) and *alkB*::Mud1(Ap^r *lac*) fusions is compared in $recF^+$ and *recF143* mutant strains, *recF143* mutants are deficient in their ability to induce expression of *alkA* and *alkB* upon alkylation treatment. Thus, the MMS and MNNG hypermutability of a *recF143* mutant strain correlates with its decreased ability to induce genes required for repair of methylation damage. The wild type *E. coli recF* gene is also required for normal induction of the SOS response. Thus, *recF* may be involved in the regulation of both the SOS and adaptive reponses. Alternatively, *recF* may affect expression of both sets of damage inducible genes in a more general fashion similar to that seen with topoisomerase or gyrase mutants. (Supported by NIH grant GM37052).

E 369 MUTANT URACIL DNA GLYCOSYLASE IN BLOOM'S SYNDROME. T. Vollberg, G. Seal, K.Brech, S. Karp, B. Cool, and M. Sirover, Temple University, Philadelphia, PA. 19140.
Three monoclonal antibodies that react with uracil DNA glycosylase of normal human placenta were tested to determine whether Bloom's syndrome can be specifically identified by an alteration in its glycosylase molecule. As defined by enzyme-linked immunosorbent assay (ELISA), one of the monoclonal antibodies, 40.10.09, did not recognize nor inhibit uracil DNA glycosylase purified from five Bloom's syndrome cell strains. Four of the cell strains were derived from Ashkenazic Jews and one from an American Black. This lack of recognition of the Bloom's syndrome glycosylase was observed in ELISA even when 10 ug (100 fold excess) of the Bloom's syndrome enzyme was used. As defined by mixing experiments, no inhibitor was present in the Bloom's syndrome enzyme preparations. In contrast, all five Bloom's syndrome glycosylases reacted with two other anti-human placental uracil DNA glycosylase monoclonal antibodies, 37.04.12 and 42.08.07. In immunoblot analyses, the denatured glycosylase protein from all five Bloom's syndrome cell strains was immunoreactive with the 40.10.09 antibody. Thus, the alteration of the uracil DNA glycosylase gene may be identical in all five Bloom's syndrome cell strains. ELISA reactivity was observed with all three monoclonal antibodies using glycosylases from five normal human cell types, as well as thirteen other cell strains derived from individuals with inherited disorders. These results suggest that a mutation in the uracil DNA glycosylase protein may be characteristic of Bloom's syndrome. The aberrant immunoreactivity of the mutant Bloom's syndrome glycosylase to antibody 40.10.09 may serve as a molecular marker with which to specifically diagnose this human genetic disease prior to the onset of clinical symptoms.

E 370 SWITCHING FUNCTIONS IN SALMONELLA ARIZONAE LACTOSE UTILIZATION GENES, Cindy C. Wang, Massachusetts Institute of Technology, Cambridge, MA 02139.
On lactose MacConkey agar, red (phenotypically Lac+) papillae formed on S. arizonae Lac- colonies after 72 hours of growth (communication from R. Fitts). The presence of these papillae suggests that the S. arizonae lac repressor switched off, allowing β-galactosidase synthesis. The papillae were found to exhibit the same β-galactosidase activity as an S. arizonae Lac+ strain using enzyme assays. This indicated that the papillae were indeed Lac+ revertants. A 3.4 KB subclone of DNA encoding lactose utilization genes from a Lac+ Salmonella arizonae strain in a pBR322 plasmid vector has been shown to encode a protein which can repress expression of the E. coli lac operon and which binds to purified E. coli lac operator DNA [R. Fitts (submitted)]. ebg, "evolved β-galactosidase," activity is not regulated by the E. coli lacI repressor, nor does the ebg repressor, ebgR, regulate lacZ expression [B. Hall, Genetics 90:673-681 (1978)]. In this study, the S. arizonae lac repressor was found to regulate the E. coli ebg operon. In E. coli lacZ-deletion-bearing strains expressing ebg and transformed with the plasmid repressor, blue and white sectored colonies were observed on media containing XGal, a β-galactosidase indicator. This sectored appearance of the colonies suggests that the S. arizonae lac repressor switches on or off to either represss or permit β-galactosidase synthesis. The DNA sequence of these S. arizonae lac genes is not presently known, but perhaps the mechanisms and regulatory functions of the switching phenomena are related. This research was conducted in the laboratory of Dr. R. Fitts and supported in part by the M.I.T. Undergraduate Research Opportunities Program.

E 371 CLONED URACIL-DNA GLYCOSYLASE INHIBITOR GENE OF BACTERIOPHAGE PBS2: EXPRESSION IN ESCHERICHIA COLI AND PROPERTIES OF THE GENE PRODUCT, Zhigang Wang, Debra A. Elleman and Dale W. Mosbaugh, Clayton Foundation Biochemical Institute and Department of Chemistry, The University of Texas, Austin, Texas, 78712.
Uracil-DNA glycosylase inhibitor gene from Bacillus subtilis phage PBS2 was expressed in E. coli cells. This inhibitor gene product completely inactivated the E. coli uracil-DNA glycosylase both in vitro and in vivo. The biological effects of the inhibitor protein on E. coli cells were studies. Expression of this inhibitor gene in E. coli cells resulted in (i) an ung phenotype; (ii) a weak mutator phenotype as determined by spontaneous mutation rate to nalidixic acid and chloramphenicol resistance; (iii) a similar sensitivity to the antifolate drug aminopterin as cells lacking the inhibitor gene; (iv) a slightly increased resistance to the lethal effects of 5-fluoro-2'-deoxyuridine; and (v) no significant change in growth rate relative to cells lacking the inhibitor gene. The nucleotide sequence of the inhibitor gene has been determined and the gene product purified and characterized. It is a heat stable acidic protein with a native molecular weight of about 18,000. It also efficiently inhibited uracil-DNA glycosylase from other sources including human placenta, rat liver nuclei and mitochondria. The mechanism of uracil-DNA glycosylase inhibition by this inhibitor protein will be discussed. These findings suggested an alternative approach for obtaining mutant cells by selectively expressing the inhibitor gene in other target cells. (Supported by grants from NIH GM32823 and BRSG RR07091.)

E 372 **CLONING OF A HUMAN GENE AND ITS cDNA THAT CORRECT THE NUCLEOTIDE EXCISION REPAIR DEFECT IN CHO COMPLEMENTATION GROUP 1.** C.A. Weber, E.P. Salazar, S.A. Stewart, and L.H. Thompson, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

Nucleotide excision repair removes bulky chemical adducts and UV-induced photoproducts from DNA. The cancer-prone genetic disorder xeroderma pigmentosum is caused by defects in the incision step of nucleotide excision repair. The incision deficient, UV-sensitive Chinese hamster ovary (CHO) cell line UV5 was used to identify a human gene that corrects this repair deficiency. Transformation of UV5 with DNA from a human/hamster hybrid resulted in repair proficient transformants. Cosmid clones containing the human gene, *ERCC2*, were isolated by using human DNA to probe a library constructed from DNA of a secondary transformant. Several cosmid clones complemented the UV5 defect. The quantitatively efficient functioning of *ERCC2* in UV5 was demonstrated by comparing genomic and cosmid transformants to wild-type CHO in terms of UV-induced cell killing, mutations at the *aprt* locus, and the rate of DNA incision. Restriction enzyme fragments of cosmid DNAs were used to screen the pCD2 human cDNA expression library (obtained from H. Okayama). A cDNA with a 2.8 kb insert, which confers primarily transient UV resistance to UV5, was isolated. DNA sequencing has located the poly-A addition site and, along with analysis of overlapping cosmids, indicates *ERCC2* is ~19 kb. Further sequencing is in progress and will be used to analyze gene structure, determine the amino acid sequence of the protein, and to assess homology with known yeast and *E. coli* excision repair genes. The ability to use CHO mutant lines to obtain clones of human DNA repair genes is of general importance to the study of DNA repair, particularly because the use of defective human cell lines in transformation experiments with genomic DNAs has proven difficult. Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.

E 373 FAULTY RADIATION-INDUCED REPAIR IN T-CELL CLONES DERIVED FROM "WASTED" MICE, Gayle E. Woloschak and Christopher Krco, Argonne National Laboratory, Argonne, IL 60439, and Mayo Clinic, Rochester, MN 55905.

Mice bearing the autosomal recessive mutation "wasted" (wst/wst) display three major abnormalities: (1) faulty repair of DNA damage in lymphocytes in response to ionizing radiation, (2) immunodeficiency at mucosal sites, and (3) neurologic dysfunction. Although this mutation maps to a single gene or several tightly linked genes on chromosome 2, the molecular basis for the combined DNA repair/immunodeficiency/neurologic abnormalities is unknown. A T-cell line was derived by stimulating ficoll-purified splenic lymphocytes from wst/wst mice with Concanavalin A (Con A), Interleukin 2 (IL2), and irradiated feeder cells. The bulk culture cell line (called wst T_1) shows typical growth-response patterns to Con A and IL2 but is nontransformed. Control Q-T T-cells derived from normal B10·D2 mice are similarly responsive to Con A/IL2/feeder cells. Cultures of $5x10^6$ wst T_1 or Q-T cells were exposed to 30, 300, 650 or 1000 cGy of ^{137}Cs γ-rays (dose rate 327 cGy/min). After exposures as low as 300 cGy, wst T_1 cells repeatedly showed blast-like responses within the first 1-4 h; by 24 h, fewer than 10^4 cells were present in the culture medium. These cells were unable to recover sufficiently to exhibit cell growth in response to IL2/Con A/feeder cells. In contrast, Q-T cells did not form blasts in response to irradiation and were able to recover from exposures of 1000 cGy. These experiments demonstrate the presence of a DNA-repair defect in Con A/IL2-responsive T-cells of wst/wst mice and provide a means by which to clone genes that correct for the defect. Supported by Fraternal Order of Eagles Grant #72, The Mayo Foundation, and the U.S. DOE under Contract No. W-31-109-ENG-38.

E 374 CHARACTERISTICS OF A HERPESVIRUS GENE ENCODING URACIL-DNA GLYCOSYLASE, Diane M. Worrad and Sal Caradonna, UMDNJ-SOM, Piscataway, N.J. 08854. Herpes simplex virus (HSV) has been found to encode a uracil-DNA glycosylase enzyme which removes uracil from DNA. Uracil-DNA glycosylase activity in type 2 (HSV-2), strain 333, is higher than its activity in HSV-1, strain F. A HSV-2 cDNA library was screened by hybrid arrest and in vitro translation. Experiments revealed one isolate, #184, which showed marked inhibition of HSV mRNA translatable uracil-DNA glycosylase activity. Isolate 184 was used to hybrid select a RNA species which, when translated, produced significant glycosylase activity. Translation of hybrid selected mRNA in the presence of ^{35}S-labeled methionine and electrophoretic analysis revealed products of molecular weight 39,000, 32,000, and 28,000. Purification of glycosylase from HSV infected cell extracts showed a monomeric molecular weight band of 39,000. This major protein component was then subjected to a renaturation protocol. Subsequently, uracil-DNA glycosylase was correlated with the major protein band of 39,000d. Northern analysis found five overlapping transcripts which hybridized to isolate 184. Isolate 184, 1.25kb, was used as a probe and mRNAs of 3.4, 2.8, 2.4, 1.7, and 1.0kb were selected. All these transcripts were generated from the same coding strand. Southern analysis of HSV-1 revealed that isolate 184 was of viral not cellular origin and that it was located between 0.065 and 0.08 map units on the prototypic arrangement of the HSV genome. The presentation will include a further characterization of these five transcripts.

E 375 NEW COMPLEMENTATION GROUPS IN REPAIR-DEFICIENT CHINESE HAMSTER CELLS AND THEIR CHARACTERISTICS, Małgorzata Z. Zdzienicka, N.G.J. Jaspers* and J.W.I.M. Simons, Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands and *Department of Cell Biology and Genetics, Erasmus University, 3000 DR Rotterdam, The Netherlands.

As a complete set of DNA-repair deficient mutants should ultimately help to recognize the mechanism of DNA repair in mammalian cells, Chinese hamster cell lines defective in cellular response to DNA damaging agents were isolated. Three different general categories of mutants: sensitive to cross-linking agents, UV- on X-ray-irradiation are being characterized. Complementation analysis of the UV-sensitive mutants revealed a new seventh complementation group in addition to the six groups described by Thompson et al. (1987). Also a phenotypic heterogeneity within the first complementation group was observed. The same degree of UV sensitivity with widely different levels of nucleotide excision repair suggests that the repair gene of this complementation group has more than one functionally important domain, with only one domain involved in incision (Zdzienicka et al., 1987). Among the X-ray-sensitive mutants at least two complementation groups were identified. One mutant belongs to the complementation group of mutants isolated by Jeggo and Kemp (1983) and appears to be phenotypically similar: defective in DNA double strand breaks repair and normal in DNA single strand breaks repair. The other X-ray sensitive mutants show lack of DNA synthesis inhibition after X-ray irradiation and therefore are the first mutants isolated in rodent cells which behave like cell lines derived from patients with Ataxia telangiectasia. One mutant sensitive to cross-linking agents and not sensitive to UV represents a new complementation group in addition to the three groups found by Robson and Hickson (1986).

E 376 REGULATION OF ABUNDANCE OF DNA POLYMERASE β mRNA AND DNA DAMAGE IN CULTURED MAMMALIAN CELLS, Barbara Zmudzka, Albert J. Fornace and Samuel H. Wilson, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

β-polymerase is a constitutively expressed "housekeeping" enzyme required for essential DNA metabolism events separate from replication of genomic DNA. DNA synthesis during DNA repair is an example of such a DNA metabolism event, and the idea that β-polymerase is involved in DNA repair has been strongly supported by inhibitor studies. We used a cDNA probe to study abundance of the β-polymerase mRNA in cultured cells. In synchronized HeLa cells, representing different stages of the cell-cycle, the mRNA level varied in a 2-fold range and was highest in mid G1. The mRNA level was sharply regulated in CHO cells treated with some, but not all, DNA damaging agents. Treatment of cells with high doses of damaging agents, bleomycin, cis Pt, adriamycin, nitrogen mustard, mitomycin-c, TPA, UV or near-UV, did not lead to an increase in mRNA level. By contrast, 4 hr after treatment of cells with 100μg/ml MMS, 0.4mM H_2O_2, 20μM AAF, 30μM MNNG, or 700 rads X-ray, the mRNA level was 3 to 5-fold higher than in untreated cells. Correlation of these differences with the level of β-polymerase enzymatic activity was studied. The results suggest regulation of β-polymerase in response to some types of DNA damage.

Mutagenesis and Tolerance Mechanisms

E 400 STUDIES OF DNA REPAIR DNA POLYMERASES ON TEMPLATES WITH DEFINED DAMAGE, John Abbotts, Gerald Zon*, and Samuel H. Wilson, National Cancer Institute and *U.S. Food and Drug Administration, Bethesda, MD 20892

The DNA repair DNA polymerases, E. coli Polymerase I large fragment (Pol I lf) and human DNA polymerase beta have been examined for their activity on template-primers with defined DNA damage. The template regions are synthetic oligomers of 24 nucleotides, each consisting of poly dT except for one damage-related alteration at a single site: O6-Me-dG, N3-Me-dT, or an OEt-phosphate group. Under conditions of processive synthesis with Pol I lf (low enzyme:template ratios and short incubation times), the methylated bases act as nearly absolute blocks for chain elongation. Higher enzyme amounts and dNTP concentrations can partially overcome this block. Pol I lf proceeds past the O6-Me-dG site with the substrate preference of dTTP>dCTP>dATP>dGTP, and proceeds past the N3-Me-dT site chiefly by inserting dATP. The ethylated phosphate group does not absolutely block processive synthesis, but does increase the frequency of termination of synthesis at the damage-site as well as several nucleotides downstream. The methylated bases serve as strong blocks to elongation by DNA polymerase beta. Even at relatively high concentrations of enzyme and with all four dNTPs, this polymerase synthesizes past the damage-site only at a very low frequency. That repair DNA polymerases "stall" at positions opposite a methylated base may provide cells with a defense against misincorporation, allowing more time for alkyl removal before the extension of synthesis. Use of such template-primers with defined damage sites allows more precise analysis of DNA repair phenomena.

E 402 ANALYSIS OF GENETIC ALTERATION AT THE APRT LOCUS IN DNA REPAIR-PROFICIENT OR -DEFICIENT CHO CELLS, Gerald M. Adair, Julia B. Scheerer, Christy MacKinnon, and Ann Brotherman, The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX 78957.

We are using the endogenous aprt locus in CHO cells to investigate spontaneous and induced mutation. Using the aprt$^{+/o}$ hemizygote CHO-AT3-2, we have isolated a series of UV-hypersensitive, DNA repair-deficient mutants which show cross-sensitivity to a variety of mutagens. These cell lines permit comparison of mutation spectra in repair-proficient versus repair-deficient genetic backgrounds. Southern blot analysis of DNAs from spontaneous AA^r mutants has revealed distinctive spectra of restriction site loss or site gain mutations, deletions, insertions, or rearrangements involving the aprt gene. Restriction site-loss or site-gain mutations have been detected at 28 different sites along the aprt gene. Analysis of 180 spontaneous AA^r mutants of CHO-AT3-2 revealed 18 site-loss or site-gain mutations and 7 deletions. Analysis of 254 mutants isolated from repair-deficient cell lines revealed 17 site-loss or site-gain mutants and 9 deletion mutants, plus 8 mutants with rearrangements involving the aprt locus. Analysis of 151 AA^r mutants induced by 254nm UV light revealed 11 with site-loss or site-gain mutations; none showed deletions. Site-gain mutations permit identification of both the site and specific base change involved, and often provide insight as to the probable mutagenic lesion. (Supported by PHS Grants CA-28711 and CA-04484).

E 403 A RAT NUCLEAR PROTEIN IMMUNOLOGICALLY RELATED TO THE E. COLI RECA PROTEIN : DETECTION AND MITOMYCIN-C INDUCTION.
Jaime F. Angulo, Patrice L. Moreau, Jean Laporte, Anne-Marie Hill and Roger Bertolotti.
Laboratoire d'Enzymologie, C.N.R.S. 91190 Gif-sur-Yvette, France.

Using immunoblotting techniques, we have shown that a polypeptide in rat FR3T3 cells was recognized by antibodies raised against E. coli RecA protein. This RecA-related-antigen (termed RRA1) had a monomeric molecular weight of about 120 KD. Cellular levels of RRA1 doubled during active proliferation as compared to resting cells. A further two-fold increase in RRA1 levels was noted following treatment with mitomycin C.

Immunocytochemical studies have shown that RRA1 was mainly concentrated at the nuclear periphery in the vicinity of the inner nuclear membrane.

E 404 PSIB POLYPEPTIDE IS AN ANTAGONIST OF RECA PROTEIN IN E. COLI, Adriana Bailone, Assar Backman, Suzanne Sommer, Jerome Celerier, Mira M. Bagdasarian, Michael Bagdasarian and Raymond Devoret G. E. M. C., Enzymologie, C.N.R.S., F-911190 Gif-sur-Yvette, France and Michigan Biotechnology Institute, Lansing, MI 48909, USA. We found a new plasmid function preventing SOS induction, and thus called Psi (Plasmid SOS Inhibition). Here we provide a mechanism for Psi function. PsiB polypeptide (12 kDa) antagonizes all the cellular functions governed by RecA protein such as intra-chromosomal recombination, cell mutagenesis, and resistance to UV-irradiation. Inhibition of RecA protein activities are dependent on the level of PsiB polypeptide, which is expressed by psiB, a gene located near oriT, the orgin of conjugal transfer, at coordinates 54.9 of plasmid R6-5. The anti-RecA protein action of PsiB polypeptide can be accounted for by an indirect effect of PsiB on the activation of RecA protein.

E 405 RecA-MEDIATED CLEAVAGE ACTIVATES UmuD FOR MUTAGENESIS, John R. Battista, Takehiko Nohmi, Lori A. Dodson, and Graham C. Walker, MIT, Cambridge, MA 02139. UmuD shares significant amino acid sequence homology with LexA repressor, which is cleaved at an ala-gly site in a RecA dependent reaction. Cleavage of LexA is believed to occur by self digestion, catalyzed by residues ser-119 and Lys-156 of LexA. RecA is considered a positive effector, which stimulates the autodigestion. Recently, evidence establishing that UmuD is also cleaved in a RecA-dependent manner has been presented. We have performed a series of genetic experiments which indicate that this RecA-mediated cleavage activates UmuD for its role in mutagenesis and that the COOH-terminal fragment of cleaved UmuD is necessary and sufficient for the role of UmuD in UV mutagenesis. Using site-specific mutagenesis, a family of mutants were constructed at the cleavage site (cys-24-gly-25 bond) and at the putative catalytic residues (ser-60 and lys-97). By analogy to similar mutants in LexA, these mutant UmuD proteins would be expected to be non-cleavable. Each of these UmuD mutations substantially reduce mutability but do not eliminate it entirely. In addition, a mutant was generated in which nucleotides 4-72 of umuD were deleted. This mutant gave rise to a protein equivalent to the COOH-terminal fragment of cleaved UmuD. The COOH-terminal fragment complements a umuD44 background and restores mutability to a recA430 background. Interestingly this does not restore mutability to a lexA(Def)ΔrecA strain suggesting a previously unknown third role for RecA in UV mutagenesis.

E 406 Sexual Reproduction as a DNA Damage Processing Mechanism, Carol Bernstein and Virginia Johns, University of Arizona, Tucson, AZ 85724. The adaptive function of sexual reproduction is a major unsolved problem in evolutionary biology. In other words, it is not known why sex exists. We recently proposed that the fundamental selective advantage of sex is its promotion of recombinational repair and hence survival of DNA in the germ line of organisms. If sex is primarily an adaptation for repairing DNA damage then a facultatively sexual/asexual organism should use the sexual cycle more frequently when DNA damage increases. Hydrogen peroxide (H_2O_2) and its free radical product ($OH^{\cdot}$) are by-products of oxidative cellular metabolism. They have been identified as an important natural source of DNA damage in cells. For instance, there are several thousands of oxidative DNA hits per cell per day from this source in humans.

The single-celled fission yeast Schizosaccharomyces pombe can use the mitotic cycle and proliferate vegetatively or use the sexual cycle to conjugate, undergo meiosis and sporulate. We treated S.pombe with either nothing or else increasing levels of H_2O_2. Treatment with H_2O_2 to yield fractional cell survivals of 0.05 to 0.9 increased the frequencies of sexual spores in populations of cells in late exponential phase and early stationary phase by 7-10 fold. The level of sporulation went from <0.1% (late exponential phase) and 4% (early stationary phase) in the untreated population to almost 1% (late exponential) and 30% (early stationary) in the treated populations. Thus, S. pombe responds to DNA damage by shifting a substantial fraction of cells into the sexual cycle. This supports our hypothesis that sexual reproduction is an adaptation for repairing damage in germline DNA.

E 407 CARCINOGEN-INDUCED HOMOLOGOUS RECOMBINATION BETWEEN DUPLICATED CHROMOSOMAL SEQUENCES IN MAMMALIAN CELLS, Nitai Bhattacharyya, Veronica M. Maher, and J. Justin McCormick, Michigan State University, East Lansing, MI 48824-1316.
A series of structurally-related nitroaromatic carcinogens, 1-nitrosopyrene (1-NOP), N-acetoxy-2-acetylaminofluorene (N-AcO-AAF), and 4-nitroquinoline-1-oxide (4-NQO) were compared with the 7,8-diol-9,10-epoxide of benzo[a]pyrene (BPDE) for their ability to cause homologous recombination between duplicated thymidine kinase (tk) genes located on a plasmid stably integrated into $tk^{-/-}$ mouse L cells (Liskay et al., C.S.H.S.Q.B., 49: 189, 1984). The target tk genes from Herpes virus are separated by a segment of DNA containing the neo gene and each contains a frameshift mutation (an 8 bp Xho I restriction enzyme site) inserted into a different place. Only by undergoing a productive recombinational event between the two non-functional genes can a functional gene product be made by the cell and the recombinant selected with HAT medium. With this system, we have demonstrated dose-dependent increases in intrachromosomal homologous recombination induced by these four agents. When the frequency of recombination was compared as a function of concentration, BPDE was the most potent, then 1-NOP, and 4-NQO and then N-AcO-AAF. When compared on the basis of equal cell killing, the carcinogen most efficient at inducing homologous recombination was 1-NOP, followed by N-AcO-AAF, BPDE, and 4-NQO. When compared on the basis of equal numbers of adducts initially bound to DNA, the most efficient agent tested was BPDE, followed by 1-NOP, 4-NQO, and N-AcO-AAF. Molecular analysis of the types of recombinational events indicated that 85% represented gene conversion events, the same as is found with this cell line for spontaneous homologous recombination. Supported by Grants DHHS CA21253 and Health Effects Institute 87-2.

E 408 MUTAGENIC CONSEQUENCES OF PURINE-STARVED DNA REPAIR, Diane Black and Andrew Collins, Department of Biochemistry, University of Aberdeen, AB9 1AS, Scotland
When the purine auxotroph CHO cell line Ade-C, defective in glycinamide ribonucleotide synthetase (GARS), is starved for purines by incubating without hypoxanthine, DNA replication soon ceases, but repair of UV-induced damage continues in an aberrant fashion. Incision, repair synthesis and ligation take place, but lesions seem to remain in the DNA. Aberrant repair is highly mutagenic. Ouabain-resistant cells arise after UV irradiation at four times the rate in purine-starved as in unstarved Ade-C cells. Partial revertants also appear among cells UV irradiated in the purine-starved state. The broad spectrum of hypoxanthine dependence seen in such cells derived from a single clone suggests the possibility of gene amplification rather than true reversion. This is supported by enzymatic analysis of GARS activity; V_{max} doubles during further selection of "revertant" cells with low hypoxanthine, and renders the cells capable of surviving without added hypoxanthine, even though the GARS activity is still only a few per cent of that in wild-type cells. Mutagenesis involving the GARS gene is of particular interest since, in humans, this gene is located on chromosome 21 close to the locus for Down's syndrome.

E 409 PROGRESS IN THE ISOLATION OF A MAMMALIAN POST REPLICATION RECOVERY GENE, S.D. Bouffler and R.T. Johnson, Cambridge University, Cambridge CB2 3EJ, England.
SVM is a UV sensitive, SV_{40} transformed Indian muntjac cell line. The UV sensitivity is accounted for in part by a poor capacity to replicate DNA on a damaged template (Pillidge et. al. 1986 Int. J. Radiat. Biol. 50 119-136). In this respect SVM may be similar to the PRR defective XP variant.

Co transfection of mouse genomic DNA and pSV2neo into SVM followed by G418 and UV selection has led to the isolation of clones with improved UV survival and PRR. One such primary transfectant contains approx 1/200th of a mouse genome. In a secondary co transfection (1° transfectant DNA + pSV2neo into SVM) experiment a clone with the improved phenotype, approx 50 neo copies and approx 1/1000th of a mouse genome was isolated. In a third round of transfection, linked transfer of pSV2neo and the putative PRR gene was achieved. The tertiary transfectant clone contains only 1 neo copy and approx 1/10000th of a mouse genome.

Currently cosmid and lambda libraries generated from tertiary transfectant DNA are being screened to allow the isolation of this mouse PRR gene. Further progress will be reported at the meeting.

E 410 INFLUENCE OF POST-REPLICATION REPAIR ON TRANSFORMATION OF HUMAN FIBROBLASTS, Jayne C. Boyer, William K. Kaufmann, David G. Kaufman and Marila Cordeiro-Stone, Department of Pathology and Curriculum in Toxicology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

We are studying the effect of post-replication repair (PRR) on transformation of diploid human fibroblasts to anchorage independence (AI). PRR may be defined as the process of growth of nascent DNA replicated from a template containing carcinogen-induced lesions which are blocks to DNA replication. This was quantified by the measurement of inhibitions of DNA synthesis and rate of DNA strand growth induced by ultraviolet radiation (UV) and benzo[a]pyrene-diol-epoxide (BPDE). Slopes determined in dose-response studies indicate that xeroderma pigmentosum variant (XPV) fibroblasts have 25%-33% of the PRR observed in normal cells after UV treatment. However, the same level of PRR was observed in both cell types after BPDE treatment. Cytotoxicity studies confirmed that XPV cells, by comparisons to normal fibroblasts, exhibit the same sensitivity to killing by BPDE but are more sensitive to UV. We are now measuring frequencies of transformation to anchorage independence in normal and XPV fibroblasts and dose-response relationships are being generated. Other laboratories have shown that XPV fibroblasts exhibit enhanced sensitivity to mutagenesis and transformation by UV but are not hypermutable by BPDE. These observations are consistent with post-replication repair involvement in increasing the probability of cell survival and also reducing the cellular risk of transformation. (Supported by PHS Grants CA20658 and CA40504)

E 411 UV MUTAGENESIS IN E.COLI, Bryn A. Bridges, MRC Cell Mutation Unit, University of Sussex, Brighton, Gt. Britain.

UV mutagenesis has been postulated to occur in two steps, misincorporation and bypass (Bridges and Woodgate, PNAS, 82 (1985) 4193-4197). Misincorporation can be seen as mutation induction in UV-irradiated umuC,D or lexA (Ind^-) strains rescued by delayed photoreversal (DPR). DPR mutagenesis exhibits the mutation frequency decline phenomenon with ochre suppressor mutations, like normal UV mutagenesis. It does not, however, require RecA protein, indeed it may be inhibited by RecA protein. With an in vitro system, no evidence was found for incorporation of bases opposite photoproducts by PolI or PolIII holoenzymes in the presence of RecA protein. In fact, when bases had been inserted opposite photoproducts by PolI under the influence of Mn^{++}, they were removed by PolIII holoenzyme in the presence of RecA protein.

E 412 GENETIC CHARACTERIZATION OF SOS INDUCED-UNTARGETED MUTAGENESIS OF PHAGE : INVOLVEMENT OF DNA POLYMERASE I, Geneviève Maenhaut-Michel and Perrine Caillet-Fauquet, Université libre de Bruxelles, B-1640 Rhode St Genèse, Belgium.

UV-induced untargeted mutagenesis of phage is the increase over the background mutagenesis observed when unirradiated phage are grown in irradiated E.coli host bacteria. It was suggested that untargeted mutagenesis results from a transiently decreased fidelity of the replication complex induced in response to the block of the replication fork by the presence of UV damage in the host genome. The expression of phage λ untargeted mutagenesis and of an altered form of DNA polymerase I (PolI) showed a great similarity of genetic requirements. This has encouraged us to study expression of phage untargeted mutagenesis in well characterized polA mutants. We have also compared the expression of untargeted mutagenesis of single-stranded and double-stranded DNA phages in different mutant hosts. Our results show that : i) the polymerase but not the exonuclease 5' → 3' activity of PolI is required for the expression of untargeted mutagenesis of phage λ and M13 ; ii) proficient excision repair is required for double-stranded but not for single-stranded DNA phage ; iii) the umuC function which is not required for untargeted mutagenesis of the double-stranded DNA phage λ , is necessary for untargeted mutagenesis of the single-stranded DNA phage M13 and ØX174 ; iv) in opposition to what was found for phage λ, untargeted mutagenesis of M13 is constitutively expressed in the recA730 mutant in which RecA protein is constitutively activated by the 730 mutation. We concluded that the process of untargeted mutagenesis involves different SOS proteins depending on whether its DNA substrate is single- or double-strand.

E 413 EFFECT OF DNA REPAIR ON SITE-SPECIFIC TRANSITIONS FROM O^6-ALKYL G:C AND O^6-ALKYL G:T
R. Chambers, E. Gojska, S. Hojatti and H. Borowski. Dalhousie Univ. Halifax, Nova Scotia, Can.
Using the site-specific mutagenesis system developed in our laboratory, we have measured the transition frequencies produced from a single O^6-MeG:C, O^6-BuG:C, O^6-MeG:T or O^6-BuG:T located at either the first or second position the third codon of gene G in ΦX174 RF I' DNA. Transitions were measured *in vivo* using spheroplasts with either normal DNA repair or a defect in excision, in recombination, or in both. Specific phenotypic screens based on well characterized mutants were used to determine the transition frequencies. In these experiments, repair by excision or recombination will decrease the transition frequency from both G*:C and G*:T (where G*=alkylG). Methyl transfer repair will decrease the frequency from G*:C, but increase it from G*:T.

Spheroplast Phenotype	BuG:C % mutants	BuG:T % mutants	MeG:C % mutants	MeG:T % mutants
$UvrA^+$ $RecA^+$	0.3	0.3	14	5
$UvrA^-$ RecA+	2	2	0.4	14
$UvrA^+$ $RecA^-$	0.4	0.4	2	11
$UvrA^-$ $RecA^-$	4	2.5	<0.2	16

As expected the mutation frequency depends upon the nature of the alkyl group and the status of DNA repair. The data indicate that BuG is repaired primarily by excision. The high mutation frequency produced by MeG in cells with normal repair and the effect seen in *uvrA* cells was unexpected since MeG believed to be repaired by alkyl transfer. We suggest that the UvrABC excision inhibits alkyl transfer repair by stalling, without cutting, at MeG. Release of this inhibition in *uvrA* cells decreases the mutation frequency from MeG:C and increases it from MeG:T. RecA protein may show a similar effect.

E 414 REPAIR OF LESIONS NEAR DNA REPLICATION FORKS REQUIRES SOS INDUCIBLE REPAIR SYNTHESIS,
Priscilla K. Cooper, Sherry Gee, and Vincent Ling, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.
SOS induction in E. coli affects two aspects of the excision repair process: levels of the uvrABC excision nuclease are increased, and the long patch pathway of repair synthesis is induced. The SOS-regulated (long patch) component of repair synthesis operates at a small fraction of damage sites whether the initial step of the excision repair is uvrABC excision or glycosylase-AP endonuclease incision. Our previous work has suggested a requirement for induced excision repair in SOS-mediated resistance to both the lethal and replication-blocking effects of DNA damage. This is true for damage introduced by treatment with alkylating agents as well as that resulting from UV irradiation, suggesting that it is the induced repair synthesis that is required. One possible model for a specialized role for this SOS repair process is that lesions introduced into DNA in the vicinity of replication forks are refractory to constitutive excision repair because of structural constraints but that the induced long patch repair synthesis pathway is able to operate at such lesions. We have developed a two-dimensional agarose gel electrophoresis method for separating DNA fragments that contain replication forks from linear DNA fragments in order to test this hypothesis. Using this technique, we have found that a significant proportion of radioactive label incorporated into long repair patches migrates with the DNA fragments that contain replication forks, whereas essentially none of the label in constitutive short repair patches is in this position. We conclude that lesions in the vicinity of replication forks are repaired by SOS-induced long patch excision repair.

E 415 CHROMATIN STRUCTURAL CHANGES PRECEDE REPLICATION IN INITIATED REPLICONS DURING INHIBITION OF DNA ELONGATION,
Joseph A. D'Anna, Deborah L. Grady, and Robert A. Tobey, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.
Partial inhibition of DNA elongation by several agents produces changes in the composition and structure of bulk chromatin that substantially exceed the extents of DNA replication. It has been proposed that the measured depletion of histone H1 and changes in chromatin structure occur preferentially in initiated replicons and that they can precede the replication fork to span a large part of or the complete replicon [D'Anna and Prentice, Biochemistry 22, 5631 (1983)]. To begin to test this notion we have synchronized Chinese hamster (line CHO) cells in early S phase by allowing G1 cells to enter S phase in the presence of 1.0 mM hydroxyurea. This procedure appears to produce an accumulation of partially elongated initiated replicons arising from early replicating DNA. Comparison of changes in the nucleosome repeat lengths of bulk chromatin, the early replicating unexpressed gene metallothionein II (MTII), and a later replicating repeated sequence between G1 and early S phase cells indicate the following: chromatin structural changes occur preferentially in the early replicating MTII region and exceed by 5-7 bp those seen for bulk chromatin or the later replicating repeated sequence. Since less than 10% of MTII is replicated in the early S phase cells, the results are consistent with the hypothesis that chromatin structural changes occur preferentially in initiated replicons and can precede the fork during inhibition of DNA elongation. These events appear to reflect an uncoupling of the unfolding of replicons from DNA synthesis at the fork and may be an important component in genomic damage and genomic rearrangements in S phase cells. (Supported by the Los Alamos National Laboratory under the auspices of the U.S.D.O.E.)

E 416 MUTAGENESIS AND REPAIR OF UV IRRADIATED BACTERIOPHAGE LAMBDA MAY REQUIRE REC DEPENDENT LEXA INDEPENDANT GENES, Patrick Calsou, Antonio Villaverde and Martine Defais, Laboratoire de Pharmacologie et de Toxicologie Fondamentales, CNRS, Toulouse, France.

The activated form of the RecA protein ($RecA^*$) is known to be involved in the reactivation and mutagenesis of UV irradiated bacteriophage lambda and in the SOS response in Escherichia coli K12. In lexA(Def) strains, in which lexA repressor is inactivated, repair and mutagenesis of damaged phage require $RecA^*$ dependent, lexA independent de novo protein synthesis. Fusion mutants isolated in the lexA(Def) background, are induced only in the presence of activated RecA protein.

E 417 SOS INDUCTION OF DUPLICATIONS IN E. COLI, Joan Dimpfl and Harrison Echols, University of California, Berkeley, CA 94720.

The SOS response in E. coli results from expression of a multioperon network, providing an inducible system for DNA repair and point mutagenesis. We have found that tandem duplications at the glyS locus rise approximately ten-fold during a constitutive SOS response, indicating an additional form of SOS-controlled mutagenesis not requiring DNA damage. Preliminary experiments suggest that the UmuC protein is not essential for the induction of duplications. These duplications could represent an important form of increased genetic variation during environmental stress.

E 418 MUTATIONAL SPECTRUM AND RECOMBINOGENIC EFFECTS INDUCED BY AMINOFLUORENE ADDUCTS IN BACTERIOPHAGE M13, Richard Doisy, Jeffrey Ross, and Moon-shong Tang, The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX.

Double stranded replicative form (RFI) DNA of bacteriophage M13 strain M13mp10 which carries partial *lacZ* gene has been modified *in vitro* to various extents with N-hydroxy-2-amin-fluorene (N-OH-AF) and then transfected into *E. coli* cells. High performance liquid chromatography (HPLC) analysis results demonstrate that the sole adduct (95%) formed in modified DNA is (deoxyguanosine-8-yl)-2-aminofluorene (dG-C8-AF). Approximately 20 adducts per RFI molecule constitute one lethal event when plaque forming ability if assayed on *E. coli* cells which have received no prior SOS induction. The mutagenicity of dG-C8-AF adducts was assayed by measuring loss of β-galactosidase activity as a function of adducts per molecule. A dose-dependent increase in Lac^- mutants was observed, with a four-fold increase in mutants per survivor at 30 adducts per molecule. The mutations produced, characterized by DNA sequencing, occur predominately at either G or C positions different from those observed in the spontaneous mutant spectrum. Restriction mapping results show that in our assay system, dG-C8-AF adducts induces a previously-unreported recombinogenic activity.

E 419 MECHANISMS OF CAFFEINE POTENTIATION OF DNA DAMAGE, C.Stephen Downes, Stephen R.R.Musk and Robert T.Johnson, Cambridge University, Cambridge CB2 3EJ, England. Caffeine potentiates the lethal and clastogenic effects of DNA damaging agents through complex mechanisms. In some cells it inhibits the post-replication recovery (PRR) system, through which DNA synthesis circumvents lesions in the template; a major mode of action is thought to be through the formation of double-strand DNA breaks (DSBs) at sites of PRR block. Also, caffeine overrides the delay in cycle progression imposed by DNA damage. We have found two lines of Indian muntjac cells, particularly suitable for chromosome studies, with complementary deficiencies in the response to caffeine after UV. In the DM line, PRR is sensitive to caffeine; cells accumulate DSBs at sites where PRR is inhibited; and chromosome aberrations and sister chromatid exchanges (SCEs) are moderately enhanced by caffeine. However, the DM cell cycle delay after irradiation is not affected by caffeine. In the line SVM84, where caffeine enormously enhances lethality, aberrations and SCE formation, the caffeine-sensitive PRR system has been lost; the remaining PRR is caffeine-resistant; but caffeine still boosts DSB formation, possibly through direct endonuclease stimulation. However, cycle delay in SVM84 is overcome by caffeine. The concentration dependence, and sensitivity to other methylxanthines, of the PRR and cycle effects are entirely different, suggesting separate mechanisms. In consequence of the cycle delay override, caffeine induces premature chromosome condensation in SVM cells, but not in DM, after DNA damage, or after DNA synthesis inhibition by other drugs. Resistance of DM to cycle override offers a method for isolating the gene(s) involved.

E 420 RECA MUTATIONS THAT DISCRIMINATE REPRESSORS.
M. Dutreix, P.L. Moreau, A. Bailone, F. Galibert and R. Devoret.
Laboratoire d'Enzymologie, C.N.R.S., 91190 Gif-sur-Yvette, France.

The RecA protein of E. coli promotes the cleavage of various repressors. We have isolated recA mutants deficient specifically in cleavage of either phage Ø80 repressor (recA1734) or LexA protein (recA1730). Mutations recA1734 (Arg243->Leu), recA1730 (Ser117->Phe) and recA430 (Gly204->Ser) (the latter mutation has been described as preventing λ repressor inactivation) could alter different sites in the RecA protein which are implicated respectively in the recognition of each type of repressor.

These mutants are defective in UV-induced mutagenesis in a lexA(Def) background and they are UV-sensitive except the lexA(Def) recA1730 strain which is UV-resistant. These results suggest that the RecA1734 and RecA430 proteins may be deficient in both recombinational and mutagenic repair processes, whereas the RecA1730 protein would be specifically deficient in mutagenic repair processes.

E 421 PYRIMIDINE DIMERS BLOCK SV40 REPLICATION FORKS,
Howard J. Edenberg, Indiana University School of Medicine, Indianapolis, IN 46223.

We have examined the immediate responses of SV40 replication forks to the introduction of cyclobutane pyrimidine dimers. We infect CV1 cells with undamaged SV40, and irradiate the cells with UV during the peak of viral DNA replication. The effects of the UV lesions upon SV40 DNA replication can then be assessed in the first hour. We have demonstrated by electron microscopy that replication fork movement is blocked by UV lesions present at the frequency of pyrimidine dimers (Berger & Edenberg, Mol. Cell. Biol. 6:3443), and that the size of the daughter strands equals the inter-dimer distance (Edenberg, Virol. 128:298). These data argue for semi-discontinuous blockage of replication forks by dimers: e.g. lesions in the template for the continuous strand block fork movement, while lesions in the opposite strand merely block completion of Okazaki fragments. Preirradiation of the host cell before infection significantly mitigates the inhibition by a challenge dose of UV; DNA synthesis after a dose of 60 Jm^{-2} to preirradiated cells equals that after a dose of 25 Jm^{-2} to non-preirradiated cells (Scaria & Edenberg, Mutat. Res. 183:265). We have shown that dimers block both replication fork movement and daughter strand elongation to the same extent in preirradiated and non-preirradiated cells (Scaria & Edenberg, Mutat. Res., in press). There is no evidence for rapid bypass or removal of dimers in preirradiated cells. There is, however, a reduced inhibition of new initiations that appears to explain the mitigation.

E 422 INDUCIBLE RESPONSES TO DNA DAMAGE IN SALMONELLA TYPHIMURIUM, Eric Eisenstadt, C.M. Smith, and Zoltan Arany, Harvard University School of Public Health, Boston, MA 02115.

UV irradiation of Escherichia coli induces activities that reactivate UV irradiated lambda phage. In Salmonella typhimurium, however, UV irradiation does not, apparently, induce activities that reactivate UV irradiated P22 phage. We have undertaken a study of the SOS response in S. typhimurium in an attempt to understand the genetic and biochemical basis for this fundamental difference between the two organisms. With the aid of lac operon fusions, we have identified genetic loci that are regulated by recA and lexA and whose expression is altered in topA mutants. We have also identified DNA sequences in S. typhimurium that are similar to E. coli umuC. The characterization of a S. typhimurium umuDC homologue should aid in our understanding of the function of umuDC in induced mutagenesis.

E 423 A GENETIC DISSECTION OF RECA PROTEIN IN SOS MUTAGENESIS, Don G. Ennis and David W. Mount, University of Arizona, Tucson, AZ 85721.

In Escherichia coli, induction of SOS functions by some DNA-damaging agents promotes an SOS mutator activity. Activated RecA protein ($RecA^*$) is believed to have at least three roles in SOS mutagenesis. First, $RecA^*$ facilitates the proteolytic cleavage of the LexA repressor thereby derepressing the genes required for mutagenesis (i.e., umuDC). Second, the newly described proteolytic activation of the UmuD protein. A third role has been defined by genetic experiments of G. Walker and coworkers. Proteolysis of UmuD protein, LexA protein and the lambda cI protein are promoted by a common self-cleavage mechanism (autodigestion), where $RecA^*$ acts as a positive allosteric effector for autodigestion. We present genetic evidence which suggests that many of these $RecA^*$ activities appear to be separable. Although cleavage of these diverse proteins are catalyzed by a common autodigestion mechanism, we infer from our data that the effector surfaces of $RecA^*$ protein required for cleavage of each protein are likely to be non-identical.

E 424 URACIL-DNA GLYCOSYLASE ACTIVITY INFLUENCES THE MUTAGENICITY OF ETHYLMETHANE SULPHONATE, Douglas Fix, Department of Microbiology, Southern Illinois University, Carbondale, IL 62901, and David Koehler and Barry Glickman, Department of Biology, York University, Downsview, Ontario M3J 1P3.

Studies of mutagenesis have revealed that ethylmethanesulphonate (EMS) specifically induces G:C -> A:T transitions. It is thought that O-6 ethylguanine (O-6EtG), a product of EMS alkylation, can mispair with thymine giving rise to these base pair changes. Alternatively, O-6EtG may promote deamination of the cytosine residue in the opposite strand through cross-strand protonation. In this scenario, G:C -> A:T transitions could result either from the replication of uracil-containing DNA or replicative bypass of apyrimidinic (AP) sites resulting from the action of uracil-DNA glycosylase. To determine whether mechanisms involving uracil might contribute to EMS-induced mutagenesis, the effect of increasing dose on mutation frequency was monitored using the lacI system of E. coli. With an excision defective (uvrB) strain, mutation frequency increased in a dose-squared manner. However, in a strain that was also defective for uracil-DNA glycosylase (uvrB ung), the response was linear with dose. Moreover, when these strains were also defective for the umuC gene product, the response to increasing dose was linear. These data strongly suggest that AP sites resulting from the action of uracil-DNA glycosylase contribute to the mutagenicity of EMS.

E 425 INTERACTIONS BETWEEN THE SOS RESPONSE AND EPSILON. P.L. Foster and A.D. Sullivan, Boston University School of Public Health, Boston, MA. 02118.
SOS mutagenic processing is currently hypothesized to involve activated RecA protein (RecA*) and one or both of the *umuDC* gene products aiding DNA polymerase III in translesion bypass synthesis. One role for these proteins may be to inhibit the proofreader function of the replication subunit epsilon (Bridges and Woodgate,1985, PNAS 82:4193; Lu et al, 1986, PNAS 83:619; Piechocki et al, 1986, MGG 202:162). We have been investigating possible interactions between epsilon and the SOS system in the generation of both spontaneous and induced mutations. The *umuDC* gene products appear to interact with epsilon since (1) *umuDC*$^+$ and *mucAB*$^+$ suppress the temperature sensitive lethality of *dnaQ49* and, (2) *umuC*$^-$ suppresses the mutator phenotype of *dnaQ49*. We have also found that introduction of a clone with the *dnaQ*$^+$-*rnh*$^+$ genes into a *recA441 lexA(Def)* strain decreases MMS and UV induced mutagenesis 10-fold. These two genes share a promoter region but are transcribed in opposite directions. Deletion mutations in either gene totally or partially abolish the antimutator effect. We are currently determining whether this finding reflects a functional or regulatory interaction between the two genes.

E 426 TEMPERATURE DEPENDENCE OF MUTATIONAL SPECIFICITY OF THE dnaQ49 ALLELE OF Escherichia coli, Robert G. Fowler and Roberta J. Isbell, Dept. of Biological Sciences, San Jose State University, San Jose, CA 95192.
The mutD (dnaQ) gene codes for the epsilon (ε) subunit of the DNA polymerase III holoenzyme in E. coli. Two mutator alleles, mutD5 and dnaQ49, of the mutD gene have been recovered which enhance spontaneous mutation frequencies 10^3 to 10^5 times wild-type levels. Mutation frequencies for the dnaQ49 allele are temperature-dependent with values 100 fold lower at 30°C than at 37°C. We have determined the mutation specificity of dnaQ49 using the well-characterized trpA reversion system. At 37°C the mutational specificity of dnaQ49 resembles that of the mutD5 allele, i.e, all base-pair substitution events are increased with a preferential enhancement of transitions. At 30°C the mutational specificity is decidedly different from that at 37°C with the favored events being transitions and transversions that occur at sites that are poorly corrected by the mismatch repair system. We suggest that at 30°C the dnaQ49 allele creates mispairings at the replication fork similar to those produced at 37°C although at a much lower frequency. At the higher temperature the mismatch repair system is saturated and fails to make a significant contribution in error avoidance at individual trpA sites while at 30°C mismatch repair removes many dnaQ49-induced mispairings before they are fixed as mutations.

E 427 MOLECULAR AND BIOCHEMICAL ANALYSIS OF RECA PROTEIN INTERACTIONS WITH LEXA REPRESSOR AND ATP, Nancy Freitag and Kevin McEntee, UCLA School of Medicine, Los Angeles, CA 90024.
The RecA protein of Escherichia coli is a multifunctional enzyme that promotes DNA pairing and repressor cleavage (phage λ CI and cellular LexA) in ATP-dependent reactions. We have investigated the interacions between RecA protein and LexA repressor by employing affinity columns to which RecA protein is covalently attached. LexA protein binds weakly to immobilized RecA protein but binds tightly to RecA protein which is complexed with DNA. Bound LexA protein is rapidly cleaved on these "activated complexes". Complexes of the mutationally altered RecA430 protein and DNA can be bound to the columns, however LexA protein neither binds to nor undergoes cleavage on these complexes. This mutation blocks LexA cleavage in vivo and, based on these studies, does so by reducing the affinity of activated RecA protein for LexA.

Site-directed mutagenesis has been used to alter the RecA protein in its ATP binding domain. A critical tyrosine residue (Tyr264) in this region has been replaced by phenylalamine, serine or glycine. The characteristics of strains carrying these mutations and the properties of the altered proteins will be presented.

E 428 STRUCTURE OF A HOT SPOT FOR AAF - INDUCED MUTAGENESIS : THE NAR I SITE , Jean François Lefèvre, Bruno Kieffer, Youri Timsit, Jocelyne Colin, Dino Moras, Patrice Koehl, Eric Westhof and Robert P. P.Fuchs, IBMC, 15 rue René Descartes, 67 084 Strasbourg Cedex, FRANCE.

The forward mutation spectrum induced by N-Acetoxy-N-2 acetylaminofluorene has shown that the sequence GGCGCC, recognized by the Nar I restriction enzyme, was a -2 frameshift mutation hot spot (1). Using both NMR and X-ray crystallography techniques, we have then undertaken structural studies on the duplex obtained from the 12 mer ACCGGCGCCACA hybridized with its complementary strand.

2D NMR spectroscopy was used to assign all the protons of the duplex molecule. The experimental NOE values were analysed by comparison with calculated ones obtained by including all proton dipole - dipole interactions. We started with a 12 mer in the B form and iteratively modified its structure to minimize the difference between experimental and theoretical NOE values. The structure of the Nar I site will be presented as a result of this minimisation, and compared with data obtained from X-rays crystallography.

A first appoach of the study of the structural modification induced by one AAF covalently bound to one of the guanine of the Nar I sequence will also be discussed.

(1) : Koffel-Schwartz *et al* , J. Mol. Biol., 177 (1984) 33.

E 429 ACTIVATION OF ALTERNATIVE SITES OF REPLICON INITIATION IN HUMAN AND RODENT CELLS DEFICIENT IN EXCISION REPAIR, T. Daniel Griffiths and Su Y. Ling, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

For several years we have been examining how mammalian cells are able to completely replicate their DNA despite the presence of lesions that block DNA fork progression. Recently, we published data (Mutat. Res. 184, 39-46, 1987) indicating that exposure of wild type (AA8) as well as excision deficient Chinese hamster ovary cells (UV-5 CHO) to UV light results in the use or activation of alternative sites of replicon initiation. This allows cells to replicate sections of DNA that do not contain a "normal" site of initiation yet contain lesions that block replication from either direction. Since the excision deficient CHO cells exhibit a more pronounced and prolonged activation of these sites, we wished to determine whether other cell lines possessing various levels of excision repair also exhibit activation of these sites. We report here that excision deficient xeroderma pigmentosum human cells (XP A) also exhibit a pronounced and prolonged activation of alternative sites of replicon initiation. Repair proficient human cells, on the other hand, exhibit little or no activation. Since normal human cells excise pyrimidine dimers faster than do CHO AA8 cells, this suggests that the extent of activation of alternative sites of initiation is inversely related to the ability of cells to perform excision repair. This work was supported by U.S.P.H.S. grant CA 32579.

E 430 FUNCTIONAL SITES IMPORTANT FOR REPLICATION FIDELITY IN THE DNA POLYMERASE FROM HERPES SIMPLEX VIRUS, J.D. Hall, University of Arizona, Tucson, AZ, 85721.

The DNA polymerase from herpes simplex virus type 1 (HSV-1) is being used to investigate what molecular interactions are important for replication fidelity. These studies employ a combined approach of computer and genetic analyses to define functional domains in this enzyme and to identify specific residues involved in recognition of nucleoside triphosphate (dNTP) substrates. First, the DNA sequence from the HSV-1 polymerase has been compared with that of other polymerase genes. This comparison reveals regions of striking homology with sequences from several animal viruses and bacteriophages. These similarities suggest a similar structural organization in these distantly related proteins and implicate the most highly conserved sites as being important for enzyme function. Second, amino acid residues from the HSV-1 polymerase have been identified which are likely to be located at the binding site for dNTPs. These residues are altered in polymerase mutants with enhanced or impaired abilities to recognize correct dNTPs (i.e., mutants resistant to nucleotide analogues and antimutators which insert fewer mis-paired nucleotides during replication than wild type). Some of these mutations also occur at or near the highly conserved regions described above. The phenotypes of these mutants and the locations of the mutant sites are useful for suggesting what polymerase-dNTP interactions may be altered by the mutations.

E 431 FIDELITY OF REPLICATION OF AN SV40-BASED PLASMID IN VITRO, Janet Hauser, Michael P. Carty, Arthur S. Levine and Kathleen Dixon, Section on Viruses and Cellular Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda MD 20892.

We have used the SV40-based shuttle vector, pZ189, in a forward mutagenesis assay to study the fidelity of replication in crude extracts of CV1 cells prepared by the method of Li and Kelly. The pZ189 vector contains the early region of SV40 (including the SV40 origin of replication), the origin of replication of pBR327, the β-lactamase gene and the supF gene, which serves as a selectable marker for mutagenesis. DNA synthesized in SV40 T antigen-dependent replication reactions is treated with DpnI to remove input molecules which have not been fully replicated and with dam methylase to prevent loss of potential mutants through methyl-directed mismatch repair; competent E. coli are then transformed with this DNA. The observed mutant frequency (about 0.02%) is low, suggesting an average error per nucleotide incorporated of less than 1/60,000. These results imply that replication of DNA in the in vitro system has a higher fidelity than DNA synthesis as measured in gap-filling reactions with purified DNA polymerases. When UV-irradiated pZ189 is added to the in vitro system, replication is inhibited in a UV dose-dependent fashion. Characterization of the structure of replicative intermediates from these reactions should reveal whether UV-damaged DNA is processed in the in vitro system as it is in vivo.

E 432 EARLY EVENTS IN ASBESTOS TREATED CELLS: IMPLICATIONS FOR BULKY DNA LESION MODEL, Robert C. Johnson, M.U.S.C., Charleston, S.C.

Asbestos alone transforms cells; however, the nature of the primary DNA lesion is unclear. Recent indirect evidence suggests that oxygen radicals are generated by asbestos and that a transformation pathway may overlap with radiation induced transformation (Hei and Hall, 2nd International Conference on Anticarcinogenesis and Radiation Protection, Gaithersburg, Maryland, 1987). In this study we demonstrate evidence that asbestos induces early events that are associated with processing of bulky lesion damage in DNA. Specifically, DNA synthesis elongation is perturbed; however, DNA synthesis is not discontinuous, and asbestos does not inhibit daughter strand gap repair of ultraviolet light damage. The augmentation of a 53.6K protein after asbestos exposure was studied. Induction of a 53.6K protein (a possible protease) may be a useful early event, specific for bulky lesion processing (Mallick et al,1982,Proc. Natl. Acad. Sci. 79:7886). Finally, radiation sensitive strains of human fibroblasts were examined for hypersensitivity to asbestos as compared to normal strains.

E 433 A POINT MUTATION IN pZ189 MADE THE SHUTTLE VECTOR PLASMID MORE SUSCEPTIBLE TO UV-B DAMAGE, F. Kanai and K.H. Kraemer, Laboratory of Molecular Carcinogenesis, NCI, Bethesda, MD 20892

The effect of UV-B photoproducts on biological function was examined with a plasmid host cell reactivation assay. pZ189, a 5504 bp shuttle vector plasmid, contains the amp^r gene, which permits selection in bacterial cells, and the 160 bp E. coli sup F suppressor tRNA gene, which serves as a mutagenesis target but is not essential for plasmid survival. UV survival of a mutant plasmid containing a point mutation in the sup F gene in which an A is replaced by a T at position 136 (p-136T) was compared to that of the wild type plasmid. The plasmids were treated with UV and introduced into E. coli. After treatment with UV-B, 295 nm (10 kJ/m^2), the number of ampicillin resistant colonies with p-136T was 5 to 80-fold lower than with pZ189. These survival differences between the two plasmids were also observed after photoreactivation with E. coli photolyase, and, at lower doses, with a repair deficient E. coli strain (uvr-B). Treatment with 254 nm or 315 nm radiation resulted in equal survival of both plasmids. We tested other point mutants in order to determine the effect of sequence, but did not find survival differences at 295nm or 254 nm. These data suggest that a lethal photoproduct is formed in the 295 nm treated mutant plasmid which is different from the pyrimidine dimer in its wavelength dependence of formation and behavior to E. coli photolyase. Candidates for this lesion are purine containing photoproducts involving AT or TA.

E 434 CONSTRUCTION AND MUTAGENICITY OF BIOLOGICALLY ACTIVE DNA THAT CONTAINS SINGLE, SITE-SPECIFIC ARYLAMINE ADDUCTS, Charles M. King[1], Nobuya Tamura[1], Pawan K. Gupta[1], Thomas M. Reid[1], Dana Johnson[2] and Louis J. Romano[2], Michigan Cancer Foundation[1] and Wayne State University[2], Detroit, MI 48201.

The oligonucleotide, 5'-ATCCGTC-3', has been modified by the addition of an arylamine (2-aminofluorene or 4-aminobiphenyl) or the arylacetamide derivative at C-8 of the single guanine. The oligonucleotide adducts were purified by reverse phase HPLC and characterized by electrophoretic, spectroscopic and hydrolytic techniques to confirm their structures. The modified oligonucleotides were hybridized and ligated into gapped heteroduplex M13mp9 DNA to place the guanine adduct at position 6253 of the minus strand [Johnson et al., Biochemistry 25:449 (1986)]. The integrity of the DNA was confirmed by demonstration of its resistance to HincII, sensitivity to BamHI, and by the sizes and relative quantities of fragments produced after restriction outside the oligonucleotide. The mutations produced by these molecules in E. coli were primarily dependent on the prior induction of SOS functions of the host cells. Phenotypic mutations, as indicated by use of a β-galactosidase complementation system, yielded frameshifts in a run of Gs starting 5 bases 5' to the site of the adduct. "Silent" mutations identified by failure of plaques to hybridize under stringent conditions to an oligonucleotide probe that spanned the position of the adduct, were the result of base substitutions at or near the adduct site. DNA constructed with unmodified oligonucleotide yielded a mutation frequency consistent similar to wild type M13mp9 DNA. 2-Acetylaminofluorene adducts induced more frameshifts than 2-aminofluorene moieties. However, the amine produced more base substitutions than the acetamide, most of which were 1 to 4 bases from the adduct site.

E 435 GENETIC CONTROL OF -AAF INDUCED MUTAGENESIS AT ALTERNATING GC SEQUENCES , Nicole Koffel-Schwartz and Robert P.P. Fuchs, IBMC,15 rue René Descartes, 67084 Strasbourg-Cedex, FRANCE

The forward mutation spectrum induced by the chemical carcinogen *N* -Acetoxy-*N* -2 acetylaminofluorene was determined using the tetracycline resistance gene inactivation assay (1). It is found that 90% of the induced mutations are frameshift mutations located at specific sequences (mutation hot spots). Two classes of mutation hot spots were found : i) -1 frameshift mutations occuring at runs of guanines (*umuDC* dependant pathway) and ii) -2 frameshift mutations occuring at alternating GC sequences (*umuDC* independant pathway).

Using a reversion assay specific for the loss of two base pairs (GpC) within a $(GpC)_3$ sequence, we analysed the genetic requirements of this -AAF induced frameshift mutation pathway. The mutation frequency in different SOS mutant strains (*lex A(def) rec A 430, lex A(def) rec A 730, lex A(def) Δrec A, lex A(ind⁻))* were studied and compared to the mutation frequency obtained in a wild type strain.

The results obtained show that the -AAF induced -2 frameshift mutation pathway is under the control of the SOS system. However, this pathway is both *umuDC* and *recA* independant. Although the RECA protein does not play an active role (i.e. full recovery of mutations in the *lex A(def) ΔrecA* strain) it is found that the non-activated form of the RECA protein inhibits this mutation pathway. The activated form of the RECA protein ($RECA^*$) is no longer inhibitory. These results will be discussed in the light of the specific structure that -AAF induces within alternating GC sequences.

1. Fuchs et.al. Nature 294 (1981) 657-659.

E 436 HERPES SIMPLEX VIRUS TYPE 2 INDUCES REPAIR REPLICATION OF CELLULAR DNA IN VIRUS INFECTED CERVICAL CARCINOMA CELLS, [1]Pirkko Kulomaa, [2]Olli-Pekka Kallioniemi, [3]Jorma Paavonen and [1]Matti Lehtinen, Depts [1]Biomed. and [2]Clin. Sci. and [3]Clin. Chem., Univ. Tampere, SF-33101 Tampere, Finland.

We have studied the mode of DNA-synthesis in HSV-2 infected cervical cancer cells (CaSki and C-33A). We used a hapten-specific monoclonal antibody for the flowcytometric determination of bromodeoxyuridine incorporation into newly synthesized DNA in the virus infected cells. After an initial decrease in the amount of DNA-synthesizing cells, HSV-2 infected CaSki cells showed an exponential increase of both the DNA-synthesizing cells and amounts of infectious virus. In the less permissive C-33A cells the of amounts DNA-synthesizing cells cycled throughout the virus-cell interaction, while low amounts of virus were syntesized only. The inhibition of viral DNA-synthesis by phosphonoformate was able to inhibit the virus induced changes in the CaSki but not in the C-33A cells. Our results suggest that following HSV-2 infection the CaSki cells exhibit exponentially increasing DNA-synthesis which corresponds to the replication of the viral DNA. In the C-33A cells the mode of the virus induced DNA-synthesis corresponds most likely to repair replication of cellular DNA.

E 437 PURIFICATION, CHARACTERIZATION AND FIDELITY OF PORCINE LIVER MITOCHONDRIAL DNA POLYMERASE GAMMA AND AN ASSOCIATED PROOFREADING EXONUCLEASE, Dale W. Mosbaugh* and Thomas A. Kunkel**, Department of Chemistry and Clayton Foundation Biochemical Institute, University of Texas, Austin, TX 78712* and Laboratory of Genetics, NIEHS, Research Triangle Park, NC 27709**.

DNA polymerase gamma has been purified 10,000-fold from porcine liver mitochondria. An exonuclease activity copurifies with the polymerase through three column chromatography steps, each based on a different separation principle (phosphocellulose, heparin agarose, ds DNA cellulose). The exonuclease digests DNA in the 3'→5' direction, releasing nucleoside-5'-monophosphates. It efficiently excises bases from a 3'-OH primer terminus with a preference for excision of mismatched bases. Under polymerization conditions mismatch excision can be inhibited using high concentrations of dNTP substrates or by adding nucleoside-5'-monophosphates. In M13mp2-based fidelity assays polymerase gamma is highly accurate. This accuracy is reduced in reactions that diminish exonuclease activity, consistent with a proofreading function for the exonuclease.

E 438 ANALYSIS OF UV MUTAGENESIS USING VECTORS CARRYING A SINGLE DEFINED LESION, Swapan K. Banerjee, Christopher W. Lawrence, Roshan B. Christensen and J. Eugene LeClerc, University of Rochester, NY 14642.

A number of interesting patterns are evident in the various sets of data obtained by sequence analysis of phage and plasmid mutations induced by exposure to UV. From the perspective of understanding the molecular mechanisms of UV mutagenesis, however, these results raise more problems than they solve. In particular, interpretation of such data requires knowledge of the relative error frequencies and mutational spectra resulting from specific photolesions located in specific sequences. To address this problem, we have devised methods for introducing a single defined photolesion at a single defined site in an M13 hybrid phage vector. Both single-stranded and double-stranded constructs can be made. Although an error frequency of over 90% is expected if random insertion of nucleotides occurs opposite a bypyrimidine lesion, preliminary data for a T-T cyclobutane dimer carried by a single-stranded vector suggests that the error frequency is five to ten fold lower than this, perhaps the consequence of residual base-pairing capacity of the dimerized thymines and polymerase bias in favor of the insertion of adenine nucleotides.

Supported by NIH Grants GM32885 and GM21858, and by DOE Grant DE-FG02-85ER60281.

E 439 ANALYSIS OF HOTSPOTS FOR UV MUTAGENESIS OF SINGLE-STRANDED PHAGE DNA IN *E. COLI*, J. Eugene LeClerc and Carol M. Kissinger, University of Rochester, Rochester, NY 14642.

Significant differences in patterns of UV-induced mutations have emerged from sequence analyses of mutations in diverse biological systems, each leading to different conclusions about preferred sites for mutations and the insertion preferences during bypass replication. Several studies have shown a preponderance of C→T transitions in cellular genes, mostly collected from the *E. coli lacI* gene, while UV-irradiated phages such as M13 and *lambda* yield roughly equal portions of T→C and C→T transitions. We have studied two hotspots for UV-induced mutation in the *lacI* gene inserted in f1 single-stranded phage DNA: C→T at the sequence TC*T (pos. 75) and T→C at TT*C (pos. 89). Infection of SOS-induced cells with UV-irradiated phage particles yielded T→C as the predominant change (66% of total $lacI^d$ forward mutations *vs.* 12% C→T) while transfection with UV-irradiated phage DNA showed C→T as the preferred mutation (52% of mutations *vs.* 9% T→C). Transfection studies using DNA isolated from UV-irradiated phage suggest that differences in the conditions of damage induction account for the varying yields of mutations in specific DNA sequences.

Supported by NIH Grant GM27817.

E 440 ELEVATED GLUCOSE 6-PHOSPHATE LEVELS ARE ASSOCIATED WITH PLASMID MUTATIONS in vivo, Annette T. Lee and Anthony Cerami, Rockefeller University, New York, NY 10021.

Previous work has shown that the incubation in vitro of plasmid DNA with glucose 6-phosphate has a mutagenic effect when transformed into wild type E.coli. To further investigate the modifications of DNA by the reducing sugar glucose 6-phosphate, we have developed an in vivo model to monitor plasmid DNA mutations. E.coli strains which are defective in phosphoglucose isomerase alone (DF40) or also defective in glucose 6-phosphate dehydrogenase production (DF2000) were transformed with a plasmid which carries the genes for ampicillin resistance and β-galactosidase production. The transformed bacteria were grown in glucose/gluconate minimal medium, then assayed for glucose 6-phosphate levels and plasmid mutation rates. An increase in plasmid mutations (6 and 13 fold) was associated with increased intracellular glucose 6-phosphate levels (20 and 30 fold) present in the DF40 and DF2000 strains, respectively. Growth of the bacteria in gluconate minimal medium does not increase the intracellular levels of glucose 6-phosphate or the rate of plasmid mutations over background. The increase in plasmid mutations as a function of increased intracellular glucose 6-phosphate levels suggests that the accumulation of adducts formed by glucose 6-phosphate and other reducing sugars over time may contribute to DNA damage in mammalian cells.

E 441 CHARACTERIZATION OF THE INTERACTION OF E. coli LEXA REPRESSOR WITH DNA; BINDING STUDIES USING MUTANT AND CONSENSUS OPERATORS, L. Kevin Lewis and David W. Mount, University of Arizona, Tucson, AZ 85721.

The interaction of LexA protein, the repressor of the SOS regulon in E. coli, with wild-type, mutant, and consensus operators has been examined. Specifically, a synthetic LexA binding site designed on the basis of sequenced operator-constitutive mutants and compiled consensus data has been tested for its ability to bind repressor in vivo. Additionally, protein-protein cooperativity has been analyzed using constitutive RecA promotor-operator:: galactokinase operon fusions which are flanked upstream by several unique cloning sites. The ability of synthetic operators placed in the upstream sites to enhance binding of repressor to the mutant operators was investigated.

E 442 IS-ELEMENTS TRANSPOSITION IS A MAJOR COMPONENT OF SPONTANEOUS MUTAGENESIS IN E. COLI Zvi Livneh*, Zehava Eichenbaum and Rami Skaliter, The Weizmann Institute of Science, Rehovot 76100, Israel.

The spectrum of spontaneous mutations in E. coli is usually considered to be composed predominantly of base substitutions, based on the extensive studies in the lacI system. The contribution of IS-elements transpositions is believed to be minor (1-4% in lacI).

We have recently developed a mutagenesis assay system based on the cro repressor gene of phage λ cloned into a plasmid. In the appropriate indicator strains, mutations in cro are scored, which reduce its binding to the O_RP_R control region of the O_RP_R - lacZ fusion in a λ lysogen.

Characterization of spontaneous mutations in cro revealed that 70% are transposition events caused by cellular IS-elements. Using restriction nuclease digestion and hybridization to IS-specific probes we have identified IS1, IS5 and an as yet unidentified element. In a different E. coli strain 35% of the spontaneous cro mutants contained IS-elements. Thus transposition by IS-elements is a major component of spontaneous mutagenesis in cro.

The nature of spontaneous mutations in E. coli has been addressed only in two systems in addition to lacI and cro. In the cI gene in a λ prophage 30-65% of the spontaneous inactivating mutations were IS-elements insertions and in phage P1 the value was up to 97%. It is thus likely that in E. coli IS-elements transpositions play a much more important role than previously thought.

E 443 UREA RESIDUES, FRAGMENTATION PRODUCTS OF THYMINE, ARE MUTAGENIC LESIONS IN A f1 lacI FORWARD MUTATION SYSTEM, Margaret Maccabee, Lynn A.Petrullo, and Susan S.Wallace, New York Medical College, Valhalla, NY 10595

Urea residues are models for thymine ring fragmentation products produced by free radical reactions. Urea residues can be selectively introduced into DNA by alkali hydrolysis of OsO_4–oxidized DNA. Using single stranded f1 transfecting DNA, we have shown that it takes between 1 and 2 urea residues per molecule to inactivate the bacteriophage. Further, urea residues appear to be mutagenic in an f1 lacI forward mutation system and like AP sites, mutagenesis was dependent on UV–induction of the SOS system of the host. In order to confirm that the mutations observed were targeted at urea residues, we are sequencing mutants using the $lacI^{-d}$ system. This sequencing data should also provide information concerning polymerase selectivity opposite a non–instructive lesion derived from thymine. These studies were supported by DHHS grant CA 33657.

E 444 CYTOSINE ARABINOSIDE(araC) IN DNA TEMPLATES PARTIALLY ARRESTS SYNTHESIS BY DNA POLYMERASE IN VITRO. Thomas J. Mikita and G. Peter Beardsley, Yale University School of Medicine, New Haven, Ct 06510.

AraC differs from deoxyC solely in the configuration at C_2' of the sugar moiety, where it carries an additional -OH group trans to the -OH group at C_3'. It is a potent anti-leukemic agent as well as a general inhibitor of DNA replication. Its mechanism of action is partially, or wholly related to its misincorporation into DNA. We have chemically synthesized DNA oligomers which contain a single araC residue at an internucleotide site. Melting temperature studies show that the araC-G base pair has comparable stability to the C-G base pair. AraC containing templates were annealed to complementary primers and used to study the effects of araC on template utilization by several DNA polymerases in vitro. AraC in the template partially arrested synthesis by the Klenow Fragment, T4 polymerase, and Hela Cell pol α2. A striking feature of this partial replication block is that it occurs at, rather than before, the template lesion site. The correct nucleotide, G, is incorporated opposite araC >95% of the time in polymerase assays. Kinetic studies show that incorporation of the next nucleotide after the lesion site is 5 to 10 fold slower than on control templates. How the structural alteration in the sugar moiety affects the catalytic function of the polymerase one nucleotide after the lesion site is not clear. Perhaps this is due to important polymerase-DNA contacts along the sugar-phosphate backbone of the template strand which are disturbed by the arabinose sugar.

E 445 EFFECTS OF OVERPRODUCTION OF SINGLE-STRANDED DNA BINDING PROTEIN ON RECA PROTEIN-DEPENDENT PROCESSES IN ESCHERICHIA COLI. Patrice L. MOREAU, Laboratoire d'Enzymologie, C.N.R.S.,91190 Gif-sur-Yvette, France

Overproduction of SSB led to a decrease in the basal level of repressor LexA . Expression of the LexA-controlled genes was increased differentially, depending on the affinity of the LexA repressor for each promoter : expression of the recA and sfiA genes was increased 5-fold and 1.5-fold, respectively. Bacteria overproducing SSB protein produced elongated cells. This effect was essentially independent of the expression of the sfiA and recA genes. These data indicate that excess SSB acts primarily by perturbing DNA replication, thereby favoring gratuitous activation of RecA protein to promote cleavage of LexA protein (J.M.B., 1987, 194, 621-634). When bacteria overproducing SSB were exposed to a DNA-damaging agent such as UV, the recA and sfiA genes were fully induced. Induction of the sfiA gene occurred, however, with a reduced rate in bacteria overproducing SSB indicating that activation of RecA was slightly hampered. High levels of SSB had a dramatic inhibitory effect on recombination between intact and UV-damaged DNA. By contrast, spontaneous and UV-induced mutagenesis frequencies were slightly increased by excess SSB.These results provide evidence that SSB modulates RecA-dependent activities in vivo. It is proposed that SSB favors the formation of short complexes of RecA and ssDNA that mediate cleavage of the LexA repressor, while it delays the formation of long nucleoprotein filaments, thereby slowing down RecA-promoted recombinational events.

E 446 PLASMID-CHROMOSOME RECOMBINATION OF IRRADIATED SHUTTLE VECTOR DNA IN MAMMALIAN CELLS, John S. Mudgett and William D. Taylor, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802

An autonomously replicating shuttle vector containing a mutant bacterial ampicillin resistance gene was used to investigate the enhancement of plasmid-chromosome recombination in mammalian host cells by ultraviolet light and gamma radiation. Sequences homologous to the shuttle vector containing a wild-type ampicillin gene were stably inserted into the genome of African Green Monkey kidney cells to act as the target for these recombination events. Shuttle vector DNA was irradiated with various doses of radiation before transfection into the transformed mammalian cells. The homologous transfer of the wild-type gene from the chromosomal insert to the shuttle vector was detected by plasmid extraction and transformation into E. coli host cells. Ultraviolet light did not enhance plasmid-chromosome recombination, but increased the levels of plasmid mutagenesis (rearrangement), which did not change with post-transfection time. In contrast, plasmid-chromosome recombination was enhanced by gamma radiation, by specific double-strand breaks, and longer post-transfection incubation in the mammalian host cells. Plasmid survival, recombination, and mutagenesis were not affected by UV irradiation of the mammalian host cells prior to transfection. The recombinant plasmid molecules were found to be mostly the result of non-conservative recombination events which involved both homologous and non-homologous interactions with the host chromosome. The observation that similar recombinant structures were obtained from all of the plasmid alterations investigated suggests a common mechanistic origin for plasmid-chromosome recombination in these mammalian cells.

E 447 CHARACTERIZATION OF A HOTSPOT FOR RECOMBINATION IN HUMAN CELLS, John P. Murnane and Michael J. Yezzi, University of California, San Francisco, CA 94143.

The human cell line LM205, transformed with the pLR309 plasmid, contains a stably integrated selectable gene marker (*neo*) without a transcriptional promoter. Spontaneous tandem duplication at the integration site relocates a Simian virus 40 transcriptional promoter to a position 5′ to the *neo* gene at a rate of 5×10^{-8} events/cell/generation, as measured by subsequent resistance of the cells to the toxic antibiotic G418. The heterogeneity in the site of recombination observed in various G418-resistant (G418-R) subclones indicated that the sequences involved have little or no homology, which has been confirmed by sequence analysis of this region. The rate of tandem duplication involving the *neo* gene was not affected by DNA-damaging agents or by inhibitors of DNA synthesis. Although these tandem duplications were relatively stable in most G418-R subclones, some G418-R subclones underwent further amplification of the *neo* gene during cloning. In one such cell line, RS-4, subclones isolated without G418 demonstrated a high degree of heterogeneity in the *neo* gene copy number (two to 20), indicating that amplification was associated with a hotspot for homologous recombination. The high rate of recombination was variable and was not a general property of all DNA in these cells, because other plasmid integration sites remained stable. The presence of deletions in the region of the *neo* gene in some cells, and the fact that amplification appeared to occur in small increments over several generations is consistent with mechanisms such as unequal sister chromatid exchange or gene conversion. Host cell DNA containing a short interspersed repetitive sequence (SINE) near the integrated *neo* gene appears to promote this recombination, which explains why cell line LM205 was the only clone out of the 30 original clones transformed with pLR309 that demonstrated spontaneous G418-R colonies. The inclusion of this SINE in the initial duplication correlates with further amplification of the *neo* gene, and therefore a propensity for recombination between these asymmetric elements could explain the high rate of duplications and deletions observed in the RS-4 cell line.

E 448 METAL CARCINOGEN-INDUCED DAMAGE RESULTING IN PERMANENT ALTERATIONS IN v-mos EXPRESSION IN CELLS INFECTED WITH THE MuSVts110 RETROVIRUS, Edwin C. Murphy, Jr., Neal W. Biggart and Susanna M. Chiocca, Dept. of Tumor Biology, Univ. of Texas System Cancer Center, Houston, TX 77030.

We have developed an assay for metal carcinogen-induced damage to a test gene in a mammalian cell by taking advantage of the growth temperature dependent phenotype of cells infected with the conditionally transformation-defective MuSVts110 retrovirus to select for mutants with metal-induced damage affecting the expression of the v-mos protein. MuSVts110 contains a frameshift deletion and requires splicing of its RNA transcript for translation of the v-mos gene product, $P85^{gag-mos}$. This splice event can only occur at 33° C. and lower; at 37-41° C., splicing does not occur. Consequently, MuSVts110-infected cells (6m2 cells) appear transformed at 33° C. and normal at 39° C. We have shown that the thermodependence lies in some cis-acting feature of the structure of the MuSVts110 transcript. In our assay, 6m2 cells are treated with a metal carcinogen and then grown at 39° C., a temperature normally nonpermissive for cell transformation. "Revertants" to the transformed phenotype at 39° C. are selected and assayed for changes in viral proteins and RNA. We have found that nickel and NMU (a chemical known to induce base substitutions) cause mutations in viral RNA splicing that allow the splice event to occur at both 33° and 39° C. Chromium and cadmium, in contrast, have no effect on splicing but induce frameshift changes in the MuSVts110 DNA that permit the translation of a 100 kd transforming protein, $P100^{gag-mos}$. Studies are in progress to determine the molecular basis of the DNA damage induced by these agents.

E 449 DNA PROOFREADING PROCESS IN MAMMALIAN CELLS: INVOLVEMENT OF DNA POLYMERASES AT G_1/S BORDER, Frank Q.H. Ngo and Jia Xian-Li, Cleveland Clinic Research Institute, Cleveland, OH 44106.
This study examines a proofreading mechanism whereby errors in DNA fragments can be recognized before replicative DNA synthesis takes place. Chinese hamster V79 cells at G_1 phase were challenged with a dose of γ-rays (20-50 Gy) and DNA single-strand (ss) and double-strand (ds) breaks were assayed at various times postirradiation. The techniques employed included alkaline filter elution (pH 12.1), neutral filter elution (pH 9.6 and pH 7.2), and hydroxylapatite uncoiling chromatography. While the relative amount of DNA breaks postirradiation decayed with a fast component and a slow component (time constants of 2-5 min and 1-2 hrs, respectively), we detected a small but significant wave of new breaks which occurred much later and were observed only when cells were allowed to progress through G_1/S border. The amount of these cell-phase specific breaks (PSSB) appeared to be approximately proportional to the dose size, and accounted for about 2-5% of the initial breaks. Aphidicolin (5 μg/ml) or ß-arabinofuranosyladenine (150 μM) given during the same entire period of repair incubation was capable of inhibiting the newly-formed breaks. Furthermore, aphidicolin, given during but not before the G_1/S interval also showed an inhibitory effect. The formation of PSSB was not affected by caffeine, a brief treatment of anisotonic solutions, nor by novobiocine (a non-specific inhibitor of topoisomerase). These results are consistent with the hypothesis that the cell-phase specific DNA breaks are products of an error-recognizing mechanism perhaps involving action of endo- and/or exonucleases immediately before DNA polymerization. Our finding is perhaps the first evidence of a novel proofreading process of DNA replication in eukaryotes. Supported by CA 33951 of NCI, DHHS.

E 450 DOUBLE-STRAND BREAK INDUCED RECOMBINATION IN YEAST, Jac A. Nickoloff[1], Jeff Singer[2], and Fred Heffron[2], [1]Los Alamos National Laboratory, Los Alamos, NM 87545, and [2]Scripps Clinic and Research Foundation, La Jolla, CA 92037.
We have introduced double-strand breaks at defined loci within or between heteroallelic genes in yeast chromosomes. The lesion is produced by the HO endonuclease, which normally introduces a double-strand break at MAT in haploid cells, initiating mating type interconversion. We have inserted a 24 bp synthetic HO recognition sequence within or between two heteroallelic ura3 genes. HO stimulates recombination between the ura3 alleles 10- to 2500-fold over spontaneous levels. Recombination frequencies and the distribution of recombinant products are determined by the locations of the mutations within the ura3 allele, and the location of the double-strand break. Surprisingly, similar recombination frequencies are found when the double-strand break is in homologous or non-homologous regions. Gene conversion events initiated in a region of non-homology result in recombinants in which either allele has been converted. A double-strand break within a region of homology results in conversion of the allele suffering the break. We have also examined the effects of the rad52-1 allele in this system. (This work was supported by National Institutes of Health Grant RO1 GM33808-1.)

E 451 MUTATIONS INDUCED BY N-ACETOXY-2-ACETYLAMINOFLUORENE IN DNA EXCISION REPAIR-DEFICIENT AND REPAIR-COMPETENT CHINESE HAMSTER CELLS, R. T. Okinaka and G. F. Strniste, Los Alamos National Laboratory, Los Alamos, NM 87545.
DNA excision repair deficient CHO cells (UV-5) are considerably more sensitive to the cytotoxic and mutagenic effects of N-hydroxy-2-acetylaminofluorene (N-OH-AAF) and N-acetoxy-2-acetylaminofluorene (N-OAc-AAF) than are repair proficient AA8-4 cells. At equitoxic doses (37% survival) N-OH-AAF induces 6 and 30 hypoxanthine-guanosine phosphoribosyl transferase ($HGPRT^-$) mutant colonies per 10^5 surviving cells in AA8-4 and UV-5 cells, respectively. The DNA from independent $HGPRT^-$ mutants isolated from either N-OAc-AAF- or N-OH-AAF-treated UV-5 and AA8-4 cultures are currently being analyzed by restriction enzyme digestion/Southern blotting and probing techniques with a cloned hamster V79 cell HGPRT cDNA (PHT12 from Dr. J. Fuscoe, Lawrence Livermore Laboratory). The intent of these experiments is to determine whether failure to excise critical N-OH-AAF induced lesions will result in elevated levels of deletion/rearrangement events at the HGPRT locus. Preliminary analyses indicate the opposite result, i.e., none of the UV-5 (16 of 16) and only a few (4 of 29) of the AA8-4 derived mutants contain large deletion and/or rearrangements. These data indicate that failure to remove N-OH-AAF and N-OAc-AAF induced lesions results in elevated levels of point or small deletion mutations. It is possible that occasional misrepair (in repair competent cells) may be responsible for the induction of deletion mutants as previously described [Carothers et al. PNAS (1986) 83:6519]. However, our data to date is not statistically sufficient to eliminate the possibility of spontaneously arising mutants accounting for the deletion events witnessed in these experiments. (This research was supported by the U.S. Department of Energy under Contract W-7405-ENG-36.)

E 452 SITE-SPECIFIC INCORPORATION OF 2-AMINOFLUORENE (AF) - AND N-ACETYL2-AMINOFLUORENE (AAF) -DEOXYGUANOSINE TRIPHOSPHATE ADDUCTS BY DNA POLYMERASES, B.D. Preston, D. Wu, T.M. Reid, C.M. King and L.A. Loeb, Department of Pathology, University of Washington, Seattle, WA 98195 and Department of Chemical Carcinogenesis, Michigan Cancer Foundation, Detroit, MI 48201.

In order to study mechanisms of mutagenesis by chemical carcinogens, we have used DNA polymerases to site-specifically incorporate arylamine nucleotides. The C8-dGTP adducts of AF and AAF were prepared by reaction of dGTP with N-acetoxy-N-trifluoroacetyl-AF and N-acetoxy-AAF, respectively, and these adducts were tested as substrates for site-specific incorporation opposite a single dC residue in a 17mer/M13mp2 primer-template molecule. The error-prone Avian Myeloblastosis Virus polymerase (AMV pol) elongated the primer by one nucleotide (~50% yield) in the presence of either AF- or AAF-dGTP. In contrast, the highly accurate E. coli DNA polymerase I (Pol I) extended the primer in the presence of AF-dGTP (~80% yield), but not AAF-dGTP. Addition of unmodified dNTPs to each polymerase reaction resulted in further extension of the 3'-adducted primers. Preliminary analyses of the extended primers by piperidine-induced strand cleavage showed that labile nucleotides (presumably the AF- and AAF-dG adducts) were site-specifically incorporated at the pre-determined site. The inability of Pol I to incorporate AAF-dGTP correlates with the unusual structure of this adduct and suggests that the stringency of nucleotide selection during DNA polymerization is determined by both the conformation of the dNTP and the intrinsic accuracy of the DNA polymerase. These data also show that error-prone DNA polymerases can be used for the site-specific incorporation of "bulky" nucleotide adducts.

E 453 EXONUCLEOLYTIC PROOFREADING BY DNA POLYMERASE ALPHA, Mary E. Reyland, Fred W. Perrino, I.R. Lehman and Lawrence A. Loeb, Department of Pathology, University of Washington and the Department of Biochemistry, Stanford University.

The 182kD polymerase subunit of Drosophila pol-primase contains a potent 3'→ 5' exonuclease which increases the fidelity of DNA synthesis 100-fold. In order to characterize exonucleolytic proofreading we have examined the specificity of mispair excision using a primed ϕX174am3 molecule containing a 3' terminal mispaired nucleotide opposite a template A at position 587 in the amber3 codon. In the absence of polymerization both A:C (template:primer) and A:A mispairs were efficiently excised; excision of a mispaired G was 20-fold lower. During concomitant polymerization the overall efficiency of excision increases 16 to 100-fold but the relative efficiency is altered such that the A:G and A:A mispairs are efficiently edited, while A:C mispairs are edited at a 30-fold lower frequency. Excision in the absence, but not the presence of polymerization, is inhibited by dGMP. Likewise, when assayed using the ϕX174am3 fidelity assay, high fidelity DNA synthesis was not reduced by concentrations of dGMP or AMP which inhibit proofreading by prokaryotic polymerases. We have also separated the polymerase and primase activities of the calf thymus complex using the conditions described to separate the subunits of the Drosophila complex (Cotterill, et al., JBC (1987), in press). However, so far, no 3'→ 5' exonuclease has been revealed. How this mammalian enzyme achieves high fidelity DNA synthesis is currently under investigation.

E 454 ACRIDINE-INDUCED FRAMESHIFT IN BACTERIOPHAGE T4, Lynn S. Ripley, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103

The type II topoisomerase of bacteriophage T4 is a central determinant of the frequency and specificity of acridine-induced frameshift mutations. Acridine-induced frameshift mutagenesis is specifically reduced in a mutant defective in topoisomerase activity. The ability of an acridine to promote topoisomerase-dependent cleavage at specific DNA sites in vitro is correlated to its ability to produce frameshift mutations at those sites in vivo. The specific phosphodiester bonds cleaved in vivo. The cospecificity of in vitro cleavage and in vivo mutation implication acridine-induced, topoisomerase-mediated DNA cleavage as intermediates of acridine-induced mutagenesis in T4.

E 455 SOS-LIKE ENHANCEMENT OF UV-INDUCED MUTAGENESIS IN MAMMALIAN CELLS. Emmanuel Roilides, Ruth Miskin, Arthur S. Levine, and Kathleen Dixon. Section on Viruses and Cellular Biology, NICHD, NIH, Bethesda MD 20892.

We have used the SV40-based shuttle vector, pZ189, to study SOS-like enhanced mutagenesis in carcinogen-treated monkey cells. Mutations that occur in the vector's supF gene during its replication in mammalian cells can be quantitated in bacteria and the DNA sequence changes in supF can be determined. When monkey cells are treated with mitomycin C before transfection with UV-irradiated (200 J/m^2) pZ189, there is a two-fold increase (from 0.25% to 0.55%, $p<0.001$) in the frequency of supF- mutants compared with transfection of untreated cells with UV-irradiated pZ189. Preliminary DNA sequence data suggest that the spectrum of UV-induced mutations in the supF gene does not change dramatically as a consequence of pretreating the cells. When pretreated cells are transfected with unirradiated pZ189, the frequency of spontaneous mutants (about 0.04%) does not appear to increase. To determine whether enhanced mutagenesis could be mediated by an extracellular factor, we pretreated monkey cells with conditioned medium from other UV-irradiated cells before transfection with UV-irradiated pZ189. This treatment also enhanced the supF- mutant frequency almost two fold (from 0.25% to 0.43%, $p<0.05$). Further experiments with this shuttle vector system should help elucidate the role of extracellular factor(s) produced by carcinogen-treated cells in enhancing mutagenesis in mammalian cells.

E 456 Identification of a UV-induced Trans Acting Protein That Stimulates Polyma DNA Replication. Zeev A. Ronai and I.B. Weinstein. Columbia University, Comprehensive Cancer Center, 701 West 168th Street, New York, N.Y. 10032

We have employed the asynchronous replication of polyoma DNA (APR) in a polyoma transformed rat fibroblast cell line (H3) as a model system for understanding the mechanism of DNA amplification. We found that APR can be induced in H3 cells either by direct UV-irradiation (254nm) of these cells, or by fusion with previously irradiated normal Rat 6 cells. These and other results suggest that DNA damage induces a trans acting protein(s) that can cause DNA amplificiation (Cancer Res. 47, 4565, 1987). In the present studies we report that when cell extracts are prepared from UV-irradiated Rat 6 cells and introduced, by a red blood cell mediated insertion (RCI) technique, into unirradiated H3 cells there is a strong induction of APR. Control studies showed that extracts from unirradiated Rat 6 cells introduced into H3 cells did not induce APR. Extracts prepared from irradiated Rat 6 cells were fractionated by anion exchange and gel filtration columns and the fractions then introduced into H3 cells by the RCI method. This led to the identification of a protein fraction of about 60 Kd, which is a potent inducer of APR. This material is being further characterized.

E 458 UV-MUTAGENESIS TARGETED AT THYMINE-CYTOSINE CYCLOBUTANE DIMERS (T=C) IN E. coli, M. Ruiz-Rubio, J. Shortt and R. Bockrath, I. U. Med. Sch., Indpls., IN 46223.

Analyses of prototrophic mutants produced by germicidal UV from ochre-defective auxotrophic strains allow measurements of events producing backmutations at the ochre defect (5'-TTA-; transcribed strand) or glutamine tRNA ochre suppressor mutations in the seven-tRNA operon (5'-ATCAAAA- or 5'-ATTAGAA to 5'-ATTAAAA-; transcribed strand for the anticodon loop). We present data on problems primarily regarding the suppressor mutations:

(a) amplified DNA photolyase (in dark) eliminated mutations targeted at T=C suggesting that the T=C + enzyme complex blocks DNA replication essential only for misincorporation, (b) the extent of photoreversal (PR) of mutations in a lexA71::Tn5 recA730 strain was used to estimate suppressor mutations targeted at T=C or T(6-4)C before and after mutation frequency decline (MFD) and the kinetics of MFD were measured before and after PR to indicate that both lesion types were sensitive to MFD but dimers were more sensitive, and (c) delayed PR mutagenesis, which follows UV, incubation and PR in umuC cells, was considered as the result of deamination at cytosine in T=C followed by trans-U DNA replication; the expts., including challenge with preliminary deamination and studies of physiologically dependent deamination rates with umuC ung strains lacking uracil DNA glycosylase activity, suggested a role for the deamination model (rather than misincorporation). The T=C photoproduct appears to target the majority of glutamine tRNA ochre supressor mutations and is a focus point for understanding MFD and delayed PR mutagenesis, phenomena strongly associated with the production of these suppressor mutations. Research supported by N.I.H. grant GM21788.

E 459 ACTIVATED AFLATOXIN INDUCES GUANINE-TARGETED TRANSVERSIONS AND TRANSITIONS WITH EQUAL EFFICIENCY IN PHAGE M13, Sambamurti, K., Callahan, J., Perkins, C., Jacobsen, J.S., Humayun, M.Z., UMDNJ - New Jersey Medical School, Microbiology and Molecular Genetics, Newark, NJ 07103.

The mutagenic consequences of aflatoxin B_1-2,3-dichloride (AFB1C12) were examined using a forward mutagenesis assay system. When replicative form (RF) DNA carrying primary AFB1C12-guanine lesions or the secondary ring-open formamidopyrimidine-AFB1C12 (FAPY) derivatives were transfected into UV irradiated or unirradiated excision-deficient E. coli carrying the mutator plasmid pGW270, the following results were obtained. 1. AFB1C12 and FAPY adduction enhanced mutation frequency 5 fold without prior SOS induction and 7 fold after SOS induction. 2. Sequence analysis of induced mutations showed that 90% of the base substitutions were targeted to guanine residues suggesting that mutations were targeted to putative AFB1C12 lesions. Ten to 20% of the point mutations were frameshifts. 3. A preference for guanines on the viral (plus) strand was observed for both AFB1C12 and FAPY lesions in non-SOS induced, but not in SOS-induced cells. 4. On the plus strand, both AFB1C12 and FAPY lesions induced mutations targeted to predicted hot spots (Muench et al., 1983, PNAS 80, 6-10) for AFB1 damage. Mutations on the minus strand were under-respresented at predicted AFB1 binding hot spots. 5. Sequence analysis indicated that GC to AT transitions as well as GC to TA transversions were induced by AFB1C12 in equal proportions.

E 460 MECHANISM OF *mutT* MUTATOR IN *ESCHERICHIA COLI*

Roel M. Schaaper and Ronnie L. Dunn, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

is a strong mutator with a unique specificity: only A•T→C•G transversions are induced. We have studied the mechanisms of *mutT* action using single-stranded phage M13mp2. *In vivo*, this phage is subject to *mutT* action with the expected specificity: A•T→C•G transversions, which are increased 50-100 fold compared to wild-type strains. We have also performed *in vitro* DNA replication studies using ss mp2 DNA and cell-free extracts from mutT strains. These extracts replicate DNA with a fidelity which is specifically reduced for A•T→C•G transversions (error frequency being increased about 50-fold above background). From the site-specificity of the mutations we have been able to conclude that *mutT* cells are specifically deficient in the prevention of A•G mismatches during DNA replication (as opposed to the mutationally equivalent T•C mismatches). We have also studied the possible interactions between the *mutT* and *mutHLS* mutation-avoidance pathways with respect to A•T→C•G transversions. It is suggested that more than one pathway exists for their generation.

E 461 A ROLE FOR MOBILE GENETIC ELEMENTS IN THE EVOLUTION OF THE umuDC OPERON, Steven Sedgwick, Genetics Division, National Institute for Medical Research, London, UK.

The umuDC operon of Escherichia coli encodes mutagenic DNA repair activity. The assimilation of umuDC into the E.coli genome as part of a mobile genetic element is suggested by the following observations: i) umuDC is part of a 12.5 kbp block of restriction enzyme sites which in multiple isolates of E.coli is flanked by polymorphisms reminiscent of transposon activity. Similar conserved tracts of enzyme sites with flanking polymorphisms can be also seen in multiple isolates of Escherichia dispar, Escherichia alkalescens and a single stock of Escherichia aurescens. Restriction site polymorphisms were not found around the recA or polA genes. ii) Direct repeats of Tn3-like terminal sequences were found at the predicted termini of the conserved region encompassing umuDC. iii) umuDC-like sequences were only detected in four species of Escherichia and not in five others. It cannot be completely excluded that umuDC has diverged so that in some species it fails to hybridize and provides little or no mutagenic DNA repair. Nevertheless the limited appearance of the sequence within the genus Escherichia is consistent with the invasion of a mobile genetic element into some branches of an evolutionary tree and not others. It is suggested that primordial E.coli acquired the umuDC region by either transposition or by replicon fusion with one of the many plasmids which are known to encode mutagenic DNA repair.

E 462 DIMER AND NON-DIMER PHOTOPRODUCTS ARE MUTAGENIC IN XERODERMA PIGMENTOSUM CELLS, S. Seetharam, D.E. Brash, M.M. Seidman*, and K.H. Kraemer, NCI, Bethesda, MD 20892 and *Otsuka Pharm. Co, Rockville, MD 20892

Xeroderma pigmentosum (XP) cells exposed to UV are hypersensitive to killing and hypermutable but the molecular lesions leading to these effects are largely unproven. Propagation of UV treated shuttle vector plasmid, pZ189, in XP complementation group A (XP-A) (with <2% residual repair activity) revealed markedly reduced plasmid survival and greatly increased frequency of G:C to A:T transition mutations cells (PNAS 83: 8273, 1986). In the present experiments we selectively removed 99% of the cyclobutane dimers, by photoreactivation (PR) of UV exposed pZ189 prior to transfection. Plasmid survival in XP-A cells increased 5- to 10-fold and mutation frequency declined 5- to 10-fold indicating that cyclobutane dimers contributed to a major portion of lethality and mutagenicity. Since most mutations involved G:C base pairs, C- rather than T- containing dimers are implicated. The non-dimer photoproducts that remained after PR were also mutagenic: The spectrum of mutations contained the same transition hotspots as seen without PR indicating that non-dimer photoproducts were minor contributors to transition mutations. The proportion of transversion mutations with XP-A increased from 6% (4/71) to 36% (16/45) ($p<0.0003$) after PR (a proportion similar to that with normal) indicating that non-dimer photoproducts are major contributors to transversion mutations. This data demonstrates that both dimer and non-dimer photoproducts are mutagenic lesions in XP cells.

E 463 GAMMA RADIATION ENHANCES THE FREQUENCY OF METHOTREXATE RESISTANCE IN CHINESE HAMSTER OVARY CELLS, Rakesh C. Sharma and Robert T. Schimke, Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Mammalian cells are known to develop resistance to various chemotherapeutic drugs including methotrexate (MTX) and its analogs. These chemotherapeutic drugs are also used in combination with radiotheraphy. Therefore, we are studying the effect of gamma radiation on the frequency of MTX resistance and dihydrofolate reductase gene amplification. Gamma irradiation of Chinese hamster ovary AA8 cells resulted in enhancement of the frequency of MTX resistant colonies (maximum enhancement: 2000-fold after 1000 rads of gamma irradiation). The enhancement of MTX resistance was dependent on the dose of gamma radiation and increased with time after irradiation; a maximum enhancement was observed when MTX was added at around 18 h after irradiation . At this time we observed cells accumulating at the G2/M phase as measured by flow cytometric analysis. These results suggest that the cells that survive irradiation have very high probability of becoming drug resistant. We have isolated MTX resistant colonies and are in the process of characterizing them with regard to the mechanism for drug resistance.
Supported by research grant CA-16318 from the National Cancer Institute.

E 464 MUTAGENESIS BY IONIZING RADIATION AND ALKYLATING AGENTS IN HUMAN CELLS USING A RECOMBINANT SHUTTLE PLASMID. M. O. Sikpi*, K. H. Kramer+, R. J. Preston and S. Mitra. *Univ. of Tenn. Grad. Sch. Biomed. Sci., Biol. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831 +National Cancer Inst., Bethesda, MD 20892.

The supF-tRNA gene in the shuttle plasmid pZ190 (modified pZ189) was used as a target for mutagenesis in human cells by N-methyl-N-nitrosourea (MNU) and X-rays. After mutagenic treatment of the target DNA alone, it was religated to the vector, transfected into lymphoblastoid cells, and the replicated plasmid (amplified in E. coli) was analyzed for mutations. A 15-fold increase over spontaneous mutations (0.05%) was obtained after X-irradiation (10 Gy). 87% (13/15) of the induced mutants contained base substitutions with the majority (11/13) having multiple mutations. Only GC pairs were substituted, among which 24 out of 32 were transitions and the rest transversions. MNU induced a mutation frequency of 3.8% in GM606 (Mex^+) and 5.1% in L33 (Mex^-) cells. Among the mutants, 81 out of 133 were base substitutions, 52 were deletions, and 4 insertions. Most substitution mutations involved GC pairs and were transitions. Mutation-prone sites in the tRNA gene were observed that were different for X-rays and MNU. X-ray-induced chromosome aberrations have been analyzed in G1 and G2 phases of GM606 cells. A comparison is being made of MNU vs. X-ray-induced aberrations in identical S-phase cell populations to determine if the patterns of microscopically observed deletions parallel the spectrum of those obtained at the molecular level. (Research supported by U. S. Dept. of Energy under contract with Martin Marietta Energy Systems.)

E 465 DNA POLYMERASE I AND AMV REVERSE TRANSCRIPTASE RECOGNITION OF ETHENO BASES IN TEMPLATES, B. Singer and S. J. Spengler, University of California, Berkeley, CA 94720.

Three different bases with an additional ring, resulting from vinyl chloride metabolite reaction with A, C or G, were incorporated into polynucleotides. These templates, containing a single type of modified base, were replicated using various polymerases under conditions maximizing fidelity. The resulting changes in triphosphate incorporation indicated that a proper spatial relationship was necessary for continuation of replication. This structure can be maintained by either stacking forces or hydrogen bonding. 1,N^6-etheno A was essentially a replication block and no "basepair" with any other base could be constructed. The relatively stable hydrated form of 3,N^4-etheno C did not block replication nor lead to measurable errors. The flexibility of this ethano ring could allow hydrogen bonding with G. Upon dehydration to 3,N^4-etheno C, 1 dTTP/20 εC was incorporated. The only etheno derivatives found in doublestranded DNA, N^2,3-etheno G, has a mutagenic potential equivalent to that of O^6-methyl G. dTTP is incorporated in the complement about 20% of the time while the remaining proportion of εG acts as G. In both εG•C and εG•T basepairs, two hydrogen bonds can form, with a structure very similar to the G•T wobble pair. The high mutagenic potential of N^2,3-etheno G, coupled with its ability to form both a normal and a mutagenic basepair, makes this derivative a likely candidate for an initiator of carcinogenesis induced by vinyl chloride, bifunctional aldehydes, and other chemicals that form similar metabolites. This work was supported by Grant CA 42736 from NCI.

E 466 SPECIFICITY OF umuC-DEPENDENT AND umuC-INDEPENDENT GAMMA RADIATION MUTAGENESIS IN Escherichia coli. Kendric C. Smith and Neil J. Sargentini, Department of Therapeutic Radiology, Stanford University School of Medicine, Stanford, CA 94305.

While UV radiation mutagenesis is completely dependent on the umuC gene, gamma radiation mutagenesis shows only a partial requirement for the umuC gene. The role of the umuC gene in gamma radiation mutagenesis was determined at several sites to test whether the umuC gene plays a partial role at all sites or whether it shows a site-specific, "all or none" involvement. The results support the latter hypothesis. E. coli K-12 AB1157 wild-type and umuC122::Tn5 cells were irradiated under nitrogen, and Arg^+ revertants (of argE3 ochre) were selected. Revertants were tested with amber and ochre mutants of bacteriophage T4 to categorize the putative base changes. The production of supB, supEoc and back mutants did not require the umuC gene, while the production of supC(or U), supL(or N) and supX mutants was highly dependent on umuC. Knowledge of the base changes involved in suppressor mutation induction leads to the following conclusions for anoxic gamma radiation mutagenesis: the umuC-independent mechanism results in GC -> AT transition mutations, while the umuC-dependent mechanism results in AT or GC -> TA tranversion mutations. Irradiation in the presence of oxygen added further complexity; it produced additional mutagenesis at 5 of the 6 sites tested (i.e., at all sites except supB), and in each case this oxygen effect was completely umuC-dependent. Work supported by CA-33738, NCI, DHHS.

E 467 IN VITRO MUTAGENESIS BY CHROMIUM AND NICKEL IONS. Elizabeth T. Snow, New York University Medical Center, Institute of Environmental Medicine, P.O.Box 817, Tuxedo, NY 10987.

Although metal compounds are important environmental carcinogens, the mechanisms of metal carcinogenesis and mutagenesis are poorly understood. Chromium, for example, appears to be a classic mutagen in vivo; however, it is unknown what, if any, chromium species produce mutagenic lesions. Nickel, on the other hand, is poorly mutagenic although it may interfere with DNA polymerase fidelity in vitro. M13mp2 DNA was treated with metal ions in solution and the treated DNA was transfected into calcium-treated JM101 E. coli cells and assayed for viability and mutation induction (lacZα). Chromium-induced DNA damage, although toxic, is only marginally mutagenic in non-SOS induced E. coli. It is only slightly more mutagenic (2- to 3-fold over background) in SOS-induced cells. Nickel-induced damage is also only slightly mutagenic under these conditions. A more sensitive M13-based forward mutation system is being developed to investigate these problems and to determine the relative roles of DNA damage versus metal-ion-polymerase interactions in metal-induced mutagenesis.

E 468 FORMATION AND ACCUMULATION OF ETHENOGUANINE IN THE TARGET TISSUE FOR ACRYLONITRILE CARCINOGENESIS, James A. Swenberg, Suzanne A. M. Koch and Vernon E. Walker, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Acrylonitrile (ACN) is an important monomer used in the synthesis of acrylic fibers, plastics and adhesives that has been shown to be carcinogenic in rats. Since 7-(2-oxoethyl)guanine (7OEG) had been qualitatively identified in brains of rats exposed to ACN and since 7OEG and N^2,3-ethenoguanine (εG) had been identified in livers of rats exposed to vinyl chloride, a structurally related chemical, we have investigated the formation of these two adducts using HPLC and fluorescence detection following treatment of calf thymus DNA with acrylonitrile epoxide (ANO) and following chronic exposure of rats to drinking water containing 500 ppm ACN. 7OEG was quantitatively reduced to 7-hydroxyethylguanine with sodium borohydride to improve its chromatography. Both 7OEG and εG were formed in DNA reacted with ANO *in vitro*. The εG/7OEG ratio was 0.12. Following 2 years of exposure to ACN, rat brains contained 1600 pmol εG per μmol guanine and had an εG/7OEG ratio of 1.25. The increase in the εG/7OEG ratio after chronic exposure results from differential loss and repair of the two adducts. These data suggest that the highly promutagenic DNA adduct, εG, is poorly repaired and accumulates in the target tissues for carcinogenesis.

E 469 CONSTRUCTION AND MUTAGENICITY OF BIOLOGICALLY ACTIVE DNA THAT CONTAINS SINGLE, SITE-SPECIFIC ARYLAMINE ADDUCTS, Charles M. King[1], Nobuya Tamura[1], Pawan K. Gupta[1], Thomas M. Reid[1], Dana Johnson[2] and Louis J. Romano[2], Michigan Cancer Foundation[1] and Wayne State University[2], Detroit, MI 48201.

The oligonucleotide, 5'-ATCCGTC-3', has been modified by the addition of an arylamine (2-aminofluorene or 4-aminobiphenyl) or the arylacetamide derivative at C-8 of the single guanine. The oligonucleotide adducts were purified by reverse phase HPLC and characterized by electrophoretic, spectroscopic and hydrolytic techniques to confirm their structures. The modified oligonucleotides were hybridized and ligated into gapped heteroduplex M13mp9 DNA to place the guanine adduct at position 6253 of the minus strand [Johnson et al., Biochemistry 25:449 (1986)]. The integrity of the DNA was confirmed by demonstration of its resistance to HincII, sensitivity to BamHI, and by the sizes and relative quantities of fragments produced after restriction outside the oligonucleotide. The mutations produced by these molecules in *E. coli* were primarily dependent on the prior induction of SOS functions of the host cells. Phenotypic mutations, as indicated by use of a β-galactosidase complementation system, yielded frameshifts in a run of Gs starting 5 bases 5' to the site of the adduct. "Silent" mutations identified by failure of plaques to hybridize under stringent conditions to an oligonucleotide probe that spanned the position of the adduct, were the result of base substitutions at or near the adduct site. DNA constructed with unmodified oligonucleotide yielded a mutation frequency consistent similar to wild type M13mp9 DNA. 2-Acetylaminofluorene adducts induced more frameshifts than 2-aminofluorene moieties. However, the amine produced more base substitutions than the acetamide, most of which were 1 to 4 bases from the adduct site.

E 470 DIFFERENCE IN UV MUTABILITY BETWEEN COMPLEMENTATION GROUP A AND C XERODERMA PIGMENTOSUM LYMPHOBLASTOID CELL LINES. Kouichi Tatsumi, Mariko Toyoda, Akira Tachibana, Izumi Arita and Hiraku Takebe, Kyoto University, Sakyo, Kyoto 606, Japan

Survival and mutation after ultraviolet (UV) light irradiation were compared among eitht human lymphoblastoid cell lines (LCLs): one LCL with normal excision repair capacity (HH4), six excision repair deficient xeroderma pigmentosum (XP) LCLs derived from three complementation group A patients (XP20S, XP15OS and XP7NI) and three group C patients (XP1BE, XP3BE and XP3KA), and one LCL from an XP heterozygote (XPF7NI, the father of XP7NI). Relative to HH4 and XPF7NI, both groups A and C cells were more sensitive to the cytotoxic effect of UV by virtue of diminished shoulders and steeper slopes in the survival curves determined by growth curve extrapolations. Both groups A and C LCLs were more sensitive than the normal LCL to UV-induced mutagenesis for 6-thioguanine resistance (TG^r) determined by the limiting dilution technique using 96 well microtiter plates. However, group A LCLs were extremely hypermutable by UV; i.e. 3.3 J/m^2 induced an approximately 500 fold increase of mutant fraction in XP7NI cells with the background of 2 x 10^{-6}. Group A XP LCLs remained much more mutable by UV even when plotted against survival, whereas the mutant fractions for group C XP LCLs and those for HH4 and XPF7NI followed the same line. The difference in mutability between group A and C XP LCLs was much less at Na-K dependent ATPase locus (ouabain resistance) than at HPRT locus. These results imply that the mechanism of mutation in group A XP LCLs may intrinsically be different from that in group C XP, XP heterozygote or normal LCL.

E 471 GENETIC ANALYSIS OF THE DNA BINDING DOMAIN OF LEXA PROTEIN OF E.coli, Andrew T. Thliveris, David W. Mount, University of Arizona, Tucson, AZ 85721.
The LexA protein of E.coli plays a major role in the SOS response. It is a repressor of at least 20 genes in the SOS regulon. We are interested in better defining the DNA binding domain of LexA repressor. Two different genetic approaches have been taken. First, several mutant repressors which are defective in DNA binding have been isolated. Genetically, the mutations generating these repressors are dominant to $LexA^+$, indicating that the mutant proteins can poison normal protein by forming defective mixed dimers. The repressors are presumably defective due to the elimination or disruption of contacts made between side chain(s) within the protein and the DNA helix. Thus, these mutations should map in the DNA binding region of the repressor. Second, a genetic selection has been used to isolate DNA binding specificity mutants. The recA operator, a known LexA binding site, has been altered in a symmetric fashion. A class of mutant repressors which restore binding to this altered operator but have little or no affinity for the wild-type recA operator were isolated. At best, this type of mutant would allow the identification of amino acids in the repressor which make specific contacts with base pair(s) in the DNA binding site. The altered amino acids in the above two classes identify a putative helix-turn-helix motif similar to regions in other repressors such as lambda cI and Cro proteins.

E 472 MOLECULAR ANALYSIS OF SPONTANEOUS AND MITOMYCIN C-INDUCED MUTATIONS IN THE AS52 CELL LINE. K. R. Tindall[1] and L. F. Stankowski, Jr.[2], [1]Laboratory of Genetics, NIEHS, Research Triangle Park, NC 27709 and [2]Pharmakon Research International, Inc., P.O. Box 313, Waverly, PA 18417. The AS52 cell line is an *hprt*-deficient Chinese hamster ovary (CHO) line that carries a single copy of the bacterial *gpt* gene functionally and stably integrated in the CHO genome. The *gpt* gene is analogous to the mammalian *hprt* locus and mutants at *gpt* can be isolated as 6-thioguanine- resistant colonies. We have used the AS52 cell line to study a variety of mutagens that generate deletions and/or putative point mutations as determined by Southern blot analysis. One agent, mitomycin-C (MMC), induces both deletions and putative point mutations. The yield of deletions or point mutations induced by MMC is dose-dependent. At high doses deletions predominate while at lower doses point mutations predominate. These data represent the first molecular evidence, in a mammalian somatic cell system, of mutant spectrum shifts as a function of mutagen dose and at least two pathways of MMC-induced mutagenesis are implied. We have begun to amplify mutant *gpt* sequences using the polymerase chain reaction (PCR) technique. The PCR technique provides a means by which mutant loci isolated in mammalian cells can be rapidly processed for DNA sequence analysis. Mutant-sequence spectra are being generated for both spontaneous and MMC-induced *gpt* mutants. In addition, analysis of the deletion mutants is progressing using the πvx-recombinational recovery system to clone and sequence deletion end-points. These data demonstrate the utility of the AS52 cell line in molecular analyses of mutation.

E 473 DETERMINATION OF THE SPECTRUM OF MUTATIONS INDUCED BY DEFINED WAVELENGTH SOLAR UVB (313 nm) RADIATION IN MAMMALIAN CELLS USING A SHUTTLE VECTOR, Rex M. Tyrrell and Stephen M. Keyse, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.
Mutations induced by UVB (313 nm) radiation, a wavelength in the region of peak effectiveness for sunlight induced skin cancer in humans, have been analysed at the sequence level in simian cells using an SV40 based shuttle vector (PZ189) which contains a bacterial suppressor tRNA gene as the target for mutagenesis. We find that monochromatic radiation at 313 nm is less mutagenic in this system (by a factor of approximately 3) when compared with germicidal UVC (254 nm) radiation at fluences which induce roughly equivalent numbers of pyrimidine dimer photoproducts. Of 226 313 nm induced mutants characterised by agarose gel electrophoresis and sequencing, 44% were found to be either deletions or insertions. The remainder were base substitutions with 31.5% containing a single change (28% single base change + 3.5% tandem double base change) and 24.5% containing multiple mutations clustered within the 143 bp region sequenced. The majority (62%) of base changes were G-C→ A-T transitions and together with the two transversions, changes at G-C base pairs account for 90% of all base substitutions. This pattern is similar to that seen after analysis of mutations induced by radiation at 254 nm. However, significant differences are seen in the distribution of mutational "hotspots" within the supF sequence when the mutational spectra at the two wavelengths are compared. Additional "hotspots" were induced by UVB radiation which arise predominantly (97%) at sites of mutations involving multiple base changes in the supF gene.

E 474 THE SPONTANEOUS MUTATIONAL SPECTRUM OF *rad52* SUPPORTS THE CHANNELLING CONCEPT, Elizabeth A. Savage, Grace S.-F. Lee, R. Gary Ritzel, and R.C. von Borstel, Genetics Dept., University of Alberta, Edmonton, Canada. The radiation-sensitive mutant *rad52* of yeast confers mutator activity (von Borstel *et al.*, 1971). Forward mutations were obtained at the *URA3*$^+$ locus (in a yeast integrating plasmid which is a pBR322 plasmid plus three yeast genes) are obtained using inositol selection followed by 5-fluoro-orotic acid (5-FOA) medium. After the cells are starved in uracil drop-out liquid medium for 4 hours, selection is done in inositol and uracil drop-out medium for 1 - 3 days. The cells are then plated on 7.3 mM 5-FOA medium at pH 3.2. The mutated *ura3*$^-$ genes have been cloned from 29 spontaneous mutations for the *RAD*$^+$ control and from 40 spontaneous mutations for the *rad52* strain.

The spectra of the two haploid strains are similar but not identical. The *RAD*$^+$ strain had 6 deletions, 1 insertion, 7 transitions, and 15 transversions. The *rad52* strain had 9 deletions, 1 insertion, 2 multiple base substitutions, 7 transitions and 21 transversions. The increase in transversion numbers in *rad52* appears primarily in the AT --> TA class.

The observation that the spectra of the *rad52* strain and the *RAD*$^+$ control are generally similar is in good agreement with the channelling hypothesis (Hastings *et al.*, 1976), and the notion that the *RAD52* system is nonmutagenic. (Supported by the NSERC of Canada.)

Literature cited:

von Borstel, R.C., K.T. Cain, and C.M. Steinberg, 1971, Inheritance of spontaneous mutability in yeast, Genetics 69: 17-27.

Hastings, P.J, S.-K. Quah, and R.C. von Borstel, 1976, Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA, Nature 264: 719-722.

E 475 SEQUENCE ANALYSIS OF POINT MUTATIONS AT THE HPRT GENE. Harry Vrieling, Małgorzata Z. Zdzienicka, Marie-Louise van Rooijen, Jo W.I.M. Simons, and Albert A. van Zeeland, State University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands. A methodology for rapid sequence analysis of base pair changes in the mammalian HPRT gene has been developed, using the polymerase chain reaction (PCR) procedure. First a cDNA copy is synthesized, using total cytoplasmic RNA, an HPRT specific oligonucleotide primer and AMV reverse transcriptase. Then a second primer is added which can anneal to the newly synthesized DNA strand at a position of about 350 bp downstream of the first primer, and a ds-DNA molecule is synthesized with the thermostable Taq DNA polymerase. After 25 cycles of denaturation, primer annealing and extension by Taq polymerase, the amplified fragment is purified, cut with appropriate restriction enzymes and cloned into M13 sequencing vectors. Starting with only 20 ug total cytoplasmic RNA we were able to amplify the entire HPRT coding region (=654 bp) in two overlapping fragments. The molecular basis for HPRT deficiency was investigated in (a) 29 N-ethyl-N-nitrosourea (ENU) induced mutants from mouse lymphoma cells, (b) 19 UV-induced mutants from V79 Chinese hamster cells, and (c) 20 UV-induced mutants from a UV^S V79 Chinese hamster cell line (V-H1), which was originally isolated and characterized by Zdzienicka et al (1987). Southern blot analysis showed that all mutants had identical restriction patterns as wild type, ruling out the occurrence of large alterations in these mutants. Sequence analysis of three of the ENU-induced mutants showed that one contains an A : T to G : C transition, another an A : T to T : A transversion and that the third is probably mutated in the 3' splice site of intron 1, since it gives rise to a mixed mRNA population missing the 2nd or 2nd and 3rd exon. Since V-H1 is 10-fold more sensitive to UV than wild type V79 cells (D_{10} value) and 7-fold more mutable at the HPRT locus, we want to investigate whether V-H1 has a changed UV mutation spectrum. 6 out of 7 mutants from V-H1 showed a G : C to A : T transition, all positioned at dipyrimidine sites. One mutant was caused by a -1 frameshift. These results indicate that also in this UV^S cell line mutations preferentially occur at dipyrimidine sites, where cyclobutane dimers and/or (6-4)-photoproducts can be formed. Our PCR technique can only be used when mutant cells still produce HPRT mRNA. Northern analysis showed that this was the case in 85% of the ENU-induced mutants and 95% of UV-induced mutants.

E 476 SUPPRESSION OF THE POSTREPLICATION REPAIR DEFICIENCY OF recF MUTANTS OF ESCHERICHIA COLI BY recA(Srf) AND recA(Tif) DEPENDS ON THE recJ FUNCTION

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The partial suppression of the UV-sensitive phenotype of recF mutants by mutations in the recA gene (such as srfA and tif; 1; 2) was examined. The mechanism of suppression by both recA mutations appears to be the same and depends on two prerequisites. First, the mutant recA protein must be synthesized in increased amounts as during SOS induction or as a result of a recAo (operator constitutive) mutation. A lexA(Ind) mutation abolished suppression except in a recA(Srf)recAo mutant. Second, suppression requires the recJ function and is independent of genes recN, dinA, dinB, dinD, and dinF in recF strains. The level of recJ expression in a lexA(Ind) strain suffices for full suppression indicating that recJ is not an SOS gene. We also show that the inefficient induction by UV of the SOS gene uvrA in a recF mutant (3) is improved by a recA(Srf) mutation to wildtype level (measured by β-galactosidase synthesis in a uvrA::Mud(Ap lac) fusion mutant). This alleviation of SOS induction does not require recJ$^+$. We suggest that recA(Srf) and recA(Tif) initiate a new pathway of postreplication repair which operates independent of recF. This pathway is less effective and depends on increased amounts of mutant recA protein plus recJ function.

1. Volkert, R.M. and Hartke, M.A., J. Bacteriol. 157, 498-506 (1984)
2. Volkert, R.M., Margossian, L.J. and Clark, A.J., J. Bacteriol. 160, 702-705 (1984)
3. Thoms, B. and Wackernagel, W., J. Bacteriol. 169, 1731-1736 (1987)

E 477 RecBCD ENZYME IS REQUIRED FOR *in vitro* RECOMBINATION BETWEEN LINEAR DNA DUPLEXES,
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Recombination between linear DNA duplexes that possess a double-strand break was assayed using unlabeled whole-length-linear duplex DNA and ^{3}H-labeled half-length-linear duplex DNA obtained from plasmid pACYC184. The appearance of ^{3}H-labeled DNA that migrated as whole-length-linear duplex DNA on agarose gels was taken as evidence for recombination. When the DNA mixture was incubated with one protein, either RecA, RecBCD, or Ssb (or any combination of two or three proteins) under a variety of reaction conditions, no recombinant DNA was detected. However, when the DNA mixture was incubated with the RecBCD and Ssb proteins briefly under the condition that allows unwinding to proceed, and then the $MgCl_2$ concentration was raised such that Ssb-promoted and RecA-promoted renaturation could occur, recombinant DNA was detected. Inclusion of the RecA protein greatly increased the rate of formation of recombinant DNA, but its presence was not essential. Deletion of either the RecBCD or the Ssb protein abolished the formation of recombinant DNA. The requirement for RecBCD in the recombination between linear DNA duplexes *in vitro* is consistent with the requirement for a functional *recB* gene in the repair of DNA double strand breaks (Wang and Smith, *J. Bacteriol.*, 156, 1093, 1983), and supports our model for the *recBC*-dependent repair of DNA double-strand breaks *in vivo* (Smith et al., *J. Photochem. Photobiol.*, 1, 1, 1987).

E 478 EVOLUTIONARY DIVERGENCE OF THE SOS-LIKE (SOB) SYSTEM OF *BACILLUS SUBTILLIS*.
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The gram positive spore forming bacterium *Bacillus subtilis* possesses a DNA damage inducible repair system analogous to the SOS response of *Escherichia coli*. This regulon, termed the SOB system, is non-functional in strains carrying mutations in the *recE*$^{+}$ gene. Analysis has revealed that the RecE protein is the effector molecule of the SOB system and that this protein is very similar to the RecA protein of *E. coli*. In fact, antibodies raised against RecA cross react with RecE and a cloned *recA*$^{+}$ gene will complement the *recE4* mutation of *B. subtilis* with regards to DNA repair capacity, genetic recombination and the induction of *din* (DNA damage inducible) genes. Interestingly, the *E. coli* RecA protein will not alleviate the inability of *recE4* strains to induce resident prophages.

Another interesting divergence of the SOS and SOB systems concerns the development of the competent state in *B. subtilis*. Essentially, a proportion of the population of a culture of *B. subtilis* will differentiate into a distinct physiological state in which they can then adsorb, transport, and utilize exogenous DNA. During this development, the SOB system is activated spontaneously. This activation is again under the control of a functional RecE protein. However, the enhanced production of the RecE protein that occurs during the development of competence is independent of the presence of a functional RecE protein. Thus, the results clearly demonstrate a dual form of regulation for the *recE*$^{+}$ gene.