REPAIR AND PROCESSING OF DNA DAMAGE

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Repair and Processing of DNA Damage

Keynote Address

C5-001 EVOLUTION AND REPAIR, John Cairns, Clinical Trial Service Unit, Harkness Building, The Radcliffe Infirmary, Oxford OX2 6HE, England.

Having been doing research since before the beginnings of molecular biology, I look at a subject like DNA repair from a somewhat different vantage point. It seems rather peculiar, for example, that Watson and Crick did not mention the possibility that the stability of genes might be achieved, in part, by DNA repair. But the history of biology is full of opportunities for the wisdom of hindsight. Keynote addresses are perhaps the right kinds of occasion for discussing past accidents and oversights.

E. coli As A Paradigm for Cellular Responses to DNA Damage

C5-002 ROLE OF DNA REPAIR IN AN INDUCIBLE RESPONSE TO SUPEROXIDE OR NITRIC OXIDE

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Oxidative damage to DNA is a common burden for aerobic organisms, and both constitutive and inducible defenses against it have evolved. A key class of enzymes is the endonucleases that attack abasic (AP) sites. In addition to the spontaneous and glycosylase-mediated reactions that generate AP sites, free radicals attack the deoxyribose moiety of DNA to displace bases a form a class of abasic sites oxidized at the 1, 4 or 5 carbons. Known AP endonucleases have varying abilities to initiate repair by cleaving at these oxidized abasic sites. The predominant AP endonuclease of *E. coli*, exonuclease III, acts poorly on these sites, while the less abundant enzyme endonuclease IV cleaves 4-oxidized abasic sites relatively well. Moreover, endonuclease IV activity can be strongly increased under the control of the *soxRS* stress regulon. The *soxRS* system is activated by agents that generate intracellular superoxide, and also by the nitric oxide produced by activated macrophages. In either case, the activation occurs in two stages, with the redox-sensing protein SoxR triggered as a transcription factor for the *soxS* gene, whose product then activates the genes for endonuclease IV and other regulon components. During phagocytosis by macrophages, the activation of the *soxRS* regulon provides substantial protection against cytotoxicity specifically during the nitric oxide-generating phase of the phagocytic attack. Notably, this protection requires the function of endonuclease IV, which implies that nitric oxide, perhaps in combination with other macrophage products, produces DNA damages that are not well repaired by other enzymes. We are investigating the nature of these damages and the specific role of endonuclease IV in repairing them.

Yeast As A Paradigm for Cellular Responses to DNA Damage

C5-003 DIFFERENTIAL EXCISION REPAIR IN *S. cerevisiae*. Richard Verhage[1], Alain van Gool[2], Jan Hoeijmakers[2], Piet van de Putte[1] and Jaap Brouwer[1]. [2]Department of Cell Biology and Genetics, Medical Genetics Center, Erasmus University, Rotterdam, The Netherlands, [1]Laboratory of Molecular Genetics, Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, 2300RA Leiden, The Netherlands.

Excision repair of UV-induced pyrimidine dimers occurs with different efficiencies for different parts of the genome. Silent, heterochromatic regions are repaired slower than transcriptionally active regions and within an active gene the transcribed strand is repaired faster than the non-transcribed strand. In yeast we have isolated some of the factors specifically involved in repair of these different regions. The *RAD7* and *RAD16* geneproducts that we showed earlier to be essential for repair of silenced chromatin are also essential for the repair of the non-transcribed strand of active genes. The repair phenotype of *rad7* and *rad16* cells closely resembles that of human cells derived from XP-C patients suggesting that *RAD7* and *RAD16* are the functional yeast homologs of the XP-C gene. We have cloned the yeast homolog of the human *ERCC6* gene that complements the UV-sensitivity of CS-B cells that are deficient in transcription coupled repair. The homolog that we have called *RAD26* shows a high degree of sequence similarity with it's human counterpart and in *rad26* cells the transcribed strand is repaired at almost the same rate as the non-transcribed strand. Surprisingly this mutant is not sensitive to UV-irradiation. Analyses of gene-specific repair in yeast mutants leads to the hypothesis that the basic excision repair machinery depends on two distinct pathways for the recognition of dimers in chromatin, a *RAD7/RAD16* dependent pathway for damage in non-transcribed DNA and a process dependent on transcription and *RAD26* for damage in transcribed DNA.

C5-004 MUTATIONS THAT BLOCK THE ABILITY TO SENSE DNA DAMAGE AND COORDINATE S-PHASE ARREST
Zheng Zhou, Tony A. Navas, James B. Allen, William Jones, Annette Alcasabas, and Stephen J. Elledge* Howard Hughes Medical Institute, Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Cells respond to DNA damage or blocks in DNA replication in two ways: They arrest the cell cycle and they induce the transcription of genes involved in DNA repair. We have found that these two responses are controlled by the same pathway. Using a genetic selection, we have identified eight *DUN* (DNA damage uninducible) genes that fail to induce *RNR3*, an alternative regulatory subunit of ribonucleotide reductase, in response to DNA damage. Several encode general members of the cellular transcription machinery. Others appear to be specifically involved in the DNA damage sensory pathway. *DUN1* encodes a protein kinase that is activated in response to DNA damage. *dun1* null mutations are viable but sensitive to MMS and hydroxyurea (HU) and fail to induce several DNA damage inducible genes. However, they have intact cell cycle checkpoints, and are thus specific to the transcriptional response.

In a second screen we searched for mutations that allow cells enter mitosis in the presence of a DNA replication block. These are called *SAD* mutants (S-phase arrest defective) and have the following phenotypes: 1) HU-sensitivity, 2) Rapid death in the presence of HU, 3) Are rescued by prior arrest with α-factor, and 4) Some show morphological phenotypes in the presence of HU that suggest entry into mitosis. The *sad1* mutants are sensitive to UV and MMS and fail to arrest the cell cycle in response to DNA damage or replication blocks. In addition to its cell cycle phenotype, *sad1* mutants are also defective in activation of the Dun1 protein kinase in response to DNA damage. *SAD1* encodes an essential protein kinase suggesting the possibility of a kinase cascade in the transmission of the DNA damage signal.

Mutants in a third gene, *DUN2*, display properties consistent with a role as a senor of replication blocks and some forms of DNA damage. *DUN2* encodes a previously identified DNA polymerase and is in a unique position to sense perturbations of replication. How these genes work together to sense and transduce DNA damage signals will be discussed.

C5-005 NUCLEOTIDE EXCISION REPAIR IN THE YEAST *Saccharomyces cerevisiae*, Errol C. Friedberg[1], Zhigang Wang[1], Roger Kornberg[2], Jesper Q. Svejstrup[2], W. John Feaver[2], Xhiaou Wu[1], Thomas Donahue[3], Steven Buratowski[4] and Alan E. Tomkinson[5] [1]Laboratory of Molecular Pathology, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75235, [2]Department of Cell Biology, Stanford University, Stanford, CA. 94305, [3]Department of Biology, Indiana University, Bloomington, IN 47405, [4]Department of Biochemistry and Molecular Pharmacology, Harvard University, Boston, MA 02142 and [5]Institute for Biotechnology, Center for Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78245.

The RNA polymerase II basal transcription apparatus of *S. cerevisiae* includes a complex designated core TFIIH/Ssl2, which consists of six proteins. Four of these are encoded by the *RAD3*, *SSL2* (*RAD25*), *TFB1* and *SSL1* genes. The genes encoding the remaining two proteins have not yet been identified, but are tentatively called *TFB2* and *TFB3*. Using a cell-free system it was previously shown that extracts of *rad3* and *ssl2* mutants are defective in nucleotide excision repair (NER) and that this defect can be fully corrected by the addition of purified core TFIIH or core TFIIH/Ssl2 complex, but not by the addition of purified Rad3 or Ssl2 proteins. We have now shown that extracts of conditional-lethal *tfb1* and *ssl1* mutants are also defective in NER at temperatures that are permissive for growth. Once again, this defect can be corrected by the addition of purified core TFIIH. However, mixtures of the two mutant extracts do not complement each other. Collectively,these results provide direct biochemical evidence for a requirement of the *RAD3*, *SSL2*, *TFB1* and *SSL1* genes in NER. All four proteins appear to function in NER as components of a stable complex (core TFIIH/Ssl2). We predict that the proteins encoded by the putative *TFB2* and *TFB3* genes will also be required for NER, bringing the number of indispensable genes for NER in *S. cerevisiae* to a minimum of eleven (*RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD10*, *RAD14*, *SSL1*, *SSL2*, *TFB1*, *TFB2* and *TFB3*). All of these proteins and possibly others as yet unidentified appear to assemble into a very large "super" complex that we designate as the nucleotide excision repairosome of yeast. Studies by other investigators suggest that at least three other genes (*RAD7*, *RAD16* and *RAD23*) are involved in NER, apparently with specialized roles that relate to chromatin conformation.

The Rad3 and Ssl2 proteins are endowed with 5'->3' and 3'->5' DNA helicase activities, respectively. The precise role(s) of these helicases in NER remain to be defined. The Rad1 and Rad10 proteins form a stable complex that was previously shown to degrade bacteriophage M13 DNA, but not double-stranded DNA. More refined studies have shown that the Rad1/Rad10 complex is a junction-specific endonuclease that recognizes duplex-3' single strand junctions in DNA. Degradation of M13 DNA presumably reflects the presence of such junctions at sites of secondary structure. This specificity predicts a role of the Rad1/Rad10 endonuclease in generating incisions 5' to sites of base damage during NER. We propose that the Rad2 endonuclease catalyze incisions 3' to such sites.

C5-006 CELLULAR RESPONSES TO DNA DAMAGE IN *S. POMBE*, Alan R. Lehmann[1], Antony M. Carr[1], Dominic F. Griffiths[1],Johane M. Murray[2], Felicity Z. Watts[2], and Shirley McCready[3], [1]MRC Cell Mutation Unit and [2]School of Biology, Sussex University, Falmer, Brighton BN1 9RR, UK, and [3]Dept. of Biochemistry, Oxford Univ., South Parks Rd., Oxford OX1 3QU, UK

The fission yeast *S. pombe* is exceptionally resistant to killing by UV and ionising radiation. Classical nucleotide excision repair genes are highly conserved in *S. pombe*, but in mutants with deletions in any of these genes, there is a substantial residual level of photoproduct removal (1). This suggests that there is a second pathway for removing UV damage. Mutants in the *S. pombe rad2, rad18* and *rhp51* genes are sensitive to UV, partially deficient in photoproduct removal and appear to define a second epistasis group involved in excision repair. *S. pombe rad2* has homologues in *S. cerevisiae* and human cells(2). This group of homologues is a member of the *S. cerevisiae RAD2* family, and the mammalian homologue encodes a nuclease which is identical to previously characterised nucleases variously designated as DNaseIV, FEN-1, MF-1. It is required for lagging strand synthesis in *in vitro* DNA replication systems, although the *S. pombe* gene is not essential for proliferation. *rad18* is an essential gene with several different structural motifs, including a nucleotide binding site, a leucine zipper and several heptad repeat regions. These features, also found in myosin - type molecules, suggest a possible motor function. *rhp51* is the *S. pombe* homologue of *RAD51*.

In addition to its direct repair capabilities, *S. pombe* has a very efficient G2 checkpoint mechanism (3), whereby damaged cells are arrested in G2 and are prevented from entering mitosis before the damage has been repaired. Several genes controlling the G2 checkpoint signal transduction pathway have been characterised and their individual functions are currently being analysed. The encoded proteins contain a number of interesting features including domain homologies to replication proteins (*rad17*), protein kinases (*chk1 rad27*) and lipid kinases (*rad3*).

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Mechanisms of Excision Repair

C5-007 ENZYMES FOR BASE EXCISION REPAIR: STRUCTURAL AND MECHANISTIC CONSIDERATIONS, Maria M. Thayer[1], Clifford D. Mol[1], John A. Tainer[1], Holly Ahern[2], Dongxia Xing[2], and Richard P. Cunningham[2], [1]The Scripps Research Institute, La Jolla, CA and [2]The University at Albany, State University of New York, Albany, NY.

Two base excision repair enzymes from *Escherichia coli* have been crystallized and the X-ray structures determined to 1.85Å for endonuclease III and to 1.7Å for exonuclease III. Endonuclease III is an elongated bilobal molecule with a deep cleft separating two similarly sized domains with primarily α-helical secondary structure. The enzyme contains a [4Fe-4S] cluster which is ligated by four cysteines with the spacing (Cys- X6-Cys-X2-Cys-X5-Cys) which is unlike that of any other [4Fe-4S] protein of known structure. Difference Fourier maps of thymine glycol soaked crystals located a substrate binding site which contains a seven residue β-hairpin. Electrostatic calculations show that the surface of the molecule near the cleft between the two domains is positively charged. The positive charge and the sequence homology with *E. coli* MutY, *Salmonella typhimurium* MutB protein and *Methanobacterium thermoformicicum* ORF10 implicate this surface as the location of the active site. Two novel DNA binding motifs exist at either end of the groove and we have named them the Helix-hairpin-Helix motif and the [4Fe-4S] cluster ligand motif. Site-directed mutagenesis studies have identified candidates for the active site residues for the AP lyase activity. Exonuclease III is a two-fold symmetric, four-layered α-β protein in which two closely packed six-stranded β-sheets are flanked by two pairs of α-helices. At one end of the molecule is a groove between the ends of the β strands. This surface also has several exposed loops which may be significant for DNA binding. The structure of a ternary complex with metal and dCMP bound have been used to identify the active site. This structure suggests that the enzyme hydrolyzes a phosphodiester bond via the nucleophilic attack of a water molecule activated by a single metal ion. The enzyme appears to use a single active site and identical reaction mechanisms for exonuclease, AP endonuclease, phosphatase and RNase H activities. Site-directed mutagenesis has been used to confirm the identity of active site residues.

C5-008 REPAIR GENES IN *E. COLI* AND THE SEARCH FOR THEIR HUMAN HOMOLOGS, Jeffrey H. Miller, Malgorzata Mroczkowska, Claudia Scheimer, and Robert Lloyd, Department of Microbiology and Molecular Genetics, and the Molecular Biology Institute, U.C.L.A, Los Angeles, CA 90024.

We have characterized several mutator genes in *E. coli.* Two of these, *mutY* and *mutM*, are part of the GO repair system, preventing mutations resulting from the 8-oxodGuanine lesion. In collaboration with Dr. Ying-Fei Wei of Human Genome Sciences, Inc., we have detected and are characterizing a human cDNA clone containing a large segment of the homolog to *mutY*. We will describe progress in analyzing this gene and other human homologs to GO system proteins. We will also discuss the cloning and sequencing of two additional mutators in *E. coli*, *mutA* and *mutC*.

C5-009 THE BIOCHEMISTRY OF DNA NUCLEOTIDE EXCISION REPAIR IN MAMMALIAN CELLS, Richard D. Wood, Abdelilah Aboussekhra, Rafael R. Ariza, Maureen Biggerstaff, Jonathan G. Moggs, Anne O'Donovan, and Mahmud K.K. Shivji, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms EN6 3LD, United Kingdom

The most versatile strategy for repair of damage to DNA is nucleotide excision repair. In mammalian cells, the complete mechanism involves more than 25 polypeptides, and defects in many of these are associated with various inherited disorders in humans. The syndrome xeroderma pigmentosum (XP) is associated with mutagen hypersensitivity and increased cancer incidence, and analysis of the nucleotide excision repair defect in XP has been most informative.

We have been studying this repair pathway in a cell-free system. Purified and partially purified proteins are used, as well as antibodies and other inhibitors. A current view of the course of events can be summarized as follows. Damage is located by binding of damaged DNA recognition proteins including the XPA protein. Local unwinding of the duplex flanking the damaged site, or conversion to an open structure, is mediated by a protein complex (TFIIH or a closely related assembly) that includes the XPB and XPD DNA helicases. Incisions are introduced on the modified strand, one 3' to the lesion by the XPG nuclease and one 5' to the lesion by the ERCC1/ERCC4/XPF nuclease. These are "structure-specific" nucleases that can cleave near the border between duplex and single-stranded DNA. The precise location of the incision sites seems to be dictated by the extent of opening or unwinding of the DNA around the lesion. In nontranscribed DNA, the XPC protein complex also appears to be needed for at least one of these incisions. XPC protein complex can bind tightly to single-stranded DNA. The single-stranded DNA binding protein RPA is also required during the first stage, either during recognition of damage or to stabilize the incised site and mediate the transition to the repair synthesis stage. PCNA-dependent DNA polymerase (ε or δ) then performs gap-filling DNA repair synthesis. PCNA is loaded onto the DNA by RFC. A repair patch of ~30 nucleotides is formed, and a DNA ligase completes the process. A protein phosphatase is needed for optimum repair of UV-damaged DNA *in vitro*, emphasizing that polypeptides besides those mentioned participate in the repair reaction by modulating the activity of other components.

Repair and Processing of DNA Damage

Recombinational Repair and the Processing of Strand Breaks

C5-010 MOLECULAR STAGES IN V(D)J RECOMBINATION, Martin Gellert[1], Moshe Sadofsky[1], Joanne E. Hesse[1], J. Fraser McBlane[1], and David B. Roth[2], [1]Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD 20892 and [2]Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030

An early step in V(D)J recombination appears to involve double-strand breakage at the border between the signal sequence and its neighboring coding sequence. We recently found two distinct species of molecules broken at this site. Broken signal ends associated with V(D)J joining at the TCR beta or delta locus are quite abundant in mouse thymus DNA, and their structure is simple. Most of them terminate exactly at the signal border on both strands and carry a 5'-phosphoryl group. These ends seem likely to be primary cleavage products.

Broken ends on the adjoining coding DNA are harder to find and have a more unusual structure. So far, they have only been found in DNA from mice with the scid mutation, which interferes with the completion of coding joints. These ends are sealed back on themselves as DNA hairpins. Such a DNA species is a plausible intermediate in V(D)J recombination, because an off-center nick in a hairpin would explain the short self-complementary (P nucleotide) insertions often found in coding joints. We thus believe that these hairpins must occur transiently also in wild-type cells.

In attempting to understand the roles of RAG-1 and 2 in V(D)J joining, we have made numerous mutations in both genes. One class of altered RAG-1 proteins is particularly interesting. These mutants allow efficient recombination of substrates with some coding sequences but are totally inactive (1000-fold down) on others, although both types work well with normal RAG-1. A closer analysis using randomized coding flanks shows that it is the first two nucleotides adjacent to the signals that are crucial. These mutations provide the first evidence for interaction of RAG-1 with DNA at the site of recombination, and may help to define the initial steps of the reaction.

C5-011 RECOMBINATION IN YEAST AND ITS RELATIONSHIP TO DNA REPAIR, Hannah L. Klein, Hua-ying Fan, Kenneth Chang, Dessislava Dimova, Anuradha Thangavelu, and Marilena Aquino de Muro, New York University Medical Center, New York, NY 10016.

Mitotic recombination is one mechanism that is used to repair DNA damage. Hyperrecombination mutants either create a recombinogenic substrate or fail to repress mitotic recombination. Mutations in genes involved in DNA repair or replication often result in a hyperrec phenotype. A search for mitotic hyperrec mutants, *hpr* mutants, identified mutations in DNA polymerase genes as well as mutations in novel genes. One of these genes, *SRS2(HPR5)*, encodes a DNA helicase that is homologous to *uvrD*. This helicase functions in the error-prone DNA repair pathway to channel repair intermediates into the pathway. We propose that the helicase acts in an antirecombinase fashion to disrupt heteroduplex intermediates which can become recombination substrates. The *SRS2/HPR5* helicase gene also has an essential role during meiotic recombination and delays the appearance of recombination products. A model of the role of the helicase in recombination will be presented. Another hyperrec mutant, *hpr1*, identified a gene that has homology to DNA topoisomerases. However, no topoisomerase phenotype of function has been demonstrated. We believe that the *hpr1* mutant leads to the accumulation of a recombinogenic substrate which is not a double strand break. Suppressors of the *hpr1* mutant are in genes required for transcription. Data suggesting the existence of a novel DNA repair complex associated with the basal transcription machinery will be presented.

C5-012 THE FORMATION AND RESOLUTION OF HOLLIDAY JUNCTIONS DURING THE RECOMBINATIONAL REPAIR OF DNA DAMAGES, Stephen C. West[1], David Adams[1], Richard Bennett[1], Fiona Benson[1], Adelina Davies[1], Hazel Dunderdale[1], Kevin Hiom[1], Boris Kysela[1], Christine Mézard[1], Alison Mitchell[1], Carol Parsons[1], Rajvee Shah[1], Irina Tsaneva[1], Andrzej Stasiak[2], Edward Egelman[3], [1]Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K., [2]University of Lausanne, CH-1015 Lausanne, Switzerland, [3]University of Minnesota, Minneapolis MN55455.

The *ruv* locus of the *E. coli* chromosome encodes three proteins that interact with Holliday junctions during genetic recombination and the recombinational repair of DNA. Two of these proteins, RuvA and RuvC, form part of the DNA-damage inducible (SOS) repair system. The 22 kDa RuvA protein binds specifically to Holliday junctions and targets RuvB protein (37 kDa) to the site of the junction. RuvB assembles on DNA to form ring structures that have been visualized by electron microscopy. Structural analysis indicates that there are 6 RuvB monomers per ring. Images of the rings have been cylindrically averaged and 3-D reconstructions indicate that the RuvB rings contain a deep hollow core through which the DNA lies. Using model Holliday junctions, we have shown that RuvAB catalyze Holliday junction movement in vitro. This process, known as branch migration, occurs at the expense of RuvB-mediated ATP-hydrolysis and leads to the formation of heteroduplex DNA. RuvAB-Holliday junction complexes have been analyzed by DNAseI footprinting and by electron microscopy. RuvA protein binds all four strands of DNA at the crossover point, whereas RuvB binds the junction asymmetrically. The polarity of branch migration is defined by the asymmetric assembly of RuvB with respect to RuvA. A model for branch migration will be presented in which RuvB drives branch migration by helical rotation coupled with DNA unwinding as the DNA passes through the RuvB ring structure.

Following branch migration, the production of mature recombinant DNA molecules requires RuvC-mediated resolution of Holliday junctions. RuvC is a junction-specific endonuclease which catalyzes resolution via the introduction of nicks into two DNA strands of the same polarity. The cuts are placed with perfect symmetry in each duplex to produce nicked duplex products that are repaired by DNA ligase. The resolution reaction can be sub-divided into three distinct steps: (i) structure-specific recognition of the junction by RuvC, (ii) DNA distortion, and (iii) sequence-dependent cleavage. Analysis of Holliday junction resolution by RuvC indicates the presence of resolution 'hotspots' which correspond to a tetranucleotide sequence 5'-$^{A}/_{T}$TT$^{G}/_{C}$. The combined roles of RuvA, RuvB and RuvC in the post-replicational repair of DNA damage will be presented.

DNA Repair, Replication and Transcription

C5-013 TRANSCRIPTION-COUPLED DNA REPAIR, Philip Hanawalt[1], Fred Christians[2], Brian Donahue[1], Ann Ganesan[1], Ángel Islas[3], Lori Lommel[1], Allen Smith[1], Graciela Spivak[1], and Kevin Sweder[1]. [1]Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, [2]University of Washington, Seattle, [3]University of California, San Francisco.

Preferential repair of expressed genes in a wide range of species, from *Escherichia coli* to human cells, has been shown to be largely due to the efficient removal of transcription-blocking lesions from the transcribed DNA strand (1, 2). The non-transcribed strand does not exhibit transcription-coupled repair (TCR). No significant variation in TCR is noted through the normal human cell cycle for a continuously expressed gene. In yeast TCR operates equally well on plasmid-borne and chromosomal genes. Pol I transcribed ribosomal genes are not subject to TCR (3). We don't yet know the extent to which the excision-repair enzymes participate in the detail of lesion recognition at the site of an arrested RNA polymerase. An actively elongating RNA polymerase II transcription complex is required as TCR is eliminated at the restrictive temperature in pol II^{ts} mutants of yeast and by the transcription elongation inhibitor α-amanitin. Although a bacterial transcription-repair coupling factor (Mfd) has been shown to disengage a stalled polymerase from the DNA at a lesion site (4) we suggest an alternative model for eukaryotes, in which the abortion of transcripts in the typically large genes could be problematic. The transcription elongation factor SII has been shown to catalyze RNA transcript cleavage by pol II at the site of a transcription-arresting cyclobutane pyrimidine dimer in the template DNA strand, without release of the polymerase or the truncated RNA product (5). We suggest that this process may be an early step in eukaryotic TCR, prior to interaction with the coupling factor (likely, ERCC6) thought to recruit the excision-repair enzymes to the lesion site. At least four protein components of the TFIIH basal transcription initiation factor are essential for both TCR and overall genomic excision-repair, thereby serving roles in both transcription and repair (6). Additional experimental data relevant to models for TCR will be reviewed.

TCR may be involved in the phenomenon of adaptive mutagenesis. We postulate that "gratuitous TCR" events might occur at some lesion-free sites that pose transient blocks to transcription elongation. Reiterative DNA turnover at such sites in expressed genes could eventually result in mutagenic events in quiescent cells.

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Eukaryotic Transcription

C5-014 TRIPLE ROLES OF TFIIH: RNA POLYMERASE TRANSCRIPTION FACTOR, NUCLEOTIDE EXCISION REPAIROSOME, AND CDK-ACTIVATING KINASE, Jesper Q. Svejstrup[1], William J. Feaver[1], Zhigang Wang[2], Xiaohua Wu[2], N. Lynn Henry[1], Errol C. Friedberg[2], and Roger D. Kornberg[1], [1]Dept. of Structural Biology, Stanford University, School of Medicine, Stanford, CA 94305, [2]University of Texas Southwestern Medical Center, Dallas, TX.

TFIIH, a multisubunit protein conserved in structure and function from yeast to man, plays three widely disparate roles:

1) Essential RNA polymerase II transcription factor. Resolution of holoTFIIH into 5-subunit core TFIIH, 3-subunit TFIIK, and Ssl2 protein reveals that all three components are required for transcription.
2) Nucleotide excision repairosome. A complex of core TFIIH, Ssl2 protein, and the products of all additional *RAD* genes known to be required for nucleotide excision repair in yeast (*RAD1, 2, 4, 10, 14*) is especially active in repair *in vitro*.
3) CDK-activating kinase (CAK). The kinase subunit of TFIIK has been identified as Kin28 protein, homolog of the human CAK component M015. Others have shown in a 2-hybrid screen that Kin28 interacts with the cyclin Ccl1, homolog of the human CAK component cyclin H.

C5-015 CHROMATIN STRUCTURE AND GENE EXPRESSION, Alan P. Wolffe, Geneviève Almouzni, Philippe Bouvet, Stefan Dimitrov, Jeffrey J. Hayes, Nicoletta Landsberger, Karl Nightingale, Dmitry Pruss, and Kiyoe Ura, Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, NIH, Bethesda, MD. 20892.

A chromatin environment provides many advantages to the protein machines involved in DNA metabolism, including the regulation of transcription. Nucleosome formation can alternately either repress (1) or potentiate transcription (2). Nucleosome formation can also facilitate retroviral integration events (3, 4). The three dimensional folding of DNA in chromatin has an important structural and regulatory role. We have characterized the influence of individual histones and their domains on nucleosome structure, nucleosome positioning and the capacity of nucleosomes to repress transcription (5, 6). These in vitro experiments have been extended to chromatin function in vivo. Replication coupled chromatin assembly is required for the general repression of basal transcription in Xenopus oocyte nuclei (7). In spite of complete chromatin assembly, certain transcription factors (eg GAL4/VP16, HSF) activate transcription from the initially repressed state. These transcription factors have two functions: the relief of repression (chromatin disruption) and the activation of the transcription process (recruitment of the basal transcription machinery). We have examined the relative importance of these two functions during vertebrate development as the structural components of chromosomes are modified. These modifications include the replacement of linker histones eg B4, H1, H1° and alterations in histone acetylation (8-10). Some of the consequences of removal or overexpression of the linker histones for gene expression in the developing embryo have been determined.

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Mutagenesis

C5-016 STUDIES OF DNA REPLICATION FIDELITY IN HUMAN CELL EXTRACTS, Thomas A. Kunkel, David C. Thomas, Jayne C. Boyer, Dana T. Minnick, Shunji Izuta, John D. Roberts, Asad Umar, Shang Yin and Dinh C. Nguyen, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709

We are attempting to understand the mechanisms by which mutations are generated or avoided during replication of eukaryotic genomes. The approach is to measure the fidelity of SV40 origin-dependent replication of double-stranded DNA in extracts of human cells, using as a mutational target the α-complementation domain of the *E. coli LacZ* gene in bacteriophage M13mp2 DNA. Studies of replication of undamaged DNA (1) suggest that : i) fidelity is high for replication of both the leading and lagging strands, ii) alterations in the relative and absolute concentrations of dNTPs affect replication error rates, iii) exonucleolytic proofreading of errors occurs on both strands, iv) replication error rates are highly sequence-dependent, v) fidelity on the leading and lagging strands is similar for certain errors but different for others, and vi) the leading and lagging strand replication complexes can proofread the same mispair with different efficiency. Data have also been obtained for replication of DNA containing adducts of known carcinogens (2). Although both AAF adducts and cyclobutane pyrimidine dimers inhibit replication, mutagenic translesion synthesis does occur during both leading and lagging strand replication. At some template positions, the cyclobutane pyrimidine dimer-dependent replication error probability is higher during lagging strand replication than during leading strand replication, while at other sites the opposite is observed. Thus the relative fidelity of leading and lagging strand translesion synthesis varies by position. Finally, in an attempt to establish the biochemical defect(s) responsible for the genetic instability associated with cancer cells, replication fidelity and the efficiency of repair of heteroduplexes containing mismatches and loops is being examined in tumor cell lines derived from a variety of cancers (3).

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C5-017 MUTAGENESIS IN *E. coli*, Melissa Lee, Sumati Murli, Timothy Opperman, Angelina Guzzo, and Graham C. Walker, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Although it is widely thought that most UV and chemical mutagenesis results from a process of translesion bypass, elucidation of the physiologically-relevant mechanisms responsible for such mutagenesis has proved a formidable challenge. In the cases of both *Escherichia coli* and *Saccharomyces cerevisiae*, the isolation of mutants that are deficient in UV and chemical mutagenesis has indicated that specialized cellular functions are required for this phenomenon. In *E. coli*, the products of the *umuD*, *umuC*, and *recA* genes are needed. The *umuDC* operon is repressed by the LexA protein and is induced as part of the $recA^{+}lexA^{+}$-dependent SOS response. The SOS response is induced when RecA, activated by the single-stranded DNA produced by a cell's attempts to replicate damaged DNA, mediates the proteolytic cleavage of the Ala84-Gly85 bond of LexA by facilitating an otherwise latent capacity of LexA to autodigest. Activated RecA also activates UmuD for its role in mutagenesis by mediating the posttranslational cleavage of UmuD at its Cys24-Gly25 bond to yield UmuD'. In addition, mutagenesis requires a function of RecA that is distinct from its roles in mediating LexA and UmuD cleavage. Genetic studies have indicated that neither DNA Pol I or Pol II are required for mutagenesis whereas DNA Pol III has been positively implicated. In addition, *umuDC*-dependent mutagenesis, but not mutagenesis mediated by *mucAB* (a plasmid-borne *umuDC* homolog) requires functions of the GroEL/GroES molecular chaperones. The addition of purified UmuD', renatured UmuC, and RecA has been shown to permit DNA Pol III holoenzyme to carry out limited bypass synthesis on a primed DNA substrate with a single abasic site in the template strand. Since it has proved very difficult to purify soluble UmuC from cells that overproduce UmuC, we are employing an alternative approach which involves the modest overproduction of a derivative of the protein carrying a histidine tag, accompanied by the simultaneous production of other SOS-regulated proteins and activated RecA. Several approaches are being taken to analyze the nature of the protein-protein interactions that are necessary for $umuD^{+}C^{+}$-dependent bypass to take place. For example, these interactions are being explored by the use of affinity chromatography and by the use of glutathione S-transferase fusions. Furthermore, biologically-active UmuD derivatives that contain a single Cys residue have been constructed and are being used in a variety of experimental approaches. For example, the reactivity of the unique thiol group of each unique Cys has been used to assess its solvent exposure. In addition, the ability of the thiol group within each Cys residue to form disulfide bonds within the UmuD homodimer has been examined to assess the relative proximity of various positions to the dimer interface. A strategy of attaching photocrosslinking agents to these thiol groups is being used to analyze the interaction between UmuD and other proteins. Consistent with an interaction of UmuD' and UmuC with the replication apparatus are the observations that a strain overproducing the *umuDC* operon is cold-sensitive and that the conditional lethality is associated with an inhibition of DNA synthesis. The nature of this replication defect is being investigated by analyzing the interactions between UmuD'/UmuC and the subunits of the DNA polymerase III.

Genomic Instability

C5-018 DNA METHYLATION AND GENOME STABILITY IN FUNGI, Eric U. Selker, Institute of Molecular Biology, University of Oregon, Eugene OR 97403

What, if anything, counters changes to genomes caused by spontaneous chromosomal rearrangements and transposons? In the filamentous fungus Neurospora crassa, repeated sequences are specifically recognized and subjected to mutation and DNA methylation in the sexual cycle by a process named RIP (repeat-induced point mutation; see 1). Relics of transposons that have been inactivated by RIP have been found in N. crassa and other species of Neurospora and a newly introduced multicopy transposon is efficiently inactivated by RIP in the laboratory (2). Similarly, in the fungus Ascobolus immersus, a process closely related to RIP, MIP (methylation induced premeiotically; see 3) methylates, but does not mutate, duplicated sequences. Both RIP and MIP operate on both linked and unlinked duplications, whether created spontaneously or by DNA-mediated transformation. Since duplications can lead to translocations, the action of RIP and MIP on duplications may prevent the establishment of rearrangements. Mutations and/or methylation resulting from RIP can extend hundreds (and probably thousands) of bp into unique sequences adjacent to the repeated sequences, potentially reducing the fitness of strains harboring inactivated sequences (4). Preliminary studies suggest that changes to the genome can also be countered by methylation in the vegetative phase in Neurospora. Transforming DNA sequences sometimes become methylated and/or silenced, especially when multiple copies integrate into the genome (5-8). I will present our current understanding of the control and function of DNA methylation in fungi. Processes similar to RIP, MIP and de novo methylation of transforming DNA have been observed in higher eukaryotes (e.g. see 9). Thus, information from Neurospora and other simple systems may shed light on general mechanisms stabilizing eukaryotic genomes.

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Repair and Processing of DNA Damage

Molecular Pathology of Human Diseases With Defective Processing of DNA Damage

C5-019 MOLECULAR GENETICS OF FANCONI ANEMIA. Ming Chen[1], Rob Cumming[1], Flora Krasnoshtein[1], Anna Savoia[2], Jeff Lightfoot[1], Claudia Santos[1], Linda Parker[1], Jasmine Wong[1], Alexandra Joyner[3] and Manuel Buchwald[1], [1]Hospital for Sick Children and University of Toronto, Toronto, CANADA, [2]Casa Sollievo della Sofferenza, Foggia, ITALY and [3]New York University Medical School, New York.

Fanconi anemia (FA) is an autosomal disorder characterized by bone marrow failure, a variety of congenital malformations and an increased predisposition to the development of malignancies, especially AML. FA cells show chromosome fragility, an increased sensitivity to the cytotoxic action of DNA cross-linking agents such as mitomycin C (MCC) and diepoxybutane (DEB) and hypomutability. Cytokine abnormalities have also been noted in FA patients and their cells. At least 4 complementation groups have been identified, labelled A to D. The basic defect in the disease is not known. We have exploited the sensitivity of FA cells to DNA cross-linking agents to isolate a set of complementing cDNAs that correct the cellular sensitivity *of FA(C), but not* FA(A),(B) or (D), cells. The cDNAs code for a novel protein of 558 amino acids with no known homologies to sequences in the data bases. Mutations have been identified in the DNA of the cell line used to isolate the cDNAs, confirming that the cDNAs are coded for by the gene defective in FA(C). The gene has been name *FAC* and maps to 9q22. Much of the genomic structure of the gene has been elucidated. The coding sequence is divided into 14 exons which span more than 100 kb. Three alternative polyadenylation sites are used, leading to messages of three different lengths. The gene is ubiquitously expressed in human and mouse tissues. *FAC* is also expressed at high levels during mouse development in mesenchymal tissues and in areas of bone that are undergoing proliferation and differentiation. Two alternative 5' exons, that do not alter the coding region, are located approximately 300 bp from each other. The more 5' exon is constitutively expressed while the other appears to be expressed after treatment with DNA damaging agents. The region upstream of the most 5' exon shows promoter activity *in vitro*. The mouse and bovine homologs have been isolated and shown to have about 75% amino acid similarity to each other and to the human homolog. Analysis of DNA from eukaryotes such as zebrafish, *Drosophila* and *C. elegans* shows cross-hybridizing bands, suggesting that *FAC* homologs may also be present. These are currently being isolated. Antibodies to FAC reveal both nuclear and cytoplasmic localization. Because of the morphological and hematological features of FA, we have used gene targeting to establish a mouse model for FA(C). A targeting construct that removes exon 8 and changes the reading frame was constructed, electroporated into embryonic stem cells and recombinants isolated. Aggregation chimeras were created and transmission of the mutant allele through the germ line was identified by Southern blotting. Homozygous defective mice have been born and they are currently being studied. (Supported by MRC Canada, NCIC, NIH and FA Research Fund).

C5-020 NUCLEOTIDE EXCISION REPAIR DEFECT AND CARCINOGENESIS IN *XPA*-KNOCKED OUT MICE, Kiyoji Tanaka, Yoshimichi Nakatsu, Masafumi Saijo, Isao Kuraoka, Toshiro Matsuda, Takehiro Kobayashi, Hiroaki Murai, Hironobu Nakane, Institute for Molecular and Cellular Biology, Osaka University, Osaka 565, JAPAN.

Group A XP gene (*XPA* gene) encodes a hydrophilic protein with 273 amino acids and C4 type zinc-finger motif. We analysed DNA repair functions of the *XPA* gene *in vitro* and *in vivo*. The XPA protein (XPA) preferentially bound to DNA damaged by UV, cisplatin or osmium tetraoxide, suggesting that XPA is involved in the damage recognition step of nucleotide excision repair (NER) processes. We found that the XPA polypeptides of 122 amino acids containing C4 type zinc-finger motif are sufficient for the activity of preferential binding to damaged DNA. A comparison of the amino acid sequences of the *XPA* homologues revealed that the N-terminal region is not well conserved except NLS and the E-cluster region, whereas the C-terminal region is highly conserved. The E-cluster and the C-terminal region are not necessary for DNA binding activity. These results suggest that XPA might have an important DNA repair function other than DNA binding. We speculated that XPA might have domains for protein-protein interactions to coordinate NER processes. We therefore searched proteins which bind to XPA using yeast two hybrid system, and found that p34 subunit of RPA (Replication Protein A) bound to XPA. The direct association between XPA and RPA complex was confirmed by the *in vitro* experiments. Furthermore, RPA complex was co-immunoprecipitated from HeLa whole cell extracts with XPA by anti-XPA antiserum, suggesting the association of XPA and RPA complex *in vivo*. It has been shown that RPA is involved in the early step of NER processes. These results taken together indicate that XPA might cooperate with RPA for damage recognition. Then, we developed *XPA*-deficient mice by gene targeting in mouse ES cells. The *XPA*-deficient mice were defective in NER, but showed neither obvious physical abnormalities nor pathological alterations. *XPA*-deficient mice that were treated with chemical carcinogen 9, 10-dimethyl-1,2-benz[a]anthracene developed severe skin ulcers, which subsequently developed into papillomas at a high frequency. These results provide direct *in vivo* evidence that XPA protects mice from tumorigenesis initiated by carcinogen-induced DNA damage(s). The *XPA*-deficient mouse provides an excellent *in vivo* model system to study carcinogen-induced tumorigenesis and mutagenesis.

Carcinogenesis

C5-021 p53 DNA REPAIR AND CARCINOGENESIS

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The p53 tumor suppressor gene product has pleiotropic functions including control of the cycle G1 checkpoint, maintenance of genomic integrity and involvement in one or more pathways of apoptosis. We are currently investigating the role of p53 in DNA repair and apoptosis (programmed cell death) in human cells. Wild-type and mutant p53 can bind in vitro to several BTF2-TFIIH associated factors, including human transcription-repair factors, XPD(ERCC2) and XPB(ERCC3), as well as Rad3, the yeast homologue of human XPD, and CSB(ERCC6), which is involved in strand-specific DNA repair. All these factors belong to recent identified DNA and RNA helicase superfamily. We also mapped the region of XPB that interacts with p53 to an indispensable domain conserved among the helicase superfamily. The C-terminal p53 domain involved in the regulation of its activity is required for the interaction. Furthermore, wild-type but not 273^{his} mutant p53 inhibited XPD and Rad3 helicase activities, and to a lesser extent XPB helicase activity. p53 did not alter the BTF2-TFIIH associated basal transcription activity nor the ATPase activity of BTF2-TFIIH or Rad3. Moreover, *Li-Fraumeni syndrome cells that* are heterozygous for the p53 mutant allele repair UV-induced pyrimidine dimers in the DHFR gene at a slower rate than normal human cells. These and other results indicate that p53 may play both an indirect (G1 checkpoint function) and a direct role in modulating nucleotide excision repair pathways.

Wild type p53 is also involved in certain pathways of apoptosis, e.g., in response to DNA single strand breaks. We are investigating the pathway of p53-dependent apoptosis and its modulation by mutant p53 and p53 binding proteins, e.g., hepatitis B viral X protein. The implications of these results in carcinogenesis will be discussed.

C5-022 DNA MISMATCH REPAIR IN YEAST AND MAMMALS. R. Michael Liskay, Sean Baker, Eric Bronner, Tomas Prolla, and Qishen Pang, Department of Molecular and Medical Genetics, L103, Oregon Health Sciences University, Portland, OR 97201-3098.

Mutations in any one of four DNA mismatch repair gene homologs, *hMSH2, hMLH1* , *hPMS1* and *hPMS2* can cause hereditary nonpolyposis colorectal cancer in humans. It has been estimated that HNPCC accounts for 4-13% of colorectal cancers in the Western World, making it the most common form of hereditary colon cancer. One characteristic of DNA mismatch repair deficiency in bacteria, yeast and mammalian cells, is instability of short tandem repeat or microsatellite sequences. Microsatellite instability has been demonstrated not only in tumours from HNPCC patients, but also in a subset of sporadic colorectal and other cancers. These findings have led to a renewed interest in understanding the mechanism of DNA mismatch repair in eukaryotes. In the yeast *Saccharomyces cerevisiae*, DNA mismatch repair requires the MSH2, MLH1 and PMS1 proteins. Experiments from our lab indicate that the yeast MLH1 and PMS1 proteins associate *in vivo* and *in vitro*, possibly forming a heterodimer. Additionally, the yeast MLH1 and PMS1 proteins act in concert to bind a MSH2-heteroduplex complex containing a G-T mismatch. Therefore, these studies have shown that the MSH2, MLH1 and PMS1 proteins are likely to form a ternary complex during the initiation of eukaryotic DNA mismatch repair. We are presently defining the domains of the yeast and human MLH1 and PMS1 proteins that are responsible for this heteromer formation, and for interaction with the MSH2 protein. In addition, we are using the yeast "two hybrid" system to find other proteins that interact with the MutL homologs MLH1 and PMS1. Such proteins will likely represent other members of the mismatch repair pathway and/or proteins of "interacting" pathways. Besides our interest in the mechanism of mismatch repair we are also interested in the dynamics of tumor formation in individuals carrying DNA mismatch repair mutations. Toward this goal we have derived, via gene targeting of mouse ES cells, mice which are heterozygous for a deletion mutation in the mouse *PMS2* gene. At present we are following such heterozygous mice for the expected tumor formation, and determining whether the homozygous mutant mice are viable.

Late Abstract

RECONSTITUTION OF HUMAN DNA REPAIR EXCISION NUCLEASE FROM PURIFIED COMPONENTS, **David Mu, Chi-Hyun Park, Tsukasa Matsunaga, David S. Hsu, Joyce T. Reardon, and Aziz Sancar, Department of Biochemistry and Biophysics, Unviersity of North Carolina School of Medicine, Chapel Hill, North Carolina USA 27599-7260.**

In *Escherichia coli*, studies have shown that a multi-subunit excinuclease, composed of UvrA, UvrB, and UvrC proteins, removes the DNA damage by incising the 8th phosphodiester bond 5' and the 5th phosphodiester bond 3' to the lesion. By using a cell-free system, it has been demonstrated that the human excision repair enzyme removes a 27-29 oligomer containing the damaged nucleotide by shifting the 5' incision site farther from the lesion . However, in contrast to the Uvr (A)BC-directed DNA damage excision, in humans it requires approximately 15-20 proteins to make the dual incisions. Given the complexity of human DNA excinuclease and the possibility that more excision repair factors are yet to be discovered, we decided to reconstitute the human excision nuclease *in vitro*. HeLa cell-free extract was fractionated into five fractions of high purity which contain all the currently known repair proteins. The results show that these five fractions are necessary and sufficient for excision repair, establishing the first reconstituted system of human excision nuclease *in vitro* and providing an answer to the question regarding its minimal composition. Using the reconstituted excinuclease, we found that the excised fragment remains associated with the post-incision DNA-protein complex, suggesting that accessory proteins are needed to release the excised oligomer.

DNA Repair I

C5-100 Identification and Characterization of A Repair Endonuclease in Deinococcus Radiodurans.

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Gram positive bacteria of the genus *Deinococcus* possess extraordinary resistance to the lethal and mutagenic effects of most agents that damage DNA, including ultraviolet (UV) radiation and the genotoxin mitomycin C (MMC). The extremely efficient ability to repair DNA damage induced by UV and MMC has been attributed, through complementation analysis, to two repair endonucleases (UV endonuclease-α) and (UV endonuclease-β) that incise deinococcal chromosomal DNA at or near sites of DNA damage, thereby initiating enzymatic excision of the DNA lesions.

The (UV endonuclease-α) gene encoded by *mtcA* and *mtcB* was found by DNA sequence analysis to be a single gene which is over 60% homologous with *E. coli* and *M. luteus uvrA* nucleotide sequences. Interestingly, a gene adjacent to and divergently transcribed from UvrA in *E. coli* and *M. luteus,* single-stranded DNA binding protein (ssb), was not detected within 1,500 bases from the UvrA homolog in *D. radiodurans*.

UvrA is one protein of the UvrABC excinuclease complex in *E. coli* that recognizes thymine dimers and other bulky damage on nucleotide strands after UV induced DNA damage. *E. coli* UvrA expressed in *mtcA, mtcB* strains of *D. radiodurans* were able to restore UV resistance to wild type levels in *D. radiodurans*. This data suggests that another repair pathway in *D. radiodurans* is responsible for Deinococcus' extraordinary resistance to UV irradiation.

C5-101 THE EVOLUTION OF EXTREME IONIZING RADIATION RESISTANCE IN THE *DEINOCOCCACEAE*,

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Members of the family *Deinococcaceae* are distinguished by their extraordinary resistance to the lethal and mutagenic effects of many DNA damaging agents including mitomycin C, ultraviolet light, and ionizing radiation. As part of our studies, we have tried to develop a plausible explanation for why the deinococci evolved such high tolerance to DNA damage. Since the existence of a terrestrial environment with radiation fluxes in excess of 5000 Gy is unlikely, we assumed that the DNA repair capability of the deinococci arose in response to an environmental stress that the deinococci routinely encounter. We have found a correlation between the deinococci's ability to survive ionizing radiation and their capacity to survive prolonged desiccation. Forty ionizing radiation sensitive mutants were isolated and divided into 17 linkage groups. Each mutant was evaluated for its ability to survive six weeks desiccation. Thirty nine of the forty mutants representing 16 of the 17 linkage groups exhibited sensitivity to desiccation. Corollary studies show that the DNA isolated from desiccated *D. radiodurans* is extensively damaged and that the pattern of damage is similar to that observed following γ irradiation.

C5-102 ISOLATION AND ANALYSES OF A GENOMIC DNA FRAGMENT THAT RESTORES RADIORESISTANCE TO SCHIZOSACCHAROMYCES POMBE rad23-1 cells,

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Schizosaccharomyces pombe rad23-1 cells are highly sensitive to ionizing radiation and UV light. To clone wild-type rad23, a genomic library made from the DNA of $rad23^{+}$ cells was used to transform a mutant cell population. Of approximately 27,000 transformants screened, one was UV resistant, relative to rad23-1 cells. Plasmid DNA was isolated from the transformant and was able to confer radioresistance upon mutant cells. The plasmid restored nearly wild-type levels of UV resistance to rad23-1 cells and did not effect the resistance of $rad23^{+}$ cells to this type of radiation. However, the plasmid only partially restored ionizing radiation resistance to mutant cells and reduced the resistance of wild-type cells to gamma-rays. The differential ability of the plasmid to influence radioresistance suggests that the requirement of the plasmid encoded protein for repair of damage induced by UV versus ionizing radiation is different. Since the gene is on a multi-copy plasmid, the protein product is overproduced and may not be at optimum levels for all types of repair. In support of this hypothesis, we found that rad23-1 cells containing one or two integrated copies of the plasmid insert exhibit wild-type levels of UV and gamma-ray resistance. The plasmid contains an 8.8 kb genomic DNA fragment. The active region is currently being more precisely defined for DNA sequence determination and other analyses. (This work was supported by NIH grants CA12536 and CA54044)

C5-103 CLONING OF THE GENE CODING FOR *ESCHERICIA COLI* ENDONUCLEASE VIII AND CHARACTERIZATION OF THE ENZYME,

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Endonuclease VIII, a protein of 29kDa, was originally identified in an *E. coli* strain which lacked endonuclease III with which it shares substrate specificity. In order to obtain homogeneous enzyme for protein sequencing, reduced AP site-containing DNA-cellulose affinity chromatography was used after radial flow S-Sepharose, Mono S, Phenyl Superose and Hydroxylapatite FPLC. Amino acid sequences were obtained and used to design degenerate oligonucleotides. A PCR fragment was obtained from *E. coli* genomic DNA and then used to probe the Kohara Library. The gene coding for endo VIII, *nei,* located at 16.7 min in the neighborhood of *suc A* , was sequenced. The protein has 268 amino acid residues and shares significant homology with FAPY DNA-glycosylases (FPG) from *E. coli, Lactococcus lactis, Streptococcus mutans* and *Bacillus firmis*. 23-27% identity and 35-50% similarity of the N-terminal 76 amino acid sequence and 26-28% identity and 43-46% similarity of C-terminal 112 amino acid sequence are seen between endo VIII and FPG. Like FPG, endo VIII also contains a zinc finger motif at its C-terminus which is the putative DNA binding domain.

Endo VIII cleaves DNA containing pyrimidine oxidation products such as thymine glycol, urea, dihydrothymine, 5-hydroxycytosine, 5-hydroxyuracil and abasic sites, releasing the lesion as a free base. Like FPG but in contrast to endo III, endo VIII cleaves the DNA backbone by βδ-elimination. The k_{cat} for cleavage by endo VIII of the thymine glygol-containing oligonucleotide, considered the preferred substrate for endo VIII and endo III, is about 300 min^{-1} and the Km is about 8 nM whereas the k_{cat} for endo III is 1 min^{-1} and the Km is about 5nM.

In view of the much higher k_{cat} for endo VIII than that for endo III, endo VIII may play a main role in removing pyrimidine oxidation products in vivo explaining the lack of sensitivity of *nth* null mutants to ionizing radiation and oxidant treatments which produces damages that block DNA replication and are substrates for endo III. *nei* null mutants and/or *nth nei* double mutants are being constructed to address the cellular function of endo VIII and its relationship to endo III.

C5-104 TRANSCRIPTIONAL REGULATION BY ADA PROTEIN OF THE E. COLI ada AND aidB GENES,

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The E. coli aidB gene is part of the adaptive response to alkylating agents. Genes belonging to this DNA repair pathway are positively regulated by the Ada protein, in its methylated form (^{me}Ada). Methylated Ada protein is able to bind to a sequence in a region of the aidB gene between 40 and 60 base pairs upstream of the transcriptional startpoint. This region shows only poor homology with the proposed consensus sequence for the Ada binding site (AAANNAAAGCGCA). Binding to the aidB gene requires a higher concentration of ^{me}Ada than for the ada promoter, suggesting lower affinity of the protein for the aidB Ada-binding site. Common features in the Ada-binding regions of ada and aidB are high A/T content and the presence of an inverted repeat sequence. Through DNAaseI protection experiments we also show that RNA polymerase can bind to both the ada and aidB promoter regions in an area partially overlapping the Ada-binding site; the binding appears to be specific, and can occur in the absence of methylated Ada. Based on these findings, we suggest that RNA polymerase is able to bind to the A/T rich sequences upstream of the ada and aidB promoters; in the presence of ^{me}Ada the nature of the RNA polymerase:promoter interaction is modified, possibly through a conformational change in RNA polymerase, resulting in transcriptional activation.

C5-105 A UNIFIED CATALYTIC MECHANISM FOR DNA GLYCOSYLASE-AP LYASES,

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Some DNA repair glycosylases have an associated abasic site lyase activity whose intrinsic catalytic efficiency is approximately equal to that of the glycosylase activity. A molecular explanation of the distinction between these glycosylases and those which do not have the lyase activity has not previously been available. Our studies on the catalytic mechanism of T4 endonuclease V have been central to the development of a chemically-based unifying hypothesis which explains this distinction.

Previous studies have revealed that an imino intermediate is formed between the N-terminal α-NH_2 group of T4 endonuclease V and Cl' of the 5'-deoxyribose in its pyrimidine dimer substrate. Thus, the hypothesis was formulated that glycosylases with no associated AP lyase activity, such as adenine DNA glycosylase, uracil DNA glycosylase, and N-methylpurine DNA glycosylase, catalyze the removal of altered bases via a nucleophilic attack that does not involve an imino intermediate. In contrast, all glycosylases with an associated AP lyase activity, such as endonuclease III, Fpg and *M. luteus* UV endonuclease would catalyze base removal by nucleophilic attack that proceeds via an imino intermediate. Experiments to test for the existence of imino intermediates have been performed on these enzymes, with the overall conclusions confirming this hypothesis.

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C5-106 REPAIR OF UV-INDUCED DNA DAMAGE BY THE SPORE PHOTOPRODUCT LYASE (*spl*) SYSTEM DURING GERMINATION OF *Bacillus subtilis* SPORES,

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Bacterial endospores are 1-2 orders of magnitude more resistant to UV than their exponentially-growing counterparts, due to their novel DNA photochemistry and DNA repair mechanisms. Upon UV irradiation, *B. subtilis* spore DNA accumulates as its major photoproduct the thymine dimer 5-thyminyl-5,6-dihydrothymine ("spore photoproduct"; SP), and not cyclobutyl pyrimidine dimers. The major route of SP repair during spore germination utilizes an SP-specific enzyme called SP lyase, encoded by the *spl* gene. SP lyase in *B. subtilis* is encoded by the second cistron of a bicistronic operon. We cloned and sequenced the *B. subtilis spl* operon and demonstrated that the *spl* cistron encodes a putative 40 kDal protein which exhibits regional amino acid sequence homology to DNA photolyases (Fajardo-Cavazos et al., J. Bact. 175: 1735. 1993). We determined that expression of the *spl* operon is apparently not DNA damage-inducible, but is transcriptionally activated during sporulation in the developing forespore compartment, resulting in packaging of the enzyme within the core of the dormant spore (Pedraza-Reyes et al., J. Bact. 176: 3983. 1994). The first cistron of the operon consists of an open reading frame (*orf*) which encodes a putative 9 kDal protein of unknown function. The *orf* product may participate in SP repair, however, as the wild-type *spl* cistron alone supplied in *trans* can complement point mutations within the *spl* cistron but not an *in vitro*-generated deletion of the entire operon. Experiments are currently underway to overexpress and purify the proteins encoded by both cistrons and to reconstruct the SP repair reaction *in vitro*.

C5-107 URACIL DNA N-GLYCOSYLASE AND ENDONUCLEASE IV FROM *ESCHERICHIA COLI* BOTH INTERACT DISTRIBUTIVELY WITH DNA.

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In general, a site-specific DNA recognizing enzyme may locate its target sites in the large background of nonspecific DNA by 1) random intermolecular collisions followed by complete dissociation from DNA after each catalytic act, and random search for the next recognition site (the distributive mode); 2) initial nonspecific binding to the DNA and subsequent translocation along the DNA molecule from one specific site to the next without apparent dissociation (the processive mode) or 3) nonspecific binding to DNA, translocation to the first specific site and dissociation after the catalytic act (the intervening mode). Using double stranded concatemeric polynucleotides of defined length, with deoxyuridine or a tetrahydrofuran residue at every twentieth position as a substrate, and a computer model utilizing a distributive or a processive search mode, it was shown that Uracil DNA N-glycosylase (UDG) and Endo IV both translocate from one specific site to another via complete dissociation from DNA after each catalytic act. The time course for uracil removal by UDG or abasic (AP) site cleavage by Endo IV measured for the DNA fragments of different lengths (23, 223 and 1047 bp) containing only one deoxyuridine (or AP site) showed a decrease in the rate of both enzymatic reactions with an increasing amount of nonspecific DNA. The results of two experimental approaches are consistent with a distributive mechanism of target location by UDG and Endo IV.

C5-108 MUTANT FPG PROTEIN FROM *E. COLI* WITH A SMALL N-TERMINAL DELETION EXHIBITS DIMINISHED N-GLYCOSYLASE AND AP-LYASE ACTIVITIES,
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Formamidopyrimidine DNA N-glycosylase (fpg protein) is a base excision repair enzyme with associated abasic (AP) activity. It recognizes a variety of oxidation and ring fragmentation products derived from adenine and guanine. Excision of 7-hydro 8-oxoguanine (8-oxoG) has been postulated to be physiologically relevant based on the relatively high 8-oxoG levels in cellular DNA, the known capacity of 8-oxoG to base-pair with adenine, and the observed increase in G to T transversions in *E. coli* cells lacking mutM, the gene encoding fpg protein. We have cloned the fpg gene into the pET22b vector downstream of the T7 promoter to establish IPTG-inducible expression via activation of the T7 RNA polymerase gene engineered into the genome of BL21(DE3) cells. Furthermore, the construct codes for a soluble fpg protein modified by the addition of 6 C-terminal histidine residues (w.t. C-$(His)_6$) to permit purification of the enzyme to near homogeneity on a Ni^{2+} affinity column. Importantly, this procedure separates the activity of the plasmid-derived fpg protein from chromosomally encoded fpg protein, a feature that is essential for unambiguous analysis of low-activity mutant enzymes. Like the native enzyme, the w.t. C-$(His)_6$ enzyme purified in this fashion displays a specific activity of 54 nmol/min-mg protein (turnover number of 1.7 min^{-1}) using supercoiled double-stranded SK-containing 1-2 methyl formamidopyrimidine sites per plasmid and catalytically removes a unique 8-oxoG or AP residue from oligonucleotides, generating the expected 3' phosphorylated fragment. Cloning and overexpression of a modified fpg gene directs formation of a 29.5 kDa protein with a small N-terminal deletion and the C-$(His)_6$ addition (designated Δ2-15) that is clearly smaller on SDS polyacrylamide gels than the w.t. C-$(His)_6$ enzyme (31 kDa). Interestingly, induction of the mutant Δ2-15 protein slows the growth of BL21(DE3) cells so that after a 2 hour induction initiated in late log phase, the resulting cell density is 65% of that for the strain lacking plasmid or harboring the w.t. C-$(His)_6$ construct. Preliminary analysis of the Δ2-15 fpg protein indicates that the mutant possesses less than 1% of the wild type activity with oligonucleotides containing a single 8-oxoG or AP site, suggesting that this evolutionarily conserved amino acid sequence is required for efficient functioning of fpg protein.

C5-109 SINGLE AMINO-ACID CHANGES ALTER THE REPAIR SPECIFICITY OF *DROSOPHILA* Rrp1: ISOLATION OF MUTANTS DEFICIENT IN REPAIR OF OXIDATIVE DAMAGE
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Drosophila Rrp1 has several tightly associated enzymatic activities, including 3'-exonuclease, apurinic/apyrimidinic (AP) endonuclease, 3'-phosphatase and 3'-phosphodiesterase. The carboxy-terminal third of Rrp1 is homologous to *Escherichia coli* exonuclease III and several eukaryotic AP endonucleases. We previously reported that this carboxyl-terminal region of Rrp1 is sufficient to repair lesions caused by DNA damaging agents *in vivo* in *E. coli.* We mutagenized this region of the Rrp1 gene by PCR mutagenesis and isolated three mutants with altered DNA repair capacity. These mutants complement the hypersensitivity of an *xth nfo* mutant of *E.coli* to MMS, but not to $tBuO_2H$, while wild-type Rrp1 confers resistance to both reagents. A single amino acid change was identified for each mutant. T462 (mutated to A) and K463 (mutated to Q) are highly conserved residues found in a cluster of 5 conserved amino acids. The third mutation identified in the screen, L484P, identifies a poorly conserved leucine residue.
L484, T462, K463 and the adjacent conserved residues Q460 and E461 were altered by site-directed mutagenesis using a plasmid construct including the entire Rrp1 gene. The complementation phenotypes and enzymatic properties of these mutants were then studied. The AP endonuclease activity of E461 mutants is reduced 30-fold and this mutant is MMS-sensitive, while all other mutants have normal levels of AP endonuclease and are MMS resistant. A 10-200-fold reduction of the phosphodiesterase activity was found for the mutants sensitive to $tBuO_2H$ (E461, T462 and K463).
The data suggests that E461 is essential for Rrp1 nuclease function, while T462 and K463 seem to modulate its substrate specificity. In addition, strong suggestions are made concerning the lesions responsible for MMS, $tBuO_2H$ and H_2O_2-induced lethality.

C5-110 CLONING ENTEROBACTERIAL HOMOLOGS OF ENDONUCLEASE III USING PCR,
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Base excision repair is an important pathway for the removal of numerous pyrimidine and purine damages in DNA. *Escherichia coli* endonuclease III (endo III) is a well-characterized base excision repair enzyme which is responsible for the repair of many pyrimidine base lesions caused by ionizing radiation or oxidative damage. Endo III, encoded by the *nth* gene, has a DNA glycosylase/AP lyase activity and the gene and protein structure have been previously determined. Redoxyendonuclease is a functionally similar eukaryotic analog to endo III found in *Saccharomyces cerevisiae*. The purification of this protein has proven difficult, therefore, cloning the gene encoding redoxyendonuclease would be a great asset in the eventual expression, purification and characterization of this enzyme. Degenerate oligonucleotide primers to regions of the *nth* gene were employed to amplify and clone *nth* homologs in several enteric bacterial species using PCR methodologies. The results of these studies will be discussed with regard to the identification and characterization of these related genes. This work was supported by NIH grant CA42607.

C5-111 FUNCTIONAL COOPERATION OF *mutT*, *mutM* AND *mutY* PROTEINS IN PREVENTING MUTATIONS CAUSED BY SPONTANEOUS OXIDATION OF GUANINE NUCLEOTIDE IN *ESCHERICHIA COLI*,
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8-Oxo-dGTP (8-oxo-7,8-dihydrodeoxyguanosine triphosphate) is a potent mutagenic substrate for DNA synthesis. The accumulation of 8-oxo-dGTP in the nucleotide pool induces G:C to T:A transversion as well as A:T to C:G transversion, and *Escherichia coli* cells possess mechanisms for preventing such mutations. The *mutT* gene product specifically hydrolyzes 8-oxo-dGTP to the the monophosphate form while the *mutM* and the *mutY* gene products function to correct mispairs caused by incorporation of 8-oxoguanine into DNA. We constructed a series of strains carrying combinations of *mutT*, *mutM*, and *mutY* mutations and investigated mutator phenotypes and 8-oxoG contents in DNA. Based on analyses of forward mutations induced in the mutator strains, the unidirectional nature of *mutT* mutator effect was attributed to the cooperative functions of MutY and MutM proteins, one suppressing the G:C to T:A transversion through correction of 8-oxoG:C mispair and the other promoting the A:T to C:G transversion through fixation of 8-oxoG:A mispair. In mutator strains lacking MutT and/or MutM proteins, 8-oxoguanine of DNA increased to a concentration expected from the increased frequency of mutation. Evidence was presented that the MutT and MutM proteins cooperate to prevent accumulation in the DNA of oxidized guanine residues.

C5-112 REPAIR OF DNA METHYLATION DAMAGE IN *SACCHAROMYCES CEREVISIAE*. Wei Xiao, Lane Rathgeber, Barbara Chow and Treena Fontanie, Department of Microbiology, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0

Treatment of cells with S_N2-type DNA methylating agents such as MMS predominantly produces 7MeG and a lethal lesion 3MeA in DNA. All the organisms examined so far contain an alkylation-specific base excision repair pathway via a 3MeA DNA glycosylase that removes the damaged base, followed by an AP endonuclease that cleaves the DNA strand at the abasic site, for the subsequent repair. Furthermore, a number of DNA radiation repair mutants are sensitive to MMS, suggesting that these radiation repair genes are also involved in the repair of DNA methylation damage. To understand how these genes are involved in DNA methylation repair, we performed epistatic analysis by combining yeast *mag1* and *apn1* mutations with various mutations involved in *RAD6* and *RAD52* groups. The *rad18* mutant had a slightly increased sensitivity to MMS than wild type cells. However, *rad18 mag1* and *rad18 apn1* double mutants were much more sensitive than either of the corresponding single mutants. Combinations of *mag1* and *apn1* with *rev3* mutation had similar results as with *rad18*. This additive effect indicates that MAG1 glycosylase is a major pathway for the removal of 3MeA and that if the base excision repair pathway is defective, the RAD6 pathway may play some important role in cell survival, possibly by replicative bypass of the damaged templates. MMS is believed to produce single-strand breaks and considered an x-ray mimetic agent. However, whether MMS induces a single-strand break directly or via base-excision pathway is not clear. We found that the *rad50* and *rad52* single mutants are even more sensitive to MMS than the *mag1* mutant; nevertheless the double mutants still showed an additive effect, indicating that the base excision repair is not the only pathway by which MMS treatment produces DNA strand breaks. Thus recombination repair must be involved in a separate pathway instead of acting downstream of the base excision repair.

C5-113 A YEAST GENE REQUIRED FOR REPAIR OF BLEOMYCIN DNA LESIONS, Dindial Ramotar and Jean-Yves Masson, CHUL, Health and Environment, 2705 Boul. Laurier, Ste-Foy, Quebec, Canada, G1V 4G2.

Bleomycin is an antitumour drug commonly used for the treatment of several tumours including lung, neck, and head. It acts via a free radical mechanism to create a narrow set of toxic lesions in the cellular DNA. These lesions produce mutations if they are not corrected. Thus, the normal cells of cancer patients treated with bleomycin must be able to efficiently repair bleomycin-induced DNA lesions in their chromosomal DNA. Cells that cannot repair these lesions are at a severe disadvantage and perhaps accumulate mutations at a high rate when cancer patients are given bleomycin treatment. The enzyme that repairs bleomycin-induced DNA lesions in eukaryotic cells has never been identified. It is an important endeavor to search and isolate the enzyme that repair bleomycin-induced DNA lesions for two reasons (i) the enzyme may be present at a high level in tumours that are resistant to bleomycin treatment, and (ii) it may play a central role in repairing lesions produced by other oxidants such as ionizing radiation, hydrogen peroxide, normal aerobic metabolism, and other antitumour agents such as neocarzinostatin.. To understand how eukaryotic cells repair bleomycin DNA lesions, we have used *Saccharomyces cerevisiae* as a model system to isolate a mutant that is hypersensitive to bleomycin. The sensitivity to bleomycin was due to the mutant inability to repair bleomycin-DNA lesions. We have subsequently cloned the gene by complementation of the mutant and the biological role of the encoded protein will be presented.

C5-114 **STRUCTURE OF THE GENE FOR HUMAN URACIL-DNA GLYCOSYLASE AND ANALYSIS OF THE PROMOTER FUNCTION**, Hans E. Krokan, Terje Haug, Frank Skorpen and Henning Lund, UNIGEN Center for Molecular Biology, University of Trondheim, N-7005 Trondheim, Norway

The gene for human uracil-DNA glycosylase (UNG) contains 4 exons and has an approximate size of 13 kb as determined by extensive restriction mapping. The promoter is very GC rich and lacks a TATA box. Furthermore, the upstream sequence is part of a CpG island, and an additional CpG island was identified in the first exon. These findings are characteristics of a housekeeping gene. The promoter contains the following putative transcription factor binding elements (from the transcription start region and upstream): SP1, SP1, c-MYC, E2F, SP1, CCAAT, CCAAT, CCAAT, AP2, AP1 and PEA3. The promoter sequence (approximately 400 bp) was linked to luciferase as a reporter gene and this construct was used to analyse the function of the various promoter elements after transient transfection of HeLa cells. The two SP1 elements proximal to the transcription initiation region were sufficient to support some 27% of the promoter activity as compared to the full-length construct. A clone that in addition contained the elements c-MYC/E2F/SP1/CCAAT increased expression to almost 90% of the full-length construct, whereas a region upstream of these elements appears to exert a negative control function. In addition, the more distant AP1 element, or unknown elements close to it, stimulated transcription 2-fold. The E2F element is probably involved in the previously demonstrated cell cycle regulation of human uracil-DNA glycosylase.

C5-115 RELATION BETWEEN SPECIFIC PROCESSING OF DNA DAMAGE AND THE INDUCTION OF THE *RNR2* GENE IN *SACCHAROMYCES CEREVISIAE*, Dietrich Averbeck and Simone Averbeck, Institut Curie-Section de Biologie, URA 1292 CNRS, 26, Rue d'Ulm, F-75231 Paris Cedex 05, France

Using an *RNR2-lacZ* fusion strain of *Saccharomyces cerevisiae* the induction of the *RNR2* gene (Elledge, S.J. and R.W. Davis, Mol. Cell. Biol. 7, 2783-2793 (1987) was studied in relation to the repair of various types of DNA damage. Following treatments with mono-and bifunctional furocoumarins (inducing specifically monoadducts or monoadducts and interstrand cross-links), benzo(a)pyrene and 1,6-dioxapyrene plus UVA (inducing oxidative damage), 254 nm UV radiation and gamma-radiation cell growth, survival responses and *RNR2* gene induction were determined (Averbeck, D. and S. Averbeck, Mutat. Res. 315, 123-138 (1994). The time course of *RNR2* induction as revealed by measures of beta-galactosidase activity indicated that gene induction took place during the repair period before the resumption of cell growth. The amplitude of induction increased with increasing equitoxic dose levels. As shown by pulsed field gel electrophoresis analysis, treatments leading to the formation of double-strand breaks as repair intermediates were most effective for gene induction, apparently as a consequence of the induction of DNA lesions such as interstrand cross-links or closely located bulky lesions such as monoadducts and pyrimidine dimers. Oxidative damage was surprizingly ineffective. On the basis of studies on the mutagenicity of the different treatments used it appears that the specific processing of DNA lesions is not only related to the induction of mutations but also to gene induction.

C5-116 NUCLEASE SENSITIVE GENOMIC REGIONS ARE REPAIRED WELL IN COCKAYNE SYNDROME AND NORMAL HUMAN FIBROBLASTS, Suzanne A. Bastin and G. J. Kantor, Department of Biological Sciences, Wright State University, Dayton, OH 45435
We have examined the repair of three genomic areas, identified by the ß-actin gene, the insulin gene and the 754 locus, representing transcriptionally active and inactive regions respectively in human fibroblasts. The active ß-actin region and the inactive insulin region are repaired at the same rate as the genome overall in Cockayne syndrome (CS) cells. The ß-actin region is a preferentially repaired region in normal cells while the insulin region is repaired at the genome overall rate. The inactive 754 locus is repaired at an equally slow rate in both cell strains. These results clearly define the hierarchy for repair of different genomic regions. We propose that the differences in repair rates between genomic areas are due to, in addition to a transcription-repair coupling factor, varying degrees of chromatin compaction. We have attempted to test this by examining the general sensitivity of these genetic areas to DNaseI digestion. Preliminary results show that the well repaired ß-actin and insulin regions are more sensitive to digestion with DNaseI than the poorly repaired 754 region. This correlation between repair rates and nuclease sensitivity suggests that chromatin accessibility is an important factor in controlling DNA excision repair in human cells. Since the repair defect in CS cells is limited to the preferential repair of active genes, the differential repair observed for the two inactive regions in both CS and normal cells must be due to another factor, possibly chromatin accessibility.

C5-117 ACTIVITY BLOTTING METHOD FOR DETECTING DNA-MODIFYING (REPAIR) ENZYMES, Kosuke Akiyama*, Shuji Seki*, Sekiko Watanabe*, Ken Tsutsui*, Yasuhiro Mitsui**, Keiko Takatori** and Shinichiro Yamamoto**, *Department of Molecular Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700, and **Department of Medicine, Kawasaki Medical School, Kurashiki 701-01, Japan
The activity blotting method in which DNA-modifying (repair) enzymes electrophoresed on a gel are blotted and detected on a damaged or native DNA-fixed nylon membrane was studied in comparison with the conventional activity gel method in which the substrate DNA is immobilized in the electrophoresis gel. In the former method, enzymes, either homogeneous or crude, having priming activity for DNA polymerase on DNA were electrophoresed on a gel, renatured, and blotted on a damaged or native DNA-fixed membrane. The sites primed by the activity blotting were demonstrated by DNA synthesis in the presence of either radioactively or non-radioactively labeled substrate. Activities of 5'AP endonuclease, DNA 3' repair diesterase and DNase I in either crude extract or purified preparations were effectively detected by the activity blotting method, although the former two enzyme activities were difficult to demonstrate by the activity gel method. The activity blotting method is expected to have wide application in DNA modifying enzyme detection by selecting or contriving the damaged DNA-fixed membrane and detection procedure. It is especially applicable to the detection of various DNA repair enzymes. The blotted membranes can be handled easily (washing, incubation, activity detection and so on), and non-radioactive detection is possible.

C5-118 CONTRIBUTION OF SPECIFIC DNA REPAIR PATHWAYS TO THE CHEMORESISTANCE OFHUMAN LYMPHOCYTES TO ALKYLATING DRUGS, C. Buschfort, C. Lensing, F. Seiler, M.R. Müller, J. Thomale, S. Seeber, and M.F. Rajewsky, Institute of Cell Biology and Department of Internal Medicine, West German Cancer Center, University of Essen Medical School, 45122 Essen, Germany.

Primary and acquired resistance of cancer cells to the cytotoxic effect of chemotherapeutic drugs represent a major obstacle in clinical oncology. To elucidate whether efficient repair of drug-induced cytotoxic lesions in genomic DNA may contribute to a drug-resistant phenotype it is necessary to measure DNA repair processes in small cell samples of human bioptic material. Two sensitive analytical methods to visualize and quantify specific DNA lesions at the level of single cells were applied to measure the DNA repair in normal and leukemic lymphocytes and in blast cells. After *in vitro* pulse exposure to N-ethyl-N-nitrosourea we have measured the elimination of O^6-Ethylguanine (O^6-EtGua) by the Immuno Cytological Assay (ICA) using specific anti-(O^6-EtGua) monoclonal antibodies (Seiler *et al.*, Carcinogenesis (1993), 9, 1907-1913). In parallel secondary DNA lesions were monitored in single cells by alkaline unwinding and *in situ* electrophoresis (Single Cell Gel Electrophoresis; "Comet assay"). The occurrence and disappearance of abasic sites and single strand breaks in nuclear DNA, predominantly induced by DNA repair processes, were measured in individual cell specimen.
The results of DNA repair analysis are correlated to other factors involved in cellular drug resistance, to *in vitro* cytotoxicity profiles and to clinical response.
Ref.: Thomale *et. al.* (1994), Br. J. Cancer, 69: 698-705.
Müller *et. al.* (1994), Cancer Res., 54: 4524-4531.

C5-119 REPAIR OF ALKYLATION DAMAGE BY DIFFERENTIATING MOUSE TERATOCARCINOMA CELLS
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Alkylating agents are toxic, mutagenic, carcinogenic in mammalian cells and tissues. The consequences of alkylation are cell- and tissue specific and depend on the different repair capabilities in the targets. The mouse teratocarcinoma cell lines allow the analysis of very early commitment and differentiation events, that are likely to be similar to those operating in the early mouse embryo. We have previously characterised the excision repair capabilities of these cells after ultraviolet light irradiation and found that differentiation is accompanied by reduction of excision repair. In the present study we examine the O^6-alkylguanine DNA alkyltransferase (AT-ase) activity in two mouse teratocarcinoma cell lines, namely PCC7, P19 and in transfected P19 cells with constructs harbouring a human AT-ase cDNA driven by a housekeeping promoter.
The two teratocarcinoma lines express different AT-ase specific activities, PCC7 cells exhibit higher, while P19 cells considerable lower, in the transfectants the basic activity is 1.5-3 fold higher than in the nontransfected parental cells. In the case of both cell lines and the transfectants also the AT-ase activity is reduced during differentiation. The transfectants were used to examine in detail the possible reasons for the reduction of AT-ase activity by comparing the undifferentiated and differentiated state at the mRNA level in Northern blott analysis and at the protein level in Western blotting.
In spite of the fact that in P19 transfectants the AT-ase activity decreases significantly during differentiation, differences were not found by mRNA and protein quantitation.

C5-120 ANALYSIS OF DNA ADDUCTS IN N-OH-AAF-TREATED REPAIR-PROFICIENT MICE BY IMMUNOFLUORESCENCE MICROSCOPY AND ^{32}P-POSTLABELING. Anita F.W. Frijhoff, Len Roza and Robert A. Baan, Department of Genetic Toxicology, TNO Nutrition and Food Research Institute, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands

As part of a collaborative study in which DNA-adduct formation and removal will be investigated in repair-deficient (transgenic) mice which are currently being generated, repair-proficient Ola129 mice were treated with N-hydroxy-acetylaminofluorene, N-OH-AAF, a metabolite of the model carcinogen 2-AAF. N-OH-AAF induces different proportions of deacetylated (AF-C8-dG) and acetylated (AAF-C8-dG, AAF-N^2-dG) adducts in DNA in mammalian cells. These adducts are removed by the nucleotide-excision repair pathway. Induction and removal of DNA damage in N-OH-AAF-treated mice were studied at the cellular level by quantitative immunofluorescence microscopy, with the use of adduct-specific (AF-C8-dG, AAF-C8-dG) monoclonal antibodies and FITC-conjugated second antibodies. To study DNA-damage removal in white blood cells (WBC) and liver, the ^{32}P-postlabeling assay was used as well.

Ola129 mice were injected intraperitoneally with N-OH-AAF (300 mg/kg b.w.). At days 1, 3 and 7 after treatment blood, livers and brains were collected. Fixed WBC and cryostat sections of liver and brain were subjected to immunocytofluorometry with AAF-dG-specific and AF-dG-specific antibodies, 6b and 9f1, respectively. In WBC, DNA damage could be detected only with 9f1; a distinct difference in damage-associated fluorescence between control and treated mice was observed at day 1. DNA damage was removed gradually to nearly background levels at day 7. In liver cryostat sections AF-dG adducts could be detected as well, however, no significant decrease in damage-associated fluorescence was observed. In brain cryostat sections AF-dG adducts were observed at day 1. Removal of DNA damage was slower in brain compared to WBC: at day 7 some DNA damage was still present.

DNA isolated from WBC and liver was tested in the ^{32}P-postlabeling assay. The results showed that the acetylated adduct (AAF-dG) is formed as well, the level in liver DNA being markedly higher compared to that in DNA from WBC. This may be due to a lower deacetylase activity in liver compared to WBC, which influences the extent of induction of AAF-dG and AF-dG adducts.

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C5-121 AP ENDONUCLEASE: A POSSIBLE TARGET FOR A NOVEL TUMORICIDAL COMPOUND, Robert H. Grafström, Tong Sun, and Debra Doleniak. The Dupont Merck Pharmaceutical Company, Glenolden PA 19036.
DMP840 {(R,R)-2,2'-[1,2-ethanediylbis[imino-(1-methyl-2,1-ethanediyl)]]-bis[5-nitro-1H-benz[de]isoquinilone-1,3-(2H)-dione]dimethylsulfonate)} is a novel, symmetrical, bis-naphthalimide compound currently in Phase II anticancer clinical trials. DMP840 cleaves abasic sites via a ß-elimination mechanism. Abasic site cleavage is dependent on both the length of the linker and also the number and type of linker amino group. DMP840 also inhibits the cleavage of abasic sites by the Human AP endonuclease I *in vitro*. Two bacterial AP endonucleases (ExoIII and EndoIII) and the mouse liver AP endonuclease are also inhibited by DMP840. This inhibition probably occurs by binding to the abasic site in DNA and preventing access to the site by AP endonuclease. Data comparing numerous analogs of DMP840 suggest that the best tumoricidal compounds have weak abasic site cleavage activity (< 20%) but are good inhibitors (>80%) of the Human AP endonuclease. Together these data suggest that the *in vivo* mechanism of action of DMP840 is linked to the repair of abasic sites.

C5-122 PURIFICATION AND DEVELOPMENT OF A SPECIFIC ACTIVITY ASSAY FOR YEAST REDOXYENDONUCLEASE; Laura Augeri and Paul Doetsch, Departments of Biochemistry and Radiation Oncology, Emory University School of Medicine, Atlanta, GA 30322
Yeast redoxyendonuclease (YRE) is a base excision repair enzyme that is functionally analogous to *E. coli* endonuclease III, the product of the *nth* gene. YRE functions as an N-glycosylase, recognizing a wide variety of oxidative DNA base damages. Removal of the damaged base results in an apurinic/apyrimidinic (AP) site. YRE processes the damage site further via an AP lyase activity cleaving the phosphodiester backbone at the AP site via beta-elimination. We have developed a highly specific activity assay for the detection of YRE utilizing synthetic oligonucleotides containing a single dihydrouracil. This assay is currently being used in a large-scale purification from a mutant strain of yeast deficient in APN1, the yeast major AP endonuclease. YRE has been extensively purified and enzyme activity correlates with a protein of 40kDa as detected by SDS-PAGE. This study is supported by NIH grant CA42607.

C5-123 GENE TARGETING OF THE AP ENDONUCLEASE(APE) GENE IN A HUMAN LYMPHOBLASTOID CELL LINE.
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APE, the apurinic/apyrimidnic (AP) endonuclease, plays an important role in cellular protection against genotoxic agents, that induce AP sites and strand breaks in DNA, such as, alkylating compounds, ionizing radiation, and oxidative stress. Moreover, the mammalian APE (also called Ref-1) is involved in reductive activation of the transcription factor AP-1.

In order to assess various biological roles of the APE (Ref-1) as well as of the unrepaired AP sites, it is necessary to isolate mutant mammalian cell lines with little or no APE protein. Our objective is to isolate a human cell line with null mutation in the APE gene. We have chosen TK6, a human lymphoblastoid line, as the parent cell, for several reasons. First, this line is being used of extensively for mutagenesis studies with various genotoxic agents. Second, this is one of the few established human lines with stable, near-diploid karyotype. Third, this line has very good growth characteristics which will be important for enzymological experiments. Construction of the gene targeting vectors and other preliminary experimental data for knocking out the APE gene in TK6 will be discussed.

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C5-124 QUANTITATION OF ABASIC SITES IN MAMMALIAN CELLS BY THE ALDEHYDE REACTIVE PROBE,

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The abasic site (apurinic/apyrimidinic site) is the most common lesion in DNA and is suggested to be an important intermediate in mutagenesis and carcinogenesis. We have recently reported a *novel* assay for the detection and quantitation of abasic sites in DNA. In this assay, the aldehyde group in an abasic site is first modified by a probe bearing a biotin residue, called the Aldehyde Reacive Prove (ARP) and then the tagged biotin is quantified by an ELISA-like assay. To elucidate the specificity of ARP to DNA damages, ARP was incubated with a variety of bases or nucleosides and the reaction mixtures were analyzed by HPLC. Of the 14 compounds tested, 2-deoxyribose and 5-formyluracil reacted with ARP. The results indicate that ARP is specific to damages having an alkyl or allyl aldehyde group. The repair of abasic sites in MMS-treated HeLa (RC-355) cells were quantitated after repair incubation (0-24h). The number of abasic sites in the DNA extracted from the cells about 10 % of that of total mathylated bases. The abasic sites in the DNA were eliminated slowly and a half life of the damage was about 24h. Adenine has been known as a potent inhibitor of mammalian AP endonuclease. When the MMS-treated cells were incubated in the presence of this compound (5 mM), the repair of abasic sites was inhibited almost completely, but no accumulation of the damages was observed. Though adenine itself did not inhibited the activity of partially purified huma MPG, it is possible the derivatives of adenine affect the enzyme activity.

C5-125 TOXICITY AND REPAIR OF PSORALEN ADDUCTS IN BACTERIOPHAGE T7,

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Psoralens, photoreactive molecules which interact primarily with thymine residues in DNA, form interstrand crosslinks as well as two types of monoadducts, the furan-side adduct and the pyrone-side adduct. To investigate the relative roles of these adducts in cellular toxicity, we have studied the interaction of 4,5',8-trimethylpsoralen (TMP) and 8-methoxypsoralen (8-MOP) with bacteriophage T7. These two derivatives differ in the fraction of pyrone-side monoadducts formed, with TMP producing very small amounts of this type of adduct. Purified phage was reacted with TMP or 8-MOP and irradiated for various times with long-wave UV light. DNA crosslinks produced in the intact phage were measured by analytical ultracentrifugation. In some experiments with 8-MOP, samples were re-irradiated after removal of the unreacted psoralen derivative to transform monoadducts into crosslinks. Biological activity was assessed by measurement of phage survival on repair-proficient (uvr^+) and repair-deficient (uvr^-) hosts. Our results can be summarized as follows: 1. At equivalent numbers of crosslinks/DNA molecule, phage survival was the same for the two psoralen derivatives. 2. The survival fraction of treated phage was significantly lower than the fraction of DNA molecules with no crosslinks; at 37% phage survival, over 60% of the phage population was free of crosslinks. 3. Phage survival decreased after the secondary irradiation. 4. The absence of excision repair in host bacteria led to decreased survival after both the primary and secondary irradiations. 5. High multiplicity of infection did not increase survival. We conclude that, although crosslinks can kill phage, as evidenced by secondary irradiation results, they are not sufficient in number to explain psoralen toxicity after primary irradiation. Therefore monoadducts, both of the furan-side and the pyrone-side types, must be in large part responsible for phage inactivation.

C5-126 INTERACTION OF T4 ENDONUCLEASE V WITH DNA CONTAINING A PYRIMIDINE DIMER OR TETRAHYDROFURAN RESIDUE,

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T4 endonuclease V is a 16 kDa DNA *N*-glycosylase/ abasic (AP) site lyase that is specific for ultraviolet light induced pyrimidine dimers. Mechanistic studies have demonstrated that the N-terminal (Thr-2) αNH_2 group of the enzyme initiates glycosidic bond scission by forming an imino intermediate with the C-1' of the 5' sugar within the pyrimidine dimer, and that Glu-23 is important for both the glycosylase and AP lyase activities. $NaBH_4$ has been shown to reduce the imino intermediate to a covalent dead-end DNA-enzyme product, which is useful for studying endonuclease V - DNA interactions.

Wild-type endonuclease V was found by electrophoretic mobility shift assay to bind only as a monomer to oligonucleotides containing either a site specific thymine dimer or tetrahydrofuran residue. In contrast, one and two molecules of an E23Q mutant were found to bind to these same substrates. DNase I and 1,10-phenanthroline-copper were used as footprinting reagents to map the DNA contacts made by these enzymes around a thymine dimer and tetrahydrofuran residue. $NaBH_4$ was used to reduce the imino intermediate formed between wild-type enzyme and thymine dimer containing DNA. Results from footprinting the wild-type enzyme on dimer containing DNA demonstrated that the enzyme protected ~9 bases of DNA on the strand opposite a dimer, but only 1-2 bases of DNA on the dimer-containing strand. The protection pattern was asymmetrically arranged to the 3' side of the dimer. These results, in combination with results from methylation protection experiments, indicate that the wild-type enzyme primarily contacts DNA on the strand opposite a pyrimidine dimer via the minor groove. Supported by T32-ES-07254 and ES04091.

C5-127 THE HMG-DOMAIN PROTEIN IXR1 BLOCKS REPAIR OF CISPLATIN-DNA ADDUCTS BY YEAST EXCISION REPAIR PROTEINS RAD2, RAD4, AND RAD14, BUT NOT RAD1 OR RAD10,

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Cisplatin is a potent antitumor drug which forms adducts on DNA. Intrastrand cisplatin-DNA adducts are specifically recognized by the yeast protein Ixr1 and other proteins with HMG domains. When the gene for Ixr1 is disrupted, the *ixr1* cells are more resistant to cisplatin treatment. One hypothesis to account for the difference in cisplatin cytotoxicity between *IXR1* and *ixr1* cells is that Ixr1 may prevent excision repair proteins from correcting the cisplatin damage. Various excision repair genes were disrupted in both *IXR1* and *ixr1* backgrounds. The difference between the *IXR1* and *ixr1* strains was greatly diminished in the *rad2*, *rad4*, and *rad14* strains. Since this difference is missing in the *rad2*, *rad4*, and *rad14* strains, it must depend on these proteins. These three proteins are all involved in excision repair, so Ixr1 probably blocks the repair of cisplatin-DNA adducts. In the *rad1* and *rad10* strains, however, the difference was still present, indicating that the five Rad proteins perform different functions. This conclusion is supported by in vitro studies done in other laboratories. Disruption of the *RAD52* gene caused the *IXR1* and *ixr1* cells to become more sensitive to cisplatin. Rad52 is involved in double-strand break repair, and is presumably involved in the repair of interstrand adducts. *rad52 ixr1* is significantly more resistant to cisplatin treatment than *rad52 IXR1*. This result indicates that Ixr1 does not efficiently block double-strand break repair.

C5-128 p53 MODULATION OF BTF2-TFIIH ASSOCIATED NUCLEOTIDE EXCISION REPAIR ACTIVITY, X.W. Wang[1], H. Yeh[1], L. Schaeffer[2], R. Roy[2], V. Moncollin[2], J-M. Egly[2], Z. Wang[3], E. C. Friedberg[3], M.K. Evans[4], B.G. Taffe[4], V.A. Bohr[4], G. Weeda[5], J.H.J. Hoeijmakers[5], K. Forrester[1], and C.C. Harris[1], [1]Lab of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland 20892-4255, USA; [2]UPR 6520 (CNRS), Unite 184 (INSERM), Faculte de Medicine, 11 rue Humann, 67085 Strasbourg Cedex, France; [3]Lab of Molecular Pathology, Dept of Pathology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235, USA; [4]Lab of Molecular Genetics, National Institute of Aging, NIH, Baltimore, Maryland 21224, USA; [5]Dept of Cell Biology and Genetics, Medical Genetics Center, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherland.
The p53 tumor suppressor gene product has pleiotropic functions including control of genomic plasticity and integrity. Here we report that wild-type and mutant p53 can bind to several BTF2-TFIIH associated factors, including human transcription-repair coupling factors, XPD and XPB, as well as Rad3, the yeast homologue of human XPD, and CSB, which is involved in strand-specific DNA repair. All these factors belong to the recently identified DNA and RNA helicase superfamily. We also mapped the region of XPB that interacts with p53 to an indispensable domain conserved among the helicase superfamily. The C-terminal p53 domain involved in the regulation of its activity is required for the interaction. Furthermore, wild-type but not 273[his] mutant p53 inhibited XPD and Rad3 helicase activities, and to a lesser extent XPB helicase activity. p53 did not alter the BTF2-TFIIH associated basal transcription activity nor the ATPase activity of BTF2-TFIIH and Rad3. Moreover, Li-Fraumeni syndrome cells that are heterozygous for the p53 mutant allele repair UV-induced pyrimidine dimers at a slower rate than normal human cells. These results suggest that p53 may play a direct role in modulating nucleotide excision repair pathways.

C5-129 STUDY OF *E.COLI* UVR(A)BC ENDONUCLEASE STRUCTURE-FUNCTIONAL INTERMEDIATES BY ANTI-UVR PROTEIN MONOCLONAL ANTIBODIES, Oleg Kovalsky and Lawrence Grossman, Department of Biochemistry, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205
Uvr(A)BC endonuclease of *E.coli* is a complex of three proteins initiating nucleotide exision repair pathway by incising a DNA chain on both sides of damage. The mechanism of damage recognition and incision by this repertoire of proteins is a complex one. It includes several intermediates characterized by different conformation of their macromolecular constituents, overall architecture and stoichiometry. In order to gain insights into these structural rearrangements we generated and purified monoclonal antibodies (mAbs) to UvrA, UvrB and UvrB*, the proteolytic fragment of UvrB lacking 43 C-terminal amino acids. MAbs recognizing distinctive epitopes were selected by additivity tests. Western blot analyses and solution competition assay of deletion and point mutants of UvrA and UvrB allowed for localization of the epitopes of two anti-UvrA, four anti-UvrB and three anti-UvrB* mAbs. A quantitative study of the solution interaction of these mAbs with respective Uvr proteins revealed: (i) nucleotide cofactor-induced structural transition of UvrA dimer and (ii) different conformations of UvrB and UvrB*, presumably related to induction of the cryptic ATPase of UvrB. Some of the mAbs were further characterized in terms of their influence on functional intermediates and the overall incision of Uvr(A)BC. These studies allowed for elucidation of the regions involved in the formation and function of Uvr(A)BC and its intermediates. The model of structural changes accompanying Uvr(A)BC functioning will be discussed.

C5-130 NUCLEOTIDE EXCISION REPAIR IN *E. COLI*: HOW ARE LESIONS RECOGNIZED? W. Dean Rupp and Irina Gordienko, Departments of Therapeutic Radiology and of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510
The dual incision at lesions in damaged DNA by the UvrABC nuclease of *E. coli* proceeds by a cascade of sequential reactions that provides an overall specificity much greater than that of any of the individual steps. We have studied several reactions that we believe represent specific key steps in the overall process. We have studied the release of short oligonucleotides with or without damage and have observed that there is a sharp limit in the length of the oligonucleotide released by UvrAB and that damaged oligos are released more readily. We suggest that the main role of this 'helicase' activity is not to translocate the repair complex to sites of damage, but rather that this reaction is due to localized action of the enzyme at a site in DNA to probe the interior hydrophobic region of the DNA in ascertaining whether there is actually damage present and if there is, to position the complex in exactly the right position for subsequent incision with UvrC. We have observed that mismatches on the oligo result in its removal as readily as the presence of the bulky AAF adduct . One interpretation of this observation is that UvrAB specifically interacts with mismatches much as it does with bulky lesions, but that the subsequent steps in the cascade are less efficient so that incision at mismatches does not occur at a high frequency even though an initial recognition step may have occurred. We are currently testing this hypothesis. During these studies, we noticed that 3' recessed ends are subject to specific incision 7 nucleotides from the 3' end and believe that this reaction may be a consequence of the second incision that normally occurs at DNA lesions on the 5' side of the lesion only after an initial incision has already occurred on the 3' side. The 5' extended single strand next to a duplex region may provide a binding site for UvrAB that allows a "second" incision to occur even though no damage is present.

C5-131 REPAIROSOME FOR YEAST NUCLEOTIDE EXCISION REPAIR, Zhigang Wang[1], Jesper Q. Svejstrup[2], Xiaohua Wu[1], William J. Feaver[2], Roger D. Kornberg[2] and Errol C. Friedberg[1], [1]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75235 and [2]Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.
Nucleotide excision repair (NER) is an important cellular defense mechanism against mutagenesis and carcinogenesis by environmental agents. Eukaryotic NER requires multiple gene products for the early steps of the repair pathway. We have detected a giant protein complex for NER from the yeast *Saccharomyces cerevisiae*, which we designate the nucleotide excision repairosome . It has a molecular mass of 700-1,000 kDa. The repairosome can be isolated in the absence of DNA damage by fractionation of yeast cell extracts. Components of the repairosome include the basal transcription factor core TFIIH/Ssl2 and all known gene products indispensable for NER, namely Rad1, Rad10, Rad2, Rad4 and Rad14 proteins. The repairosome is inactive for transcription *in vitro*. The active form of TFIIH in transcription is holoTFIIH, consisting of core TFIIH/Ssl2 and a three-subunit kinase (TFIIK). HoloTFIIH can be dissociated and re-associated *in vitro*. The repairosome can also be dissociated *in vitro*. Thus, the assembly of core TFIIH/Ssl2 into holoTFIIH for transcription or for NER may be a dynamic reversible process depending on and/or regulated by the physiological conditions of the cell. The composition and properties of the repairosome are under investigation.

C5-132 *Abstract Withdrawn*

C5-133 A DIRECT-ACTING 5' ENDONUCLEASE FROM *S. POMBE* THAT RECOGNIZES CYCLOBUTANE PYRIMIDINE DIMERS AND (6-4) PHOTOPRODUCTS,

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We have discovered and are in the process of characterizing a new endonuclease from *S. pombe* that recognizes the two major UV light-induced DNA photoproducts. This enzyme, which we have named DERE (for Direct Excision Repair Endonuclease), cleaves duplex DNA immediately 5' either to cyclobutane pyrimidine dimers or (6-4) photoproducts and produces strand scission products containing 3'-hydroxyl and 5'-phosphoryl groups. These, as well as other, properties suggest that DERE may represent a new class of DNA excision repair enzyme. This work was supported by American Cancer Society grant #NP-806.

C5-134 AN IN DUCIBLE DNA EXC: ;ION REPAIR PATHWAY IN THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*.

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Recently we reported on a novel UV endonuclease from the fission yeast *Schizosaccharomyces pombe (S. pombe)* named DERE (for direct DNA excision endonuclease) which cleaves immediately 5' to cyclobutane pyrimidine dimers and (6-4)pyrimidine pyrimidones leaving 5' hydroxyl and 3' phosphoryl ends. Taking advantage of a UV sensitive mutant, *rad12-502*, which lacks DERE activity, and partially purified enzyme, we have demonstrated that DERE is part of a second DNA excision repair process. In addition, when *S. pombe* cells are UV irradiated, DERE levels increase approximately 4 fold. Interestingly, DERE is inducible by UV light in *rad12-502* cells reaching a level equivalent to the UV-light-induced level of wild-type cells. This result suggests that the *rad12* gene product is involved in the regulation of DERE expression. Furthermore, we have found that the checkpoint control gene *rad9* is involved in DERE regulation. This is based on the observation that cells containing *rad9-192* or *rad9::ura4* constituitively produce DERE at the UV-light-induced levels. We propose that *rad12* is a negative regulator/modulator of *rad9* and that *rad9* negatively regulates DERE activity.

C5-135 PROTEINS INVOLVED IN INCISION/EXCISION DURING NUCLEOTIDE EXCISION REPAIR,

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We are using two different approaches to study the incision/excision step of nucleotide excision repair and to identify the roles of XP, ERCC and other proteins in this process.

An *in vivo* assay is to measure the binding of PCNA protein to nicks produced as intermediates during DNA repair. Quiescent primary human fibroblast cells are UV-irradiated and after 30 min are gently extracted and fixed with methanol. The cultures are then stained with anti-PCNA monoclonal antibody PC10 and a secondary FITC-conjugated antibody. Quantification of the immunofluorescence assay gives reflects the number of PCNA molecules bound to repair intermediates. Using this protocol, XP-A (XP25RO) and XP-G (XP2BI) cells showed no PCNA staining, while XP-E (XP2RO) and XP-V (XP115LO) cells showed staining indistinguishable from normal fibroblasts. XP-B (XP2BA), XP-C (XP1BE), XP-D (XP3NE), and XP-F (XP3YO) cells had an intermediate level of staining, indicating that some nicked intermediates were formed even in the absence of these gene products. Except for XP-B, the level of PCNA staining after irradiation correlates well with levels of unscheduled DNA synthesis reported by others for these cell lines.

For an *in vitro* assay, human cell extracts were fractionated by phosphocellulose and hydroxyapatite chromatography to locate the XP and ERCC factors, as well as other components necessary for repair such as RPA and PCNA. The full reaction could be reconstituted with RPA, PCNA, XPG, and two complex fractions. To measure specific incision of damaged DNA, a coupled assay is being used where repair synthesis is carried out by DNA polymerase I (Klenow fragment) instead of the mammalian DNA polymerases. The ability to reconstitute the core nucleotide excision repair reaction with these purified components and complex sub-fractions facilitates a fuller elucidation of details of the mechanism.

C5-136 PREFERENTIAL DNA DAMAGE IN THE P53 GENE BY BENZO(A)PYRENE METABOLITES IN XERODERMA PIGMENTOSUM GROUP A CELLS, TaiHao Quan and J. Christopher States, Center for Molecular Medicine and Genetics, Wayne State University, 2727 Second Avenue, Detroit, MI 48201.

We have reported previously that intracellularly bioactivated benzo(a)pyrene-*trans*-7,8-dihydrodiol (BPD) is more mutagenic/less cytotoxic in xeroderma pigmentosum group A (XPA) cells than extracellularly supplied benzo(a)pyrene-*trans*-7,8-dihydrodiol-9,10-epoxide (BPDE). In order to further investigate the molecular mechanisms of BP metabolite induced genotoxicity, DNA damage levels in the P53 gene (transcriptionally active) and the β-globin gene (transcriptionlly inactive) were determined by quantitative PCR after treating XPA cells with [^{3}H]BPD or [^{3}H]BPDE. DNA adduct formation in the genome overall was determined by measuring the incorporation of [^{3}H] from [^{3}H]BPD or [^{3}H]BPDE into DNA. Incorporation of [^{3}H] from [^{3}H]BPD and [^{3}H]BPDE was concentration dependent and was linear for [^{3}H]BPDE but not for [^{3}H]BPD. BPD- and BPDE-induced DNA damage was detected by QPCR in the P53 gene, but not in the β-globin gene. P53 gene PCR products decreased semilogarythmically with [^{3}H]BPD-induced adducts but not with [^{3}H]BPDE-induced DNA adducts in the genome overall. At equivalent overall levels of adducts, BPD treatment induced more damage in the P53 gene than BPDE treatment. The results suggest that the mechanism of BPDE-induced genotoxicity is depenedent on the rate and/or intracellular site of exposure and that transcriptionally active genes may be preferential targets for DNA damage by DNA reactive BP metabolites.

C5-137 GENETIC ANALYSIS OF MOUSE *ERCC-5/XP-G* GENE, Dale L. Ludwig[1], John Mudgett[2], Ana Perez[1], and Mark A. MacInnes[1]. 1. Life Sciences Division, Los Alamos National Laboratory, M888, Los Alamos, NM 87545; 2. Merck Research Laboratories, Rahway, NJ 07065.

The murine **E**xcision **R**epair **C**ross **C**omplementing gene **5** is the mouse homolog of human *XP-G*, known to be defective in the hereditary genetic disorder xeroderma pigmentosum (group-G). The *XP-G* gene encodes a structure-specific DNA endonuclease which is essential for nucleotide excision repair of damaged DNA. In mouse L cells, murine *XP-G* (*mXP-G*) expresses a mature mRNA of 4.2kb. Primer extension analysis determined that the promoter contains a single transcription start site with a leader of 180bp from the translational AUG. Using deletion analysis with an *mXP-G* promoter-luciferase reporter gene construct, we determined that the minimal sequence for constitutive promoter expression is shorter than -300bp. *mXP-G* cDNA-specific riboprobes were used for RNA hybridization analysis of mRNA expression *in situ* on thin-sections of embryonic developmental stages. Constitutive mRNA expression was found in all embryonic tissue lineages and stages examined (4 - 14 days) indicating that the *mXP-G* gene has ubiquitous function in development. We have also isolated a cosmid clone of *mXP-G* gene which complements the UV sensitivity and repair defects in CHO-UV135 cells. We have furthermore isolated cosmid clones containing an incomplete pseudogene of murine *XP-G* which contains extensive sequence homology to the *XP-G* cDNA. As a means to understand the complex pathophysiology of XP-G syndrome, and *in vivo* role(s) of *mXP-G* for excision repair, chromosome stability and cancer susceptibility, we are generating murine embryonic stem (ES) cell lines containing knockouts of *mXP-G*. The targeting construct deleted the entire 5' untranslated leader, translational start site and the first 26 amino acids (exon 1) of the coding region. This region was replaced by an insertion of the murine *pgkHPRT* minigene for positive selection in *HPRT* deficient ES cells. Negative selection against non-homologous recombination will use Herpes viral thymidine kinase gene to enrich for homologous recombination targeted clones. ES cells with homologous *mXP-G* gene replacements will be injected into blastocysts. Animals with mutations in *mXP-G* will then be generated by production of chimeric mice and subsequent germline transmission of the ES cell-derived mutant *mXP-G* gene. (This research is supported by US DOE Contract KP02-04-000.)

C5-138 XPA DEFICIENT MICE ARE HIGHLY SUSCEPTIBLE TO CARCINOGEN INDUCED SKIN CANCER

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To enable the functional analysis of the DNA nucleotide excision repair (NER) gene *XPA in vivo*, we have generated *XPA* deficient mice by gene targeting in ES-cells. Exons 3 and 4 of the *XPA* gene were deleted and replaced by a PGK-neo cassette. Gene-targeted ES-cells (E14; ola 129 derived) were injected into C57Bl/6 host embryos. Different chimaeric mice yielded offspring with the mutated *XPA* allele. *XPA*$^{-/-}$ mice, derived from crossings between heterozygous mice, develop normally (up to 8 months), but were not obtained in the expected Mendelian ratio.

From Northern blot analysis, using RNA isolated from liver, it was shown that *XPA*$^{-/-}$ mice are devoid of any detectable *XPA* transcripts. NER activity in fibroblasts of *XPA*$^{-/-}$ embryos is severely reduced, as determined by UV-induced UDS and cell survival after exposure to UV or 7,12-dimethylbenz(a)anthracene (DMBA).

The sensitivity of the *XPA*$^{-/-}$ mice for chemically induced skin cancer, was tested by treating the mice with DMBA. Within 12 wks $\pm$ 50% of the *XPA*$^{-/-}$ mice developed skin papillomas. *XPA*$^{+/-}$ and *XPA*$^{+/+}$ mice hardly responded to the same treatment.

In conclusion, the *XPA*$^{-/-}$ mice generated by us display a phenotype comparable to that found in XP-A patients, i.e. lack of capacity to repair induced DNA damage, and an increased incidence of skin tumors after dermal exposure to a carcinogenic agent. The XP-A mouse model therefore, may be useful to further elucidate the function of the XPA protein in NER, and the role of NER in the process of carcinogenesis.

C5-139 IDENTIFICATION OF MAMMALIAN MUTANTS DEFECTIVE IN NUCLEOTIDE-EXCISION REPAIR

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Two different sources of mammalian mutants defective in nucleotide-excision repair (NER) are available: mutants hypersensitive to UV (UV^S) isolated in vitro from rodent established cell lines and cells from patients affected by hereditary disorders such as xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and trichothiodystrophy (TTD). Complementation analysis based on somatic cell fusion and on the evaluation of the response to UV irradiation is a useful tool to characterize at the genetic level still unclassified mutants.

Genetic analysis of the repair defect responsible for the UV hypersensitivity in the clone CHO10PV isolated in our laboratory from the CHO-K1 prol$^-$ cell line was carried out by measuring the survival after UV irradiation in hybrids obtained after fusion of CHO10PV cells with mutants representative of the eleven groups of UV^S rodent mutants identified so far. These studies demonstrated that the mutant CHO10PV represents a new complementation group of NER defective rodent mutants, the group 12.

Recently we have studied at the cellular and genetic levels one Italian patient showing the clinical features diagnostic for CS. DNA repair investigations in in vitro cultured fibroblasts demonstrated the presence of the cellular alterations typical of CS, i. e. reduced survival and failure to recover normal rates of DNA and RNA synthesis following UV exposure. Genetic analysis based on the measurement of the recovery of RNA synthesis following UV irradiation in hybrids obtained by fusing the cells of the patient with CS cells representative of CS group A and B demonstrated the occurrence of complementation in all crosses. These results suggest that the patient under study is carrier of a repair defect different from those identified as responsible for CS pathological phenotype.

C5-140 CHARACTERIZATION OF THE HUMAN XPAC GENE PROMOTER AND COMPLEMENTATION OF XPA CELLS WITH AN XPAC MINIGENE,

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We have cloned the human XPAC gene and characterized its promoter by transient CAT expression assays in human fibroblasts. The human XPAC promoter is an extraordinarily weak promoter in fibroblasts (1% of RSV-LTR). Despite the promoter's weakness, we have localized both positive and negative regulatory elements suggesting that the XPAC promoter may be under complex regulation. We have constructed an XPAC minigene that uses a 2 kb 5' flanking genomic DNA fragment for the promoter and a 0.8 kb 3' flanking genomic DNA fragment for polyadenylation signals to express the XPAC cDNA. We have also constructed a high level XPAC cDNA expression vector using the strong RSV-LTR promoter (pRSV-XPAC). In XP2Os-SV and XP12Be-SV cells transiently transfected with either the minigene or pRSV-XPAC, host cell reactivation of a UV irradiated CAT expression plasmid is restored partially. UV survival in XP12Be-SV cells is completely restored by pRSV-XPAC but not by the minigene. In contrast, in pools of XP12Be-SV cells stably transformed with the minigene, both UV survival and host cell reactivation of a UV-irradiated CAT plasmid are restored completely. Host cell reactivation of a CAT expression vector is completely restored in only 2 of 3 individual clones of XP12Be-SV cells stably transformed with the minigene and selected for UV survival. Assays of UV survival and reactivation of a UV-irradiated expression vector measure different biological functions of repaired DNA. Our results suggest that these assays may be dependent on expression level or length of expression time and that no single measurement can reliably demonstrate complementation by a transfected gene.

C5-141 MUTATIONAL ANALYSIS OF THE HUMAN *ERCC1* GENE

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The ERCC1 protein resides in a complex which is able to correct ERCC1-, ERCC4- and ERCC11-deficient cell lines as well as XP-F cells. It is thought to perform the 5' strand incision during the process of Nucleotide Excision Repair (NER). The analogous complex of yeast (RAD1/RAD10) has an additional engagement in recombination, a process which may be necessary for the repair of crosslinks.
To identify the essential regions in ERCC1 for its repair function, mutant *ERCC1* cDNAs have been assayed for correction of a rodent group 1 mutant. The UV sensitivity of this mutant is comparable to the sensitivity of XP cells, but the sensitivity to the crosslinking agent MMC is extreme. We found that 92 N-terminal amino acids (one third of the protein) can be deleted without loss of function. Deletion of 103 residues, which could interfere with the association with XP-A, results in a non-functional protein. In contrast, shortening the C-terminal end by more than 4 amino acids resulted in complete loss of the correcting ability. The 4 amino acid C-terminal deletion confers full resistance to MMC, whereas the sensitivity to UV is corrected only partially. In an *ERCC1-UvrC* construct part of the C-terminus which shows significant homology with *E.coli* UvrC is replaced by the *UvrC* sequence. This hybrid gene however, could not correct for the mutant phenotype. Furthermore, specific missense mutations have been introduced in the highly conserved putative DNA binding domain. Western blot analysis suggests that most mutations cause protein instability. However, in those cases where protein is present a partially corrected phenotype is seen. Notably, a $P_{150}\rightarrow V$ construct restored resistance to MMC but retained significant UV sensitivity.
Our data indicate that protein levels limiting to UV repair are sufficient for the removal of crosslinks and therefore provide an explanation for the XP-F phenotype.

C5-142 FUNCTIONAL CHARACTERIZATION OF *ERCC1* IN RECOMBINATION AND REPAIR.

Rhonda L. Rolig, Gerald M. Adair and Rodney S. Nairn. Department of Carcinogenesis, The University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, Texas 78957.

The *ERCC1* (*E*xcision *R*epair *C*ross *C*omplementing, group 1) gene is the accepted mammalian homolog of the *RAD10* gene of the yeast, *Saccharomyces cerevisiae*. This assumption is based both on sequence homology of *ERCC1* and *RAD10* and on the function of *ERCC1* in nucleotide excision repair (NER) in mammalian cells. In yeast, *RAD10* is known to function in NER at the incision step as well as in mitotic recombination. We are particularly interested in these two aspects of *ERCC1* function in mammalian cells. We intend to determine functional homology between *ERCC1* and *RAD10* by testing the predicted role of *ERCC1* in mitotic recombination, and by characterizing regions within the *ERCC1* gene of functional importance in recombination and repair. Our research involves two approaches: (i) Sequence characterization of *ERCC1* mutant alleles obtained from a panel of conventionally-derived CHO *ERCC1* mutant cell lines using reverse transcriptase-polymerase chain reaction to recover mutant *ERCC1* transcripts for sequencing, and (ii) Production of *ERCC1* null CHO cell lines through targeted gene disruption using a positive/negative selection strategy. These cell lines will be used to test the effect of an *ERCC1* null mutation on homologous recombination. To date, we have identified several independent mutations in conventionally-derived CHO cell *ERCC1* mutants, and we have produced a CHO cell *ERCC1* knock-out mutant. Preliminary results of targeted correction of the CHO *APRT* locus in a CHO *ERCC1* null mutant will be presented. (Supported by NCI grant CA36361 to R.S.N.)

C5-143 EXPRESSION AND REGULATION OF THE UV-DAMAGED DNA-BINDING PROTEIN/XP-E FACTOR,

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A DNA-binding protein complex (UV-DDB/XP-E factor) with high affinity for (6-4) pyrimidine dimers has recently been purified from primate cells[1,2]. Two polypeptides (p127 and p41) have been identified, and the primary sequence of p127 was determined[3]. p127 is a member of a novel, conserved family of proteins; its homologs have been identified in slime mold and rice, and more recently in Drosophila. p127 cDNA translated *in vitro* does not bind to UV-damaged DNA, but the recombinant p127 expressed in monkey cells forms DNA-binding complexes similar to the endogenous protein. Primary skin fibroblasts from repair-deficient XP-E patients express ~50% of the p127 found in normal human cells. Two out of 3 XP-E patients studied have mutations in the p127 gene, and this, together with the lower levels of the protein, may affect the repair function of the binding complex in these patients.
Mammalian cells exposed to UV light show an immediate dose-dependent inhibition of the extractable UV-DDB activity. The recovery of UV-DDB activity post-UV correlates with the repair of (6-4) dimers in normal cells, while in severely repair-deficient cells, the inhibition persists up to two cell cycles. In untreated cells, all of the activity and 1/3 of the protein is localized in the nucleus. In UV-treated cells, ~80% of the nuclear activity is lost, and there is a marked translocation of the nuclear p127 from low to high-salt chromatin. A similar pattern of protein translocation after UV has been seen for PCNA and RP-A, two nucleotide excision repair accessory proteins. Translocation of these repair proteins and XP-E factor into high-salt chromatin at early times after UV suggests their tight association with UV-damaged genomic DNA before the onset of DNA repair synthesis.

[1]Abramic' et al., J. Biol. Chem. 266:22493,1991; [2]McKenney et al., J. Biol. Chem. 268:21293,1993; [3]Takao et al., Nucl. Acids Res. 21:4111, 1993.

C5-144 INTRANUCLEAR DYNAMICS OF HUMAN REPAIR PROTEIN XPG, Min S. Park, Mark A. MacInnes, Gary F. Strniste, Stepanie H. Pendergrass, Babetta L. Marrone and Jeffrey A. Knauf*, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM87545, *Department of Endocrinology, Cedars Sinai Medical Center, Los Angeles, CA 99028

Due to the genetic defects in nucleotide excision repair pathways, xeroderma pigmentosum (XP) patients have a high risk of skin cancers. Among different genetic complementation groups, XPG patients exhibit various pathophysiological symptoms ranging from growth and mental retardation to neurodegeneracy. The XPG gene encodes a 134 kDa nuclear protein with a structure-specific DNA endonuclease activity that is essential for removal of DNA damages including UV photoproducts and bulky adducts. Here we report intranuclear dynamics of XPG by various immunological methods. Indirect immunofluorescence and confocal laser scanning microscopy suggested presence of XPG protein as foci throughout the plane of the nucleus. Biochemical fractionation and immunoprecipitation studies indicated a tight but reversible association of XPG protein with intranuclear structure(s). We also identified potential nuclear localization signals (NLS) in the XPG protein by using a β-galactosidase reporter system. Two putative NLSs at the carboxy terminus (C-terminus) (amino acids 1029-1169, 1146-1186) of the XPG protein are shown to independently localize the β-galactosidase reporter protein to the nucleus. The evolutionarily conserved C-terminus peptide (amino acids 1146-1186) was able to further sublocalize the β-galactosidase fusion protein to subnuclear regions. Both endogeneous XPG protein and the β-galactosidase fusion protein became dissociated from the subnuclear regions such as foci and the perinucleolar region upon UV irradiation, and they completely reassociated with these structures within 24 hours post-irradiation. This research is supported by the US DOE under contract #KP0204000.

C5-145 IDENTIFICATION OF MUTATIONS WITHIN THE ERCC-5 GENE IN A XERODERMA PIGMENTOSUM GROUP G PEDIGREE, R.T. Okinaka[1], A. Perez[1], K. Laubscher[1], A. P. Sena[1], M.A. MacInnes[1], and K.H. Kraemer[2], [1]Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545 and [2]National Cancer Institute, Bethesda, MD 20892

Complementation data and genetic analysis [Nouspikel and Clarkson, *Hum. Mol. Genet.* 3:963 (1994)] indicate that the gene responsible for the Xeroderma pigmentosum (XP) group G syndrome corresponds to the rodent excision repair cross-complementing gene ERCC-5 (yeast homolog = Rad 2). This study attempts to identify specific mutations within the ERCC-5 gene to determine the genetic basis for this disease in a single XP-G patient and his family. The cDNA from the XP-G gene from coded cell lines (from K.H.K.) were analyzed by DGGE, SSCP and/or direct sequencing analysis to detect and identify mutations. The intial analysis of these cDNAs identifed the following: (1) a polymorphic G to C transversion at bp 3310 (coding change Asp_{1104} to His_{1104}); (2) a transition from C to T at cDNA bp position 138 (exon 2, His_{46} to His_{46}) and (3) a transversion G to T mutation at cDNA bp position 31 (exon 1), that resulted in a change in $codon_{10}$ (Glu_{10} to the termination codon, UAG, $Stop_{10}$). Further analysis to determine the distribution of these mutations within the pedigree indicated the following: (a) the father was heterozygous for the nonsense mutation, (b) the mother and an unaffected sibling were heterozygous for both polymorphic markers but homozygous (WT) at bp 31, and (c) the patient was heterozygous for all three markers. Extensive analysis of the nearly 4 kb cDNA of the XP-G gene in the patient has not yet revealed inheritance of a potentially inactivating mutation from the mother. However, RT-PCR and analysis of the polymorphic region in exon 2 indicates that the mother's cDNA (containing an Nco I site) is not a prevalent species in the PCR products from the patient and the unaffected sibling (< 10%). These results suggest that both siblings in the pedigree inherited a maternal mRNA that is relatively unstable and that this may cause a significant reduction of XP-G protein expression in the patient. The basis for this unstable message is not clear. This pedigree analysis provides evidence that mutant alleles in an XP-G patient were transmitted in Mendelian fashion from the parents to the patient. [This work sponsored by the U.S. Department of Energy under contract #KP0204000].

C5-146 DETECTING AND CHARACTERISING THE INCISIONS FORMED DURING NUCLEOTIDE EXCISION REPAIR, Jonathan G. Moggs and Richard D. Wood, Imperial Cancer Research Fund, Clare Hall Laboratories, Herts EN6 3LD, U.K.

Extracts from normal cells can carry out nucleotide excision repair, but most XP cell extracts are defective in repair synthesis and excision of a damaged oligonucleotide. To investigate the incisions made during repair, DNA containing a specifically located cisplatin crosslink was incubated with fractionated cell extracts, from which PCNA had been removed, so that gap-filling DNA repair synthesis would be impaired. DNA repair synthesis was further inhibited by omitting deoxynucleotides from the reaction buffer and also by adding aphidicolin, a specific inhibitor of DNA polymerases.

The incised intermediates formed may be subjected to restriction fragment end-labelling analysis to determine the location of each incision. Incised intermediates were linearised 424 nt 3' to the lesion and end-labelled. After incubation with HeLa cell extract, a fragment was detected of the size expected from a 3' incision being made ~5 phosphodiester bonds away from a lesion. Extract from an XP-G cell line produced little or no detectable damage-dependent incision fragment while some incision fragment was produced by ERCC1-deficient extract. These data suggest that XPG protein is required for formation of the 3' incision during nucleotide excision repair. The absence of evidence for a 5' incision by the XP-G cell extract further suggests that either the 3' incision must be made prior to the 5' incision, or that both incisions are usually made in a co-ordinated fashion. The ERCC1 gene product may not be absolutely necessary for the 3' incision, but is required for repair synthesis and release of damaged oligonucleotides. Assuming that ERCC1 is part of an endonuclease by analogy with Rad10, we propose that it plays a role in the 5' incision.

A comparison of the 3' incisions made by HeLa cell extract on different cisplatin lesions showed that the 1,2-intrastrand d(GpG) platinum crosslink is poorly incised in comparison with the 1,2-intrastrand d(GpTpG) platinum crosslink, consistent with observations made using an *in vitro* repair synthesis assay.

C5-147 MOLECULAR CLONING OF A HUMAN PROTEIN WHICH BINDS TO DAMAGED DNA, IMPLICATED IN THE DEFECTS OF XERODERMA PIGMENTOSUM GROUP E CELLS, Byung Joon Hwang, Joseph C. Liao and Gilbert Chu, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305

Xeroderma pigmentosum (XP) is an inherited disease characterized by defective repair of DNA damaged by UV radiation or agents that produce bulky DNA adducts. Human cells contain a factor that is deficient in a subset of patients from XP complementation group E and binds to damaged DNA. This factor, named XPE-BF, was purified to near homogeneity. The denatured protein migrated as a 125 kDa polypeptide on SDS-PAGE. Binding to UV-damaged nucleotides was 500,000-fold greater than for intact nucleotides, explaining how a molecule with an abundance of only 1 to 2 molecules per megabase can survey the genome for damaged DNA.

Partial amino acid sequence information of XPE-BF protein was obtained from 11 internal peptides generated by an elution-digestion-sequencing (EDS) method, leading to the cloning of XPE-BF cDNA. Eleven peptide sequences derived from the p125 protein of XPE-BF were virtually identical to the translated peptide sequence from monkey UV-damaged DNA binding protein (UV-DDB) cDNA. *In vitro* transcription and translation of the p125 cDNA yielded a polypeptide which bound specifically to UV-damaged DNA. The translated amino acid sequence of XPE-BF cDNA was 99% identical to the monkey UV-DDB cDNA, despite the previously described differences of biochemical properties such as binding specificity for damaged DNA and affinity for cyclobutane dimers. In contrast to previous reports, we found that UV-DDB protein binds with high specificity for UV-damaged DNA. Thus, we conclude that human XPE-BF is homologous to monkey UV-DDB.

C5-148 DIRECT INTERACTION BETWEEN HUMAN REPLICATION PROTEIN A AND THE NUCLEOTIDE EXCISION REPAIR PROTEINS OF XERODERMA PIGMENTOSUM COMPLEMENTATION GROUPS A AND G, Zhigang He and C. James Ingles, Banting and Best Department of Medical Research and Department of Molecular and Medical Genetics, University of Toronto, Canada M5G 1L6.

The human single-stranded DNA binding protein, replication protein A (RPA) functions in DNA replication, homologous recombination and nucleotide excision repair. To identify proteins interacting with RPA during these diverse DNA transactions, this trimeric protein was expressed in and purified from *E. coli* cells and used as column ligand in affinity chromatography experiments. RPA specifically and selectively bound the xeroderma pigmentosum protein XPA present in HeLa cell extracts. This interaction between RPA and XPA was shown to be direct and involve contacts between XPA and both the RPA1 (70 kD) and RPA2 (34 kD) subunits of RPA. XPA has been shown previously to bind preferentially to damaged DNA, however we have now shown that a complex of XPA and RPA demonstrates a remarkable cooperativity in binding to N-acetoxy-2-acetylaminofluorene (AAAF) treated DNA. Affinity columns of RPA also bound a second nucleotide excision repair protein XPG, and an XPA-RPA-XPG complex was readily detected using a co-immunoprecipitation protocol. These experiments suggest that, rather than binding to single-stranded DNA generated during the incision/excision steps of repair, or facilitating repair DNA synthesis, RPA is involved with XPA in the earliest damage recognition step. RPA may also assist in targeting the endonucleolytic activity of XPG.

C5-149 MECHANISTIC IMPLICATIONS OF PROTEIN-PROTEIN INTERACTIONS IN NUCLEOTIDE EXCISION REPAIR, Lei Li and Randy Legerski, Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

We have previously reported on a specific interaction between the excision repair proteins XPA and ERCC1. This association was found both in vivo, by means of the two-hybrid system, and in vitro, by means of recombinant proteins. To further demonstrate the functional relevance of this interaction, we have mapped the region in XPA that mediates the interaction with ERCC1 and have used site directed mutagenesis to delete highly conserved motifs within this domain. Two such mutants were evaluated by in vitro interaction and were found to be in one case greatly reduced and in the other completely deficient in association with ERCC1. These two XPA mutants were also evaluated by an in vitro excision repair assay and both were found to be unable to complement XPA extracts in contrast to wild type XPA. Furthermore, the mutant that was completely deficient in binding to ERCC1 was also able to inhibit repair synthesis in wild type extracts indicating that it was a dominant negative mutant. Finally, determination of the binding affinity between XPA and ERCC1 as a function of increasing salt concentration indicates that the interaction is likely of a hydrophobic nature. Taken together these results further support a model in which one function of XPA is to recruit the ERCC1 incision complex to the site of damage.

We have also found using a two-hybrid screen and an in vitro assay that XPA interacts with the 32 kd sub-unit of replication factor A (RPA). RPA has been shown by others to be required for excision repair in vitro. The 32 kd sub-unit of RPA is phosphorylated at the G1/S boundary of the cell cycle and is also hyperphosphorylated upon UV irradiation. We have determined that the phosphorylation state of RPA does not affect the interaction with XPA. A domain in XPA that mediates the interaction with RPA has been mapped to amino acid residues 153 through 176. Efforts are underway to identify deletion mutants within this region that are defective in the interaction with RPA. A model will be presented for the functional significance of the XPA/RPA interaction.

C5-150 REMOVAL OF UVB-INDUCED CYCLOBUTANE PYRIMIDINE DIMERS IN THE MOUSE EPIDERMIS: LOSS OF T4 ENDONUCLEASE V-SENSITIVE SITES IS SIGNIFICANTLY SLOWER THAN LOSS OF ANTIBODY-BINDING SITES, [*]Rob J.W. Berg, Hendrik J.T. Ruven, [*]Arie A. Vink, C. Maud J. Seelen, [#]Jacqueline B.A. Bergen Henegouwen, [#]Len Roza, [*]Frank R. de Gruijl, Leon H.F. Mullenders, and Albert A. van Zeeland, MGC - Dept. of Radiation Genetics and Chemical Mutagenesis, Leiden University, Leiden, [*]Dermatology, Utrecht University, Utrecht, [#]Genetic Toxicology, TNO, Rijswijk, The Netherlands

We determined the removal of cyclobutane pyrimidine dimers (CPD) in DNA of the epidermis of UVB-exposed mice using an enzymatic and two immunochemical methods. Hairless albino SKH:HR1 mice were irradiated with 2.0 kJ/m^2 (250-400 nm) UV radiation from Philips TL12/40W lamps after which they were allowed to repair DNA damage for 0 hour or 24 hours. Determination of the frequency of T4 endonuclease-sensitive sites (ESS) in isolated DNA from epidermal cells using T4 endonuclease V in combination with alkaline sucrose-gradient centrifugation revealed about 9% removal of ESS at 24 hours after UVB exposure. In contrast, determination of antibody-binding sites (ABS) in the same pool of epidermal cells using a monoclonal antibody against cyclobutane thymine dimers (the H3 antibody) in combination with flow cytometric analysis of epidermal cell suspensions, or with immunoslotblot analysis of purified, epidermal DNA resulted in a removal of ABS of about 60% at 24 hours after UVB exposure.

In addition we show that cultured murine and human fibroblasts exposed to UVC show similar kinetics for loss of ABS and ESS. Hence, the difference between the enzymatic and the immunochemical method must be specific for UVB and/or for the *in vivo* situation in the murine epidermis. The observed repair kinetics may be explained by a structural modification of the CPD, causing a loss of ABS but not of ESS.

C5-151 CHARACTERISATION OF A NOVEL CISPLATIN-SENSITIVE MAMMALIAN MUTANT CELL LINE, Eva Segelov, Graham J. Mann and Paul R. Harnett, Dept. Medical Oncology, University of Sydney Westmead Centre, Westmead, NSW 2145, Sydney, Australia.

Cisplatin is one of the most effective cancer chemotherapeutic agents. It acts chiefly by causing DNA adducts, some of which can be removed by the excision repair pathway, similarly to ultraviolet (UV) radiation-induced DNA damage. We are studying cisplatin-sensitive mammalian mutant cell lines in order to elucidate mechanisms of cisplatin DNA damage and repair. The CHO-MMC6 clone was previously isolated from replica plated CHO-K1 cells, after selection for sensitivity to mitomycin C. Further sensitivities to a variety of DNA-damaging agents were determined by assay of clonogenic survival (Table). CHO-MMC6 cells were twice as sensitive to cisplatin as the parent line and showed similar increased sensitivity to adriamycin and the nitrosurea BCNU. Sensitivities to bleomycin, ionising radiation (X-rays), and the alkylator ethylmethylsulphonate (EMS) were similar to those of parental cells.

	D_{37}*		
Agent	CHO-K1	CHO-MMC6	mutant/parent
cisplatin (uM)	30.3	14.4	2.1
mitomycin C (nM)	155	62	2.5
bleomycin (ug/ml)	5.4	5.2	1.0
ionising radiation (Gy)	4.2	3.6	1.2
BCNU (ug/ml)	15.3	4.7	3.3
adriamycin (ng/ml)	23.0	11.7	2.0
EMS (ug/ml)	322	306	1.1

* dose where surviving fraction = 1/e; at least two independent experiments.

Experiments elsewhere (C. Robson, personal communication) have shown that CHO-MMC6 is no more sensitive to UV than the parent. Total intracellular platinum was measured in parent and mutant cell lines after a 2 hr exposure to 60 or 120 uM cisplatin. There was no significant difference between the cell lines (60 uM: CHO-K1 76.4 ± 22.7, CHO-MMC6 47.0 ± 0.7; 120 uM: CHO-K1 154.5 ± 49.3, CHO-MMC6 104.6 ± 19.0 pg platinum/ug protein; mean ± SEM, n=3). The unusual pattern of cross-sensitivity to agents causing DNA-distorting adducts, but not to UV, together with evidence against excessive platinum uptake by the mutant, indicate this cisplatin-sensitive cell line may carry a novel mutation of the DNA repair apparatus.

C5-152 HUMAN XPG PROTEIN INCISES AT THE 3' SIDE OF DAMAGED DNA IN THE DUAL INCISION STEP OF NUCLEOTIDE EXCISION REPAIR, Tsukasa Matsunaga, David Mu, David S. Hsu, Joyce T. Reardon and Aziz Sancar, Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599

Nucleotide excision repair (NER) is an important cellular mechanism for removing DNA damage induced by UV and other damaging agents. In human cells, at least eight genes (XPA through XPG and ERCC1) are required for the initial incision step, but the molecular details are not well understood. Recently, the XPG protein has been shown to have single stranded-DNA endonuclease activity (O'Donovan *et al.*, 1994; Habraken *et al.*, 1994). We have analyzed the functional role of the XPG protein in the dual incision step using a specific antibody and an *in vitro* excision assay.

XPG was expressed as a fusion protein with the *E. coli* maltose-binding protein and used as an immunogen to generate rabbit polyclonal antibodies. The human excinuclease activity was tested by the excision assay with the 140-bp substrate containing cholesterol or cyclobutane TT dimer in the center. When we added the anti-XPG antibody into the excision assay with HeLa cell-free extract, a novel size of excision fragment (30-mer) was observed in addition to the normal fragments (mainly 28-mer) with reduced intensity. To determine which incision site (3', 5' or both) was modified, we purified the individual excision fragments and treated with T4 polymerase 3' → 5' exonuclease which is inhibited by bulky adducts including cholesterol and TT dimer. This treatment of both fragments yielded the same size of digested products (27-mer), indicating that the unusual excision with anti-XPG antibody was caused by a shifted 3' incision. These results suggest that the XPG protein binds on the 3' side of damaged DNA and makes the 3' incision in the dual incision step of NER.

C5-153 QUANTIFICATION OF THE XPA TRANSCRIPT IN HUMAN AND MOUSE CELL LINES.
Susan K. Layher and James E. Cleaver. Laboratory of Radiobiology and Environmental Health, University of California at San Francisco, San Francisco, CA 94143

A central dogma of Nucleotide Excision Repair (NER) which arose early on in its history is the "rodent-human paradox". This paradox stems from the observation that the survival of human and rodent cell lines to UV radiation is similar, although wild-type rodent cells repair only a small fraction of the UV-induced cyclobutane dimers, whereas wild-type human cells, repair such lesions extensively. Over the years, a plethora of experimentation has revealed that NER activity for human cells, occurs in both actively transcribed and non-transcribed regions following DNA damage, although the rate of repair occurs more slowly in non-transcribed regions. Rodent cell lines predominantly express NER activity in transcriptionally active regions; whereas cyclobutane dimers in the non-transcribed part of the genome are excised slowly, if at all.

Genetic complementation experiments with somatic cell human-rodent hybrids have suggested that many of the repair proteins are highly conserved between rodents and humans. The XPA protein in human and rodent cells is involved in the initial step of NER and is likely to be involved in the recognition of DNA damage. As an initial activity in the NER pathway, the expression level of the XPA protein might be rate-limiting for the overall process. Species differences at the repair gene level could then result in selective excision of cyclobutane dimers, and repair differences could therefore be assessed by measuring the expression of the XPA transcript. Using a combination of reverse transcription of total RNA and competitive PCR, the expression levels of XPA in human and mouse cell lines has been examined and quantitatively compared. Our findings establish that the relative expression level of XPA between human and mouse cell lines is statistically indistinguishable.

C5-154 GENETIC AND MOLECULAR CHARACTERIZATION OF THE *DROSOPHILA* HOMOLOG OF THE XERODERMA PIGMENTOSUM GROUP A GENE. Houmam Araj and P. Dennis Smith, Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

Drosophila melanogaster provides a whole-organism model for the analysis of DNA repair and mutagenesis. Recent studies by Shimamoto *et al.* (BBRC 181:1231-1237 (1991)) have identified a *Drosophila* homolog of the human DNA repair gene xeroderma pigmentosum group A (*XPA*).

To determine whether *dmXPA* represents one of the more than thirty *mus/mei Drosophila* DNA repair genes, we have undertaken the cytogenetic localization of the gene. Using *in situ* hybridization of *dmXPA*.cDNA probe (kind gift from T. Shimamoto) to larval salivary gland polytene chromosomes, we have mapped the *dmXPA* gene to the 4A1,2 bands at the distal end of the X-chromosome. This locus lies in the general vicinity of the excision repair gene *mei-9*. However subsequent Northern analysis of eight *mei-9* alleles, combined with other lines of evidence, reveal that *dmXPA* and *mei-9* are highly likely to represent two distinct yet closely linked genes (estimated at be about 0.5 cM apart).

In order to determine the genomic organization of the *dmXPA* gene, including upstream-downstream regulatory sequences along with intron-exon structure, we have cloned the full length genomic *dmXPA* sequence and found it to be less than 3.5 kb in size. We are currently in the process of determining its nucleotide sequence by automated cycle sequencing. In addition we have found that the *dmXPA* gene is expressed as a single polyadenylated transcript of about 1.3 kb that exhibits dosage compensation in adult male flies and is also expressed in the embryonic stage of the fly.

C5-155 CHARACTERIZATION OF MITOMYCIN C-RESISTANT CLONES OF A FANCONI ANEMIA GROUP A CELL LINE TRANSFECTED BY A cDNA EXPRESSION LIBRARY, James A. Hejna, Robb E. Moses, Louise S. Merkens, Thomas V. Tittle, Andrew S. Friedberg and Michael A. Whitney Oregon Health Sciences University, Department of Molecular and Medical Genetics, Portland, Oregon 97201

Fanconi Anemia cells in culture show a marked sensitivity to DNA cross-linking agents such as mitomycin C (MMC) and diepoxybutane (DEB). An immortalized Fanconi Anemia (group A) cell line, GM6914, was transfected with a pCMV-based cDNA expression library from damage-induced human fibroblasts. After selection on G418, approximately 10^6 colonies were subsequently selected on MMC and/or DEB. From a total of 65 independent transfections, 5 independent clones were resistant to both MMC and DEB, and showed normal levels of chromosomal breakage after treatment with these clastogens. Each clone carries at least one cDNA integrated into the chromosome. Recovery of cDNAs was by PCR with vector-specific flanking primers. A 0.6 kb cDNA from cell line 3-D was found to be UbA_{52} in an antisense orientation. The 51-C clone was found to contain the 3' end of a much larger cDNA. The entire 4.7 kb cDNA has now been sequenced, and encodes a novel protein with a putative size of 127 kDa. Northern blot analysis indicates that the gene is expressed in a wide variety of fetal and adult tissues. The cDNA has been mapped to 11q23. Additional cDNAs were recovered from the 5 cell lines by plasmid rescue, and are currently being identified.

DNA Repair II

C5-200 PURIFICATION AND CHARACTERIZATION OF DNA LIGASE III FROM BOVINE TESTES: RELATIONSHIP WITH DNA LIGASE II AND VACCINIA DNA LIGASE. Jingwen Chen[1], Intisar Husain[1], William Burkhart[2], Mary B. Moyer[2], William Ramos[3], Zachary B. Mackey[3], Alan Tomkison[3] and Jeff Besterman[1].
[1]Department of Cell Biology, [2]Department of Bioanalytical and Structural Chemistry, Glaxo Research Institute, Five Moore Drive, Research Triangle Park, NC 27709 and [3]Institute of Biotechnology, Center for Molecular Medicine, The University of Texas Health Science Center at San Antonio, TX 78245.

Mammalian cell nuclei contains three biochemically distinct DNA ligases. In the present study, we have found highest level of DNA ligase I and DNA ligase III in bovine testes and purified the 100kDa DNA ligase III to near homogeneity from testis nuclei. The high level of DNA ligase III in this tissue suggests its potential role in meiotic recombination. In assays measuring the fidelity of DNA joining, we detected no significant differences between DNA ligases II and III whereas DNA ligase I was clearly a more faithful enzyme and was particularly sensitive to 3' mismatches. Amino acid sequences derived from DNA ligase III demonstrated that this enzyme, like DNA ligase II, is highly homologous with vaccinia DNA ligase. Since there were no differences between homologous peptides from DNA ligase II and III (10 pairs of peptides, 136 identical amino acids), it appears that these enzymes are either derived from a common precursor polypeptide or are encoded from the same gene by alternative splicing. Based on similarities in amino acid sequence and biochemical properties, we suggest that DNA ligases II and III, Drosophila DNA ligase II and the DNA ligases encoded by the vaccinia and pox viruses constitute a family of DNA ligases, distinct from the family of replicative DNA ligases, which perform specific roles in DNA repair and genetic recombination.

C5-201 DEFECTIVE REPAIR OF DNA INTERSTRAND CROSS-LINKS IN FANCONI ANEMIA COMPLEMENTATION GROUP A, Muriel W. Lambert, and Kandallu R. Kumaresan, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Fanconi anemia (FA) is a genetic disorder associated with a high incidence of aplastic anemia, congenital abnormalities and a predisposition to develop cancer. FA cells have increased sensitivity to and reduced ability to repair DNA damage produced by DNA interstrand cross-linking agents. We have isolated a chromatin-associated DNA endonuclease complex from the nuclei of normal human cells which recognizes and incises DNA containing interstrand cross-links. We have identified a damage-recognition protein associated with this complex which binds to DNA containing interstrand cross-links produced by psoralen plus UVA light and which is defective in FA, complementation group A (FA-A), cells (Hang et al., Nucleic Acids Res 21:4187, 1993). Another endonuclease complex which incises DNA containing psoralen monoadducts is not defective in FA-A cells. These complexes from normal human and FA-A cells were examined for their ability to endonucleolytically incise DNA containing site-directed psoralen interstrand cross-links and monoadducts. A 136 base pair oligonucleotide was synthesized which contained either a single site-directed monoadduct or interstrand cross-link produced by 4,5',8-trimethylpsoralen (TMP) plus UVA light. Sequence analysis showed that an extract containing both normal complexes produced an incision on both the 3' and 5' sides of the DNA interstrand cross-link and on both sides of the monoadduct as well. The FA-A extract also produced dual incisions at the site of the monoadduct but was defective in ability to incise DNA at the site of the cross-link. These results correlate with the ability of FA-A cells to repair psoralen induced monoadducts and but not interstrand cross-links. Whether in FA-A cells the defect in the ability of the endonuclease to incise DNA at sites of interstrand cross-links and the defect in the damage-recognition protein are associated with the same protein or separate proteins is under investigation.

C5-202 DNA POLYMERASE β RELEASES 5'-TERMINAL DEOXYRIBOSE-PHOSPHATE RESIDUES FROM INCISED ABASIC SITES, Yoshihiro Matsumoto and Kyung Kim, Department of Radiation Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111

DNA polymerase β is involved in base excision repair to correct a variety of modified bases in eukaryotes. In this pathway, the gap-filling reaction by DNA polymerase β follows the incision of abasic sites by class II AP endonuclease and the excision of 5'-terminal deoxyribose-phosphate residues (dRp) by DNA deoxyribophosphodiesterase (dRpase) or dRp lyase. While two bacterial proteins, RecJ and Fpg, have been identified as dRpase and dRp lyase, respectively, no such proteins had yet been isolated as a purified form in eukaryotes. We show here that DNA polymerase β can release the 5'-terminal dRp from the incised abasic sites. The *Xenopus laevis* enzyme which was purified from ovaries and the rat enzyme which was overexpressed and purified from the recJ$^-$ bacterial cells were tested and both were able to carry out this reaction. When double-stranded DNA was used as a substrate, this activity required Mg^{2+} in the reaction and was suppressed by EDTA. The analysis of the released products by Q Sepharose column chromatography in the presence of sodium thioglycolate indicated that this reaction was catalyzed via β-elimination rather than hydrolysis. Therefore it is Mg^{2+}-dependent dRp lyase that is associated with DNA polymerase β and is distinct from the bacterial dRp lyase, Fpg, which is resistant to EDTA and inhibited by Mg^{2+}. Physical association of dRp lyase and DNA polymerase activities on the single polypeptide suggests that the excision and DNA synthesis steps in base excision repair proceed in a coordinated manner. (Supported by NIH grant R29 CA63154.)

C5-203 *ESCHERICHIA COLI* URACIL-DNA GLYCOSYLASE BINDING AND INTERACTION WITH DNA, Samuel E. Bennett, Russ Sanderson, Ole N Jensen, Douglas F. Barofsky and Dale W. Mosbaugh, Departments of Agricultural Chemistry and Biochemistry and Biophysics and the Environmental Health Science Center, Oregon State University, Corvallis, OR 97331.

Escherichia coli uracil-DNA glycosylase (Ung) was photochemically cross-linked to oligonucleotide dT_{20} in order to identify amino acid residues that reside in or near the DNA-binding site. The Ung x dT_{20} complex was purified to apparent homogeneity and matrix-assisted laser desorption-ionization (MALDI) mass spectrometry revealed that Ung was covalently cross-linked to dT_{20} in 1:1 stoichiometry as a 31,477 dalton complex. Several lines of evidence inferred the specificity of Ung x dT_{20} cross-linking: 1) denatured Ung did not cross-link to dT_{20}; 2) increasing NaCl concentration inhibited both enzyme activity and cross-linking efficiency; 3) Ung x dT_{20} did not bind to single-stranded DNA; 4) cross-linking abolished uracil-DNA glycosylase activity; and 5) the addition of the uracil-DNA glycosylase inhibitor (Ugi) protein to Ung eliminated Ung x dT_{20} cross-link formation. Following trypsin digestion of Ung x dT_{20}, four distinct peptide x dT_{20} cross-links were identified. Using a novel strategy which combined amino acid sequencing and MALDI mass spectrometery, four peptides adducted to dT_{20} were unambiguously identified as Ung-tryptic fragments (T6, T18, T19 and T18/19). These peptides appear to define two DNA-binding domains in the primary sequence of Ung corresponding to amino acid residues 58-80 and 185-213. Using a defined double-stranded concatemeric (25-mer units) DNA substrate containing site-specific uracil residues, both *E. coli* and rat liver mitochondrial uracil-DNA glycosylases were shown to utilized a processive mechanism for locating the target substrate. The degree of processivity was observed to decrease with increasing NaCl concentration as expected for an enzyme that scans DNA by electrostatic interactions. (Supported by NIH grants GM32823 and ES00210).

C5-204 DNA BASE EXCISION REPAIR ACTIVITIES IN THE EXTREME THERMOPHILE *THERMUS AQUATICUS*, William A. Franklin and Margarita Sandigursky, Departments of Radiology and Radiation Oncology, Albert Einstein College of Medicine, Bronx, NY 10461

Thermostable bacteria such as *T. aquaticus* must have mechanisms to repair DNA damage expected to occur with growth at increased temperatures. For example, rates of DNA depurination would be expected to be high at 70°C, and these bacteria require an efficient base excision repair mechanism to remove high levels of apurinic/apyrimidinic (AP) sites from DNA. We have examined several components of the DNA base excision repair pathway in these bacteria. Crude cell lysates were prepared from strain YT-1 and we have examined three specific repair activities: uracil-DNA glycosylase, AP endonuclease, and a DNA deoxyribophospho-diesterase (dRpase) activity. Lysates were found to contain each of these activities, and we have further characterized the uracil-DNA glycosylase activity following purification on Superose 12 gel filtration and the dRpase activity following purification on AcA-54 gel filtration and MonoQ. The uracil-DNA glycosylase was found to have a size of ~ 30 kDA; the enzyme showed maximal activity at 70°C, and was active in the absence of divalent cations. The dRpase activity (~50 kDa) was active on M13 DNA substrates containing incised AP sites having either 5' 2-deoxyribose-5-phosphate end-groups produced by treatment with an AP endonuclease (endo IV of *E. coli*) or 3' 4-hydroxy-2-pentenal -5-phosphate end-groups produced by treatment with an AP lyase (endo III of *E. coli*). The dRpase activity was most active in the presence of 2 mM Mn^{++} cation at 70°C at pH 7.8, and the *T. aquaticus* enzyme seems analogous to those found present in *E. coli*. Strategies for the further purification and isolation of the dRpase activity, as well as the isolation of the uracil-DNA glycosylase activity following screening of a *T. aquaticus* genomic library will be described.

C5-205 MAMMALIAN DNA LIGASE I, II AND III, Rachel Nash[1], Keith Caldecott[1], Ying-Fei Wei[2], Peter Robins[1], Emma Roberts[1], Deborah Barnes[1] and Tomas Lindahl[1]. [1] Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U.K., [2]Human Genome Sciences, Inc., MD 20850-3338.

DNA strand breaks generated by DNA damaging agents, or arising as intermediates during DNA replication, DNA excision repair and genetic recombination, are joined (either directly or following local excision and DNA repair synthesis) by the action of a DNA ligase. Three DNA ligases have been identified in mammalian cells. DNA ligase I has been purified and the cDNA cloned. DNA ligase I is the major enzyme of this type in proliferating cells and its main function appears to be the joining of Okazaki fragments during lagging-strand DNA replication. The three DNA ligases have been shown to differ in their biochemical, catalytic and serological properties. Furthermore, radiolabelling of the proteins at their active sites with [α-^{32}P] ATP enabled the products of partial or complete proteolytic digestion to be visualised by autoradiography following SDS-PAGE; DNA ligases II and III were similar to each other when compared by this technique but quite different from DNA ligase I. The role of DNA ligase II in mammalian cells is unclear. Clues as to the physiological role of DNA ligase III have come from its interaction as a heterodimer with the DNA repair protein, XRCC1, and its presence in a protein complex that is active in recombination processes *in vitro*. These data suggest that DNA ligase III is active in both base excision repair and recombination. The interaction between XRCC1 and DNA ligase III has been investigated by deletion analysis and expression of the XRCC1 cDNA *in vitro*, to determine those sequences essential for the binding of DNA ligase III. A cDNA clone encoding DNA ligase III has now been obtained, allowing more detailed investigations of both the interaction with XRCC1, and its role in the cell nucleus.

C5-206 EVIDENCE FOR A REPAIR PATHWAY SPECIFICITY OF DNA LIGATION PROVIDED BY COMET ASSAY ANALYSIS OF DNA BREAK REPAIR IN NORMAL AND DEFICIENT HUMAN CELLS EXPOSED TO RADIATIONS AND CHEMICALS. Silvano Nocentini, URA 1292 du CNRS, Institut Curie, Section de Biologie, 26 rue d'Ulm, 75231 Paris cedex 05, France.

The *in vivo* DNA rejoining ability of two Bloom syndrome cell lines, YBL6 and GM1492, and of a line with a proven defect in DNA ligase I, 46BR, was compared to that of normal human 1BR/3 fibroblasts after treatment of cells with a variety of genotoxic agents whose lesions are processed by different repair pathways. This analysis was performed using the single cell gel electrophoresis assay.

Results indicate that : *i)* the DNA ligase I defect in 46BR cells severely hampers ligation of DNA strand breaks after induction of UV-photolesions but not after γ-rays, X/XO and 4NQO induced damage ; *ii)* GM1492 BS strain has a reduced DNA ligation capacity only after relatively high UV radiation doses ; *iii)* YBL6 BS strain shows altered joining especially after damage by 4NQO ; *iv)* as 46BR cells, BS cells are not particularly affected in the resealing of gaps resulting from the base excision repair process. Thus, these results are in favor of a damage or a repair pathway specificity of DNA rejoining involving different DNA ligase activities in human cells. Also, it appears that the phenotype of BS is heterogeneous from strain to strain and shares only some features with that of 46BR cells.

C5-207 REQUIREMENT OF THE PRO-CYS-HIS-ARG SEQUENCE FOR O6-METHYLGUANINE-DNA METHYLTRANSFERASE ACTIVITY REVEALED BY SATURATION MUTAGENESIS WITH NEGATIVE AND POSITIVE SCREENING, Kenji Ihara, Hisaya Kawate, Kunihiko Sakumi and Mutsuo Sekiguchi, Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812, Japan

O^6-Methylguanine-DNA methyltransferase catalyzes transfer of a methyl group from O^6-methylguanine and O^4-methylthymine of DNA to the cysteine residue of the enzyme protein, thereby repairing the mutagenic and carcinogenic lesions in a single step reaction. There are highly conserved amino acid sequences around the methyl-accepting cysteine site, through eleven molecular species of methyltransferases. To elucidate the significance of the conserved sequence, amino acid substitutions were introduced into the *Escherichia coli ogt* methyltransferase gene by site-directed mutagenesis, and the activity and the stability of mutant forms of enzyme were examined. When cysteine-139, to which methyl transfer occurs, was replaced by other amino acids, all of the mutants carried the methyltransferase-negative phenotype. Methyltransferase-positive revertants, isolated from one of the negative mutants, carried codons for cysteine. Thus, the cysteine residue is essential for acceptance of the methyl group and is not replaceable by other amino acids. Using this negative and positive selection procedure, analyses were extended to other residues near the acceptor site. At the histidine-140 and arginine-141 sites, all of the isolated positive revertants carried codons for amino acids identical to those of wild type protein. At the proline-138, five substitutions (serine, glutamine, threonine, histidine and alanine) exhibited the positive phenotype but levels of methyltransferase activity in extracts of cells harboring these mutants were very low, suggesting that the proline residue at this site is important to maintain conformation of the protein. With valine-142 substitutions there were seven types of positive revertants, among which mutants carrying isoleucine, cysteine, leucine and alanine showed relatively high levels of methyltransferase activity. These results are taken to mean that the sequence Pro-Cys-His-Arg is essential for methyltransferase to exert its function.

C5-208 DEOXYINOSINE 3' ENDONUCLEASE, A NOVEL DEOXYINOSINE-SPECIFIC ENDONUCLEASE IN *ESCHERICHIA COLI*, Min Yao and Yoke W Kow, Department of Microbiology and Molecular Genetics, The Markey Center for Molecular Genetics, University of Vermont, Burlington, VT 05405

Deoxyinosine 3' endonuclease is a novel deoxyinosine-specific endonuclease in Escherichia coli. The enzyme has been purified to near homogeneity. It recognizes deoxyinosine, AP site, urea and mismatches in DNA and cleaves the DNA at the second phosphodiester bond 3' to the lesion in a strand-specific manner. However, the lesion is not removed from the DNA. Using gel retardation and footprinting assay, we found that deoxyinosine 3' endonuclease bound specifically to double-stranded DNA containing deoxyinosine. Two distinct complexes were observed in the non-denaturing polyacrylamide gel; complex I contained one molecule of enzyme per DNA molecule, whereas complex II contained two molecules of enzyme per DNA molecule. The nicking activity was correlated with the formation of complex I. Although the nicking activity of the enzyme required Mg^{2+}, the binding affinity was not dependent on Mg^{2+}. Interestingly, deoxyinosine 3' endonuclease showed similar affinity towards the substrate (the intact deoxyinosine-containing oligodeoxynucleotide duplex) and the product (the nicked deoxyinosine-containing oligodeoxynucleotide duplex). These data suggest that deoxyinosine 3' enodnuclease also function as a lesion specific binding protein. Since the enzyme binds to DNA both before and after cleavage, no turn-over of the enzyme activity was observed. However, the enzyme could not form stable complex with DNA containing deoxyinosine if the DNA contains a nick immediately or one base 5' to deoxyinosine. It is possible that other protein(s) is required to introduce a 5' nick to the lesion, thus helping the dissociation of deoxyinosine 3' endonuclease from duplex DNA.

C5-209 MUTATIONS IN HUMAN O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE IMPARTING RESISTANCE TO O^6-BENZYLGUANINE AND EFFECTING BINDING TO DNA, Karina Goodtzova, Tina M. Crone, Sreenivas Kanugula, Suvarchala Edara and Anthony E. Pegg, Department of Cellular and Molecular Physiology and of Pharmacology, Milton S.Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA 17033

O^6-Benzylguanine (O^6bG) is a potent inhibitor of the human O^6-alkylguanine-DNA alkyltransferase (AGT) which acts as a low molecular weight substrate for the protein. It's currently entering clinical trials as an agent improving the cancer chemotherapeutic ability of alkylating agents. Human AGT as well as AGTs from other mammalian species have been shown to be sensitive to O^6bG. In contrast microbial AGTs are very resistant to O^6bG. The striking difference between the mammalian and microbial AGTs in their sensitivity to inactivation by O^6bG is quite unexpected since there is a significant similarity in these proteins in general and particularly in the sequence surrounding the cysteine acceptor site. This fact raises the possibility that some mutations in the sequence of human AGT could lead to the protein becoming resistant to O^6bG. Mutations in the human AGT sequence (Δ1-19, Δ1-10, Δ92-97, Y114E, R128A, P138A, P138K, P138A/P140A, P138K/P140A, P140A/G156A, G156A, G156W, E172Q, E172stop, G177stop, S185stop, P140A/S185stop) have been made in order to study the ability of recombinant proteins to react with O^6bG or methylated DNA substrate. The results showed that mutations P138A, P140A and G156A significantly reduced the ability to react with O^6bG. The combination of the mutations gave even greater effect. Truncation of the AGT at the position of either G177 or S185 did not affect the ability to react with O^6bG. The removal of the 35 carboxyl terminal amino acid (E172 stop) or mutation E172Q led to the loss of all activity. The results show that alterations of amino acids in region on either side of the active site sequence can lead to a change in the ability to react with O^6bG and on the stimulation of this reaction by DNA. Mutations R128A and Y114E produced an AGT that was able to react with O^6bG at a normal rate but was inactive with methylated DNA substrate. This fact indicates that the region in which R128 and Y114 are located could be involved in the DNA binding.

C5-210 REPAIR OF ABASIC SITES BY MAMMALIAN CELL EXTRACTS, E. Dogliotti[1], P. Fortini[1], O. Rossi[2], F. Carrozzino[2], A. Abbondandolo[2] and G. Frosina[2]. [1]Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanita', 00161 Roma, Italy; [2]Laboratory of CSTA, Mutagenesis, Istituto Nazionale per la Ricerca sul Cancro, 16132 Genova, Italy.

Abasic sites (apurinic/apyrimidinic, AP sites) are the most common DNA lesions generated by both spontaneous and induced base loss. In a previous study we have shown that circular plasmid molecules containing multiple AP sites are efficiently repaired by Chinese hamster extracts in an in vitro repair assay. An average patch size of 6.6 nucleotides for a single AP site was calculated (Biochem. J., 1994, in press). To define the exact repair patch, one single AP site was built into a sequence of pGEM-3Zf(+) by priming single stranded plasmid DNA with an oligonucleotide containing a single uracil residue. The circular duplex DNA was treated with uracil-DNA glycosylase immediately before the repair reaction to leave a natural AP site. The repair synthesis carried out by hamster cell extracts was determined by restriction enzyme analysis of the area containing the lesion. The results indicate that, besides the repair events involving the incorporation of a single nucleotide at the lesion site, repair synthesis also occurs 3' to the AP site and involves the incorporation of 6-9 nucleotides. Whether nucleotide incorporation occurs 5' to the AP site is currently under investigation. An SV-40 derived shuttle vector containing a single abasic site located in the same sequence used for repair studies has been constructed. This vector will be used to investigate the mutagenic properties of this lesion following repair and replication in mammalian cells.

C5-211 STRUCTURE, EXPRESSION AND CHROMOSOMAL ASSIGNMENT OF THE MAMMALIAN MAJOR AP ENDONUCLEASE (APEX) GENE, Shuji Seki*, Kosuke Akiyama*, Sekiko Watanabe*, Ken Tsutsui*, Tatsuo Oshida** and Michihiro C. Yoshida**, *Department of Molecular Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700, and **Chromosome Research Unit, Faculty of Science, Hokkaido University Sapporo 060, Japan

APEX nuclease (APEX gene product) is the mammalian major AP endonuclease, possibly involved in repair of AP sites and single-strand breaks with 3'-blocked termini and also in transcriptional regulation via redox activation of the AP-1 transcription factors. Its gene has also been called HAP1, APE and Ref-1. We cloned the APEX gene for mouse as well as human, and determined the gene structure. The gene is a relatively small gene consisting of 5 exons and 4 introns and having multiple transcription initiation sites. A region extending from a 5' flanking region to a position in the intron II belongs to a CpG island which contains putative binding sites for several transcription factors (such as ATF, NF-IL6, Sp1 and AP2) and a promoter region. The APEX gene locus was mapped to the 14C2-D1 and 14q11.2-q12 on mouse and human chromosomes, respectively. To investigate the biological roles of APEX nuclease, antisense APEX RNA was expressed at a high level in cultured rat glioma cells. The cells with antisense RNA expression showed extremely lower AP endonuclease activity compared to the control cells, and they were more sensitive to methyl methanesulfonate and hydrogen peroxide compared to the control cells. The results support the notion that APEX nuclease is involved in vivo in repair of AP sites and oxidative DNA damage.

C5-212 DETECTION OF A LARGE GENOMIC REGION THAT IS PREFERENTIALLY REPAIRED IN NORMAL HUMAN CELLS, G.A. Shanower and G.J. Kantor, Department of Biological Sciences, Wright State University, Dayton, OH 45435
Xeroderma pigmentosum complementation group C (XP-C) cells exhibit little DNA excision-repair activity after exposure to ultraviolet light (UV; 254nm). Only 5-10% of the genome is repaired after UV insult. Most of the repair takes place in large DNA regions which are at least 30-80 kb in size and contain transcriptionally active genes. The remainder of the XP-C genome is not repaired. We propose that the same large regions, referred to as repair domains, are preferentially repaired relative to the genome overall in normal human cells. Our evidence suggests that the small ß-actin gene (3.5 kb) is preferentially repaired as a large DNA region relative to the rest of the genome in normal human cells. Detection of domain-repair in normal cells involves using a sensitive assay employing denaturing electrophoresis of UV-endonuclease digested genomic-DNA from UV-irradiated normal cells. The electrophoresed DNA is subsequently southern transferred and hybridized to several DNA probes of interest. This assay detects the rapid repair of a large genomic region containing the ß-actin region relative to other genetic markers (the 754 locus and the insulin genetic region) and the genome overall.

C5-213 SEPARATION OF EXONUCLEASE ACTIVITY AND DNA REPAIR FUNCTIONS IN *REC1* OF *USTILAGO MAYDIS*.
Michael P. Thelen, Kenan Onel, Richard L. Bennett and William K. Holloman, Department of Microbiology, Cornell University Medical College, New York City 10021
The *REC1* gene of *Ustilago maydis* encodes an exonuclease with enzymatic properties resembling those of the 3'→5' exonuclease activity of the ε subunit of *E. coli* DNA Polymerase III. The exonuclease activity of proteins derived from mutants in the *REC1* gene was investigated. This study was enabled by establishing conditions for solubility of the overexpressed *REC1* gene product. The mutants analysed included a series of deletions constructed by removing restriction fragments at the 3' end of the cloned *REC1* gene and a set of mutant alleles that had been previously isolated in screens for radiation sensitivity. Members of the latter set were determined by DNA sequence analysis to be caused by mutations that introduced premature termination codons within the *REC1* gene. All of these C-terminal deletion mutants were overproduced in *Escherichia coli* as N-terminal polyhistidine-tagged proteins that were subsequently purified by immobilized metal affinity chromatography and assayed for 3'→5' exonuclease activity. The results indicated that elimination of the C-terminal third of the protein did not result in a serious reduction in 3'→5' exonuclease activity, but deletion into the midsection caused a severe loss of activity. Biological activity of the *rec1-1* allele, which encodes a truncated polypeptide with full 3'→5' exonuclease activity, and the *rec1-5* allele, which encodes a more severely truncated polypeptide with no exonuclease activity was investigated. Both mutants were equally sensitive to the lethal effect of ultraviolet light, but spontaneous mutation rate as measured by forward mutation to hygromycin B resistance was different. It was elevated 10-fold over wild type in the *rec1-1* mutant, and 100-fold in *rec1-5*. The elevated spontaneous mutation rate correlated with the ablation of exonuclease activity. The C-terminal portion of the Rec1 protein is not essential for exonuclease activity but is crucial in the role of *REC1* in DNA damage repair. This region is conserved with the *rad1* protein of *S. pombe* and includes a stretch of evenly spaced leucines that we postulate to function in a multi-protein repair complex.

C5-214 METHYL EXCISION REPAIR DEFICIENCY (MER⁻ PHENOTYPE) INCREASES WITH AGE IN THE NORMAL BRAIN OF BRAIN TUMOR PATIENTS. John R. Silber and Mitchel S. Berger, Department of Neurological Surgery, University of Washington, Seattle, WA 98195

O^6-methylguanine-DNA methyltransferase (MGMT) lowers the incidence of alkylation-induced mutations by removing promutagenic alkyl adducts from the O^6 position of guanine. To determine if reduced or absent MGMT activity is associated with tumorigenesis in human brain, we quantitated the MGMT content in histologically normal brain overlying primary brain neoplasms in 77 patients. Approximately 55% of normal brain specimens had no detectable activity (< 0.5 fmol/10^6 cells, i.e., < 300 molecules/cell) and were designated Mer⁻ (Methyl repair deficient). The incidence of Mer⁻ brain was 3-fold higher (63% vs. 21%) in adults (patients older than 20) than in children, resulting in a lower mean MGMT activity for adults (1.8 ± 0.5 vs. 5.0 ± 2.2 fmol/10^6 cells, $P < 0.01$; Mer⁻ specimens assigned a value of 0.25 fmol/10^6 cells). The incidence of Mer⁻ status showed pronounced age dependence, increasing from 21% for patients 20 years and younger to 77% for patients 60 years and older. In contrast, we found no age dependent difference in lactate dehydrogenase activity. Moreover, the incidence of Mer⁻ specimens and mean MGMT levels did not differ in the lymphocytes of adults and pediatric brain tumor patients. Our results demonstrate that histologically normal brain of adult brain tumor patients frequently lacks detectable MGMT activity and suggest that this deficiency may contribute to carcinogenesis. This hypothesis is supported by our finding that the incidence of Mer⁻ normal brain in 41 adult patients operated for epilepsy is lower (38%) than that observed for normal brain in brain tumor patients (63%) of comparable age.

C5-215 REPAIR OF URACIL-CONTAINING DNA *IN VITRO*: PURIFICATION OF REQUIRED COMPONENTS FROM A HUMAN CELL EXTRACT. Mark K. Kenny and Karin Nealon. The Picower Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030.

Although certain proteins have been implicated in base excision repair of uracil-containing DNA, the precise set of proteins and mechanisms involved is unknown. We are using HeLa cell extracts and a duplex oligonucleotide containing a single uracil residue in an *in vitro* assay to measure uracil-dependent repair synthesis. This assay has been optimized and streamlined to permit the fractionation of the extract into two required components. One of these components has been extensively purified and copurifies with DNA polymerase β activity. Furthermore, recombinant DNA polymerase β and *E. coli* DNA polymerase I can substitute for this fraction in the DNA repair assay. Additional information on the proteins and mechanisms involved in base excision repair will also be presented.

C5-216 THE TOXICITY OF SEQUENCE AND GROOVE SPECIFIC ALKYLATING AGENTS IN WILD TYPE *E. COLI* AND REPAIR DEFECTIVE MUTANTS,
Barry Gold[1], Yi Zhang,[1] Jan Williamson[1] and Jacques Laval[2], [1]Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE 68195-6805 and [2]Unité de Biochemie et Enzymolgie, Institut Gustave-Roussy, 94805 Villejuif Cedex, France
The preparation of sequence and groove specific DNA methylating agents based on N-methylpyrrolecarboxamide subunits appended with a methyl methanesulfonate (**MMS**) functionality has been described. In contrast to simple methyl sulfonate esters that predominantly react with N7-guanine, these minor groove binding methylating agents afford *in vitro* almost exclusively N3-methyladenine (**N3-mA**) lesions. This makes them potentially useful reagents to generate DNA targets with highly specific damage. To understand if the *in vivo* reactivity of these sequence specific alkylating agents would mimic that observed *in vitro*, they were tested in wild type *E. coli* and in strains that are defective in the expression of *tagA* and/or *alkA* glycosylases. The data show that: (1) the methylating agents that equilibrium bind in the minor groove of DNA inhibit growth by 2-orders of magnitude more than **MMS;** and (2) the *alkA- tagA-* double mutant show the same 100-fold enhanced sensitivity over the *tagA-* mutant. The results clearly demonstrate the importance of the **N3-mA** lesion in toxicity and the role of the inducible *alkA* gene product in the repair of this lesion. Research supported by NIH/NCI and American Cancer Society.

C5-217 GENE SPECIFIC REPAIR OF O-ALKYLATION PRODUCTS IN MAMMALIAN CELLS: RELEVANCE FOR MUTATION INDUCTION AND CARCINOGENESIS.
Jürgen Thomale, Jörg Engelbergs and Manfred F. Rajewsky, Institute of Cell Biology, West German Cancer Center, University of Essen, D-45122 Essen, Germany
We have applied a highly sensitive immunoanalytical procedure (**ALISS**-assay) to measure formation and repair rates of mutagenic alkyl adducts in single copy genes. After pulse exposure to N-ethylnitrosourea O^6-ethylguanine (O^6-EtlGua) was induced more frequently in and eliminated faster from transcriptionally active sequences of rat cells compared to silent genes or bulk genomic DNA. Initial repair rates ($t_{50\%}$) differed intragenomically by a factor of 12 in cell lines and up to 50 in cells of different rat tissues. The influence of overall and sequence specific repair of O^6-alkylguanines on tumor induction and mutation frequencies in tumor DNA is analyzed in normal and AGT-transgenic Sprague Dawley rats.

Ref.: Thomale, J., Hochleltner, K., Rajewsky, M.F. (1994) J. Biol. Chem., **269**:1681-1686

C5-218 **MAMMALIAN DNA LIGASE II IS HIGHLY HOMOLOGOUS WITH VACCINIA DNA LIGASE; IDENTIFICATION OF THE DNA LIGASE II ACTIVE SITE FOR ENZYME-ADENYLATE FORMATION.**
Alan E. Tomkinson†, Yi-Chun J. Wang†, William A. Burkhart‡, Zachary B. Mackey†, Mary B. Moyer‡, William Ramos†, Intisar Husain#, Jingwen Chen#, and Jeffrey M. Besterman#
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Mammalian cells contain three biochemically distinct DNA ligases. One of these enzymes, DNA ligase I, has been extensively studied. DNA ligase I is required to join Okazaki fragments during DNA replication and is also involved in some forms of DNA repair. Considerably less is known about DNA ligases II and III. Despite biochemical and immunological evidence, it has been suggested that these enzyme activities may be generated from DNA ligase I by proteolysis during purification. In this report we describe the purification of DNA ligase II to homogeneity from bovine liver nuclei. This enzyme interacts with ATP to form an enzyme-AMP complex, in which the AMP moiety is covalently linked to a lysine residue. An adenylylated peptide from DNA ligase II contains the sequence, Lys-Tyr-Asp-Gly-Glu-Arg, which is homologous to the active site motif conserved in ATP-dependent DNA ligases. The sequences adjacent to this motif in DNA ligase II are different from the comparable sequences in DNA ligase I, demonstrating that these enzymes are encoded by separate genes. The amino acid sequences of 15 DNA ligase II peptides exhibit striking homology (65% overall identity) with vaccinia DNA ligase. These peptides are also homologous (31% overall identity) with the catalytic domain of mammalian DNA ligase I, indicating that the genes encoding DNA ligases I and II probably evolved from a common ancestral gene. Since vaccinia DNA ligase is not required for DNA replication but influences the ability of the virus to survive DNA damage, the homology between this enzyme and DNA ligase II suggests that DNA ligase II may be involved in DNA repair.

C5-219 COMPARISON OF RATES OF EXCISION OF MAJOR UV PHOTOPRODUCTS IN THE *HPRT* GENE OF NORMAL AND XERODERMA PIGMENTOSUM VARIANT CELLS.
Beatrice S. Tung, W. Glenn McGregor, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University. East Lansing, MI
Xeroderma pigmentosum variant (XP-V) patients are predisposed to sunlight-induced skin cancer, and their fibroblasts are extremely sensitive to mutations induced by UV radiation (simulated sunlight or UV_{254nm}). However, XP-V cells have normal rates of nucleotide excision repair of cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidones (6-4's) in their overall genome (Wang et al., MCB 13:4276, 1993). To determine if this UV hypermutability reflected abnormally slow excision repair of either of these two types of UV photoproducts in the target gene for the mutagenesis studies, i.e., hypothanine(guanine)phosphoribosyltransferase *(HPRT)*, we prepared synchronized populations of XP-V fibroblasts and normal fibroblasts, irradiated them in early G_1-phase, 12 or more hr prior to the scheduled onset of S phase, harvested them immediately or after allowing various times for repair, and analyzed the DNA for photoproducts in the *HPRT* gene, using Southern blotting. To incise the DNA at CPD's, we employed T4 endonuclease V; to incise at 6-4 photoproducts, we first used photolyase and UV_{360nm} to reverse CPD's and then incised the DNA using UvrABC excinuclease. Both the XP-V and the normal cells showed rapid, preferential, strand-specific excision of CPD from the *HPRT* gene. After 6 hr for repair, 63% of the CPD had been excised from the transcribed strand; 46% from the nontranscribed strand. After 12 hr, these values were 89% and 63%, respectively. For the genome overall, these values would be 20% and 40%, respectively. Both kinds of cells showed extremely rapid excision repair of 6-4's from the *HPRT* gene. After 1 hr, 60% were excised from either strand. After 2 hr of repair, 90% had been excised from either strand. From the genome overall, these values would be 50% and 70%, respectively. These results indicate that slower repair rates in the target gene for mutagenesis cannot explain the UV hypermutability of the XP-V cells. (This research was supported by DHHS Grants CA56796 and CA21253 from the National Cancer Institute, and by Grant CA01747 to WGM.)

C5-220 PURIFICATION OF HUMAN PROTEINS INVOLVED IN CISPLATIN-DNA REPAIR, John J. Turchi and Karen M. Henkels, Department of Biochemistry and Molecular Biology, Wright State University, Dayton, OH 45435

Four proteins have been purified from HeLa cells based on their ability to bind cisplatin damaged DNA. Chromatography of crude cell extracts on a cisplatin-DNA-cellulose affinity column revealed two distinct and separable cisplatin-DNA binding activities, a DNA dependent ATPase and a DNA helicase. The two binding activities, termed DRP-1 and DRP-2, were purified by a five column process. A sedimentation coefficient of 7.8 and a stokes radius of 51.5 Å correspond to a native molecular mass of 166 kDa for DRP-1. SDS PAGE of purified DRP-I revealed polypeptides of 95 and 39 kDa suggesting DRP-I is a heterodimer. DRP-I binds to undamaged DNA and cisplatin damaged DNA with equal affinities but displays a marked increase in binding to UV damaged DNA dependent on the extent of UV exposure. These characteristics are consistent with DRP-1 being the XPE binding factor. The structural characterization of DRP-2 is underway. The DNA binding activity of DRP-2, unlike DRP-1, specifically recognizes cisplatin modified DNA and does not bind undamaged DNA or UV damaged DNA. Purification of the DNA dependent ATPase to apparent homogeneity revealed a heterodimeric protein comprised of 70 and 38 kDa subunits. ATPase activity was severely inhibited when cisplatin damaged single or double stranded DNA is used as a cofactor and only marginally inhibited by UV damaged DNA.

The DNA helicase activity identified uses ATP as an energy source to support translocation in the 3' to 5' direction and DNA displacement. Preliminary results suggest that binding of DRP-1 to cisplatin damaged DNA inhibits helicase catalyzed displacement of the damaged primer strand. Additional results will be presented detailing the interactions of these proteins on cisplatin damaged DNA substrates.

C5-221 **HOST-CELL REACTIVATION OF A SEAP REPORTER GENE INTRODUCED INTO CELLS BY ADENOVIRUS AS A CONVENIENT WAY TO MEASURE CELLULAR DNA REPAIR**, Kristoffer Valerie and Arun Singhal, Department of Radiation Oncology Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0058

In order to conveniently measure cellular DNA repair in immortalized and primary human cells we have combined the features of high cellular infectivity of adenovirus (Ad) with that of host-cell reactivation (HCR) of ultraviolet light (UV)-damaged reporter genes. We show that an Ad having the *seap* (secreted alkaline phosphatase) reporter gene under control of a strong constitutive promoter can be used to measure relative levels of DNA repair by HCR. Most importantly, the SEAP assay allows for a convenient, inexpensive, and sensitive colorimetric microtiter assay. Only a few steps are involved and it is possible to process many samples simultaneously in a relatively short time, which is not as easily done with other reporter gene assays. Furthermore, we show that co-infection of UV-damaged SEAP Ad with an Ad carrying a prokaryotic repair gene significantly increased the HCR levels in xeroderma pigmentosum (XP) cells. The Ad gene delivery system, and the SEAP assay in particular, should simplify existing HCR assays considerably and may allow for the screening/testing of clinical specimen in a highly efficient way. By using non-lytic Ad as a vehicle it should be possible to quantitatively introduce normal or dominant negative mutant DNA repair genes transiently into bulk cell populations. Quantitative transfer is necessary for DNA repair studies which required homogenous cell populations, such as cell survival.

C5-222 SIMILAR SEQUENCE-SPECIFICITY OF BINDING OF BENZO(A)PYRENE DIOL EPOXIDE TO FREE DNA , CHROMATIN DNA AND CHROMOSOME DNA, Dong Wei, Veronica M. Maher and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824

Benzo(a)pyrene diol epoxide (BPDE) is a mutagenic metabolite of the environmental pollutant benzo(a)pyrene. To determine the effect of chromatin and chromosome structures on the spectrum of the binding of BPDE to DNA in vivo, we synchronized normal human fibroblasts by releasing them from density inhibition (G_0), and treating them with 13 μM BPDE in early G_1 phase, at the onset of S phase, in G_2 phase or during mitosis, i.e., 5 hr, 17 hr, 25 hr and 30 hr after release. Cells synchronized with aphidicolin, which stops them at G_1/S border, or with colcemid, which stops them at metaphase, were also treated with 13 μM BPDE in early S phase or M phase respectively. DNA was extracted and the distribution of BPDE adducts in the nontranscribed strand of exon 3 of the hypoxanthine phosphoribosyltransferase gene was analyzed using Ligation Mediated PCR. DNA was digested with E. coli UvrABC excinuclease to excise the BPDE adducts, and a gene specific primer was annealed and extended with Sequenase 2.0 to generate a blunt end at the site of each cut. A linker was ligated to the blunt end, and the desired fragments were amplified by PCR and analyzed on a sequencing gel. The distribution of fragments of particular lengths indicated the relative number of cuts generated at those sites by UvrABC and therefore the relative number of BPDE adducts formed at those sites. The UvrABC cutting patterns from all six samples were very similar to that generated from the free DNA treated with BPDE in vitro. These results indicated that the sequence-specificity of binding of BPDE to DNA in vivo is determined mainly by local sequence context instead of nucleosomal structures or condensed chromosomal structures. (Supported by DHHS grants CA56796 and CA21253 from the National Cancer Institute.)

C5-223 CHARACTERIZATION AND IMMUNODETECTION OF MOUSE METHYLTRANSFERASE FOR DNA REPAIR, Hisaya Kawate, Kenji Ihara, Kohfuku Kohda, Akiko Shiraishi, Kunihiko Sakumi and Mutsuo Sekiguchi, Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812, Japan

O^6-methylguanine-DNA methyltransferase (MGMT) is a specific repair enzyme for alkylated DNA and protects cells from mutagenic and tumorigenic effects of alkylating agents. The enzyme transfers alkyl groups from O^6-methylguanine and other alkylated moieties of DNA to its own molecule. For investigating the role of MGMT in preventing tumors, we are constructing MGMT-deficient mice by gene targeting. As a first step, we isolated genomic DNA for mouse methyltransferase from 129SV genomic DNA library. The gene was composed of 5 exons and spanned over 150 kb. Sequences around the exon/intron junctions were very similar to those of the human gene. To obtain better understanding for mouse methyltransferase protein, it was overproduced in *E.coli* and was purified to a homogenous state. The purified mouse MGMT was used to prepare polyclonal antibodies, which was used for the estimation of the amount of the protein in culture cells and tissues. We calculated that a single cell of NIH3T3 contained 1.8×10^4 molecules of the protein and found that mouse methyltransferase exists most abundantly in the liver. Affinity of the purified enzyme with methylated bases was examined by using double-stranded oligomers containing a single O^6-methylguanine or O^4-methylthymine at the predetermined sites. The enzyme was inactivated by oligomers containing O^4-methylthymine as well as those with O^6-methylguanine. To confirm that the mouse MGMT protein can suppress transition mutations derived from these alkylated bases, we used LacZ reversion system and found that MNNG-induced A:T to G:C transitions mutation, as well as G:C to A:T transition mutations, were efficiently suppressed by mouse MGMT protein.

C5-224 OXIDATIVE DAMAGE & PREFERENTIAL REPAIR WITH CELLULAR SENESCENCE, Nicholas J. Rampino, California Institute of Biological Research, 11099 N. Torrey Pines Rd, La Jolla, CA 92037

Oxidative damage and preferential repair in young and senescent human diploid fibroblasts exposed to IC_{50} level H_2O_2 is measured by a new assay. Single-stranded DNA, capable of hybridizing to gene specific probes, is generated enzymatically by the 3'-5' exonucleolytic procession of T4 DNA polymerase. Oxidative lesions inhibit this enzyme, and decrease the amount of complementary sequence produced, when assayed by gene specific probe hybridization. With the progression of repair, increasing quantities of single stranded DNA become available for probe hybridization.

Levels of oxidative damage and repair in DUG, a human mismatch repair homolog, was measured. It was found that (I) After a 50 µM H_2O_2 exposure, senescent human diploid fibroblasts suffer a higher level of DNA oxidation, and repair their DUG gene less efficiently, (II) Without any H_2O_2 treatment, DNA from senescent cells contains more T4 DNA polymerase blocking lesions.

This difference in DNA oxidation, and lower level of preferential repair agrees with the finding that senescent cells are more sensitive to oxidative stress than young, based on cell viability by trypan blue exclusion.

C5-225 PURIFICATION AND CHARACTERIZATION OF (6-4)PHOTOPRODUCT PHOTOREACTIVATING ENZYME, Takeshi Todo, Haruko Ryo and Taisei Nomura, Department of Radiation Biology, Faculty of Medicine, Osaka University, Osaka 565, JAPAN

Pyrimidine(6-4)pyrimidone photoproduct is the second most abundant UV photoproduct in DNA. We have found that an enzyme that catalyze the light-dependent repair of (6-4)photoproduct exist in *Drosophila melanogaster* and have purified the enzyme near homogeneity. (6-4)photoreactivating enzyme react with (6-4)photoproduct induced at TT,TC and CC site. After phototreatment, UV-irradiated DNA was converted to be digestable with restriction enzyme and templating activity of UV-irradiated DNA for elongation with DNA polymerase was restored *in vitro* and *in vivo*. These results indicate that the enzyme repairs (6-4) photoproducts to original unmodified form.

C5-226 CHARACTERIZATION OF THE PROMOTER REGION OF THE HUMAN APURINIC ENDONUCLEASE GENE. L.Harrison, A.G. Ascione, D.M Wilson III, B.Demple. Harvard School of Public Health, Boston, MA 02115.

Apurinic/apyrimidinic (AP) sites, which are generated in DNA by metabolic by-products and environmental DNA-damaging agents, are mutagenic and block DNA synthesis in vitro. Repair of AP sites is thought to be initiated by enzymes such as the human apurinic endonuclease (Ape), which cleaves on the immediate 5' side of the damage.

Previously we isolated a 14-kb genomic DNA fragment that contains the 3-kb human apurinic endonuclease gene (*APE*). A 4.1-kb *Hin*dIII DNA fragment from the region upstream of *APE* and containing approximately 65-bp of the untranslated exon was ligated into a vector containing the chloramphenicol acetyl transferase (CAT) gene. Deletion into this fragment generated constructs containing sections of the *APE* upstream region varying in size from 4.1-kb to 50-bp. Transient transfection studies have established that the basal *APE* promoter is contained within a 500-bp fragment. Deletion of sequences to within 10-bp of the putative CCAAT box reduced the CAT activity to 46%, and the removal of the CCAAT box region from the 500 bp fragment only reduced the promoter activity to 9% of the maximum level. To completely abolish promoter activity it was necessary to delete a 154 bp fragment encompassing the CCAAT box. Sequence analysis of this region of DNA identified a number of putative transcription factor recognition sites, including those for Sp1 and human upstream factor (USF). Sp1 and USF are expressed ubiquitously in human tissue, as has been found for Ape. Gel mobility-shift assays showed that both USF and Sp1 are capable of binding their respective sites in the *APE* promoter. However, DNase I footprinting using HeLa nuclear extract showed that the binding of Sp1 and USF is abrogated by the binding of proteins to the CCAAT box region.

C5-227 FANCONI ANEMIA COMPLEMENTATION GROUP C (*FACC*) CARRIER TESTING AND GENETIC MAPPING Arleen D. Auerbach and Peter C. Verlander, The Rockefeller University, New York, NY 10021

Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive disorder defined by hypersensitivity of cells to DNA cross-linking agents; a gene for complementation group C (*FACC*) has been cloned and mapped to chromosome 9q22.3 by *in situ* hybridization. Two common mutations, IVS4 +4 A→T and 322delG, and several rare mutations have recently been reported in affected individuals (Verlander et al., 1994). We now report the development of amplification refractory mutation system (ARMS) assays for rapid, non-radioactive detection of these known mutations in *FACC*. We have used these assays for detection of *FACC* mutations in affected individuals in the International Fanconi Anemia Registry (IFAR), and for carrier detection in *FACC* families, enabling us to provide improved genetic counseling to relatives of FA patients. In addition, DNA samples from 2404 healthy Jewish individuals primarily of Ashkenazi ancestry were supplied to us by Dor Yeshorim. These samples were ascertained for carrier screening for genetic diseases with a high frequency in the religious Jewish community served by this organization. Samples were tested for both IVS4 +4 A→T and 322delG mutations; 27 IVS4 +4 A→T carriers were identified, for a carrier frequency of ~1%, while no 322delG carriers were found in this population. Two of these individuals who are carriers for IVS4 +4 A →T are of Sephardic Jewish ancestry. We hypothesize that IVS4 +4 A→T is a very old mutation, predating the divergence of the Ashkenazi and Sephardic populations. We have also analyzed six microsatellite markers and one RFLP on chromosome 9q in a panel of FA families from the International Fanconi Anemia Registry (IFAR) in order to place *FACC* on the genetic map. Polymorphisms were typed in 293 individuals from 49 families. *FACC* maps to a 19.2 cM interval defined by the markers D9S287, D9S12, D9S151, and D9S196. Two point lod scores between an *Eco*RI polymorphism in intron 11 in *FACC* and the microsatellite markers show that *FACC* is tightly linked to both D9S151 (θ_{max} = 0.02, Z_{max} = 10.23) and to D9S196 (θ_{max} = 0.07, Z_{max} = 7.45); multipoint analysis confirmed this location. Haplotype analysis excluded 31 families from complementation group C. These families are being used in linkage studies to localize other FA complementation groups.

C5-228 ISOLATION AND PARTIAL CHARACTERIZATION OF MAMMALIAN POLYDEOXYNUCLEOTIDE KINASE, A POTENTIAL DNA REPAIR ENZYME, Feridoun Karimi-Busheri and Michael Weinfeld. Radiobiology, Cross Cancer Institute, Edmonton, T6G 1Z2, Canada.

Included among the large variety of DNA lesions generated by ionizing radiation are DNA strand breaks with either 3'-phosphate or 5'-hydroxyl termini, or both. During the course of repair, the 3'-termini must be restored to 3'-OH while the 5'-termini must be phosphorylated in order to allow DNA repair synthesis and ligation. Polydeoxynucleotide kinase (PNK) is an enzyme that may fulfil both of these functions.

Activity gels of kinase activity in nuclear extracts from calf thymus (CT), rat liver (RL) and human placenta (HP) indicated that the major activities in CT have molecular weights of approximately 40 and 30 kDa, while those in RL are ~60, 40 and 30 kDa, and that in HP only the 30 kDa protein is present. However, following chromatographic purification, we isolated a 60 kDa DNA kinase from calf thymus and a 40 kDa kinase from rat liver, based on SDS-PAGE electrophoresis.

A comparison of a 19-amino acid N-terminal sequence for the calf thymus enzyme to other peptide sequences indicated that the enzyme has not been previously sequenced. However, there was reasonable homology with peptide sequences within several proteins, the strongest being a 9-amino acid overlap with *wis1*-protein kinase, a regulator of mitosis in *Schizosaccharomyces pombe*. We have also examined the minimum size of oligonucleotide (generated by micrococcal nuclease digestion of calf thymus DNA) that can act as a substrate. Both the rat and bovine enzymes were able to phosphorylate oligomers as short as 7-8 nucleotides in length, but the optimal length appeared to be ~ 24 nucleotides. With the same substrate, the optimal oligonucleotide length for T4 phage polynucleotide kinase was ~12.

This work was supported by a grant to MW from the Medical research Council of Canada.

C5-229 SIMULTANEOUS DETECTION OF DNA DAMAGE, REPAIR AND MUTATIONS IN A NEW TRANSGENIC MOUSE MODEL, Michaël E.T.I. Boerrigter, Hans-Jörg Martus, Martijn E.T. Dollé, Jan A. Gossen and Jan Vijg, Division on Aging, Harvard Medical School & Gerontology Division, Beth Israel Hospital, Boston, MA 02215
The capacity to correctly restore the original DNA structure after induction of DNA lesions in target genes is of paramount importance in preventing the accumulation of mutations that may give rise to gene dysfunctioning. We have constructed a transgenic mouse model carrying multiple copies of a LacZ marker gene-containing plasmid integrated in the genome. This model makes it now possible to study both DNA repair and the induction of a broad spectrum of mutations, including large deletions, in the same animal and in the same organ after treatment with low doses of a particular DNA-damaging agent. The plasmid-based shuttle vectors are integrated in a head-to-tail arrangement in all organs and tissues, including the germ cells, and can be isolated from genomic DNA with high efficiency. The capacity of this model to detect both point mutations and large deletions was verified by analyzing mutations in different organs from the mouse following whole-body X-ray irradiation. Using this system we have also monitored the induction and repair of benzo[a]pyrene DNA-adducts. Some time-course and dose-response data will be presented. This system will allow us to obtain a greater insight in the relationship between the rate of mitogenesis in different organs, DNA damage, its removal and the mutational endpoint in a particular organ or tissue.

C5-230 IDENTIFICATION OF A LARGE GENOMIC REGION IN UV-IRRADIATED HUMAN CELLS WHICH HAS A LOW FREQUENCY OF PYRIMIDINE DIMERS. George J. Kantor and Dawn Deiss-Tolbert, Department of Biological Sciences, Wright State University, Dayton, OH 45435
Size separation after UV-endonuclease digestion of DNA from UV-irradiated human cells using denaturing conditions fractionates the genome based on pyrimidine dimer content. We have examined two fractions, one containing the average size DNA and one containing the largest DNA, for content of some specific genes. The concentrations of the β-actin, p53 and β-globin genes and the 754 locus in both fractions are equal. However, the concentrations of three contiguous genes in the insulin genetic region (tyrosine hydroxylase, insulin, insulin-like growth factor II) are 2-3 times greater in the fractions containing the largest molecules. The latter results indicate that a large genomic region, estimated to be at least 50-80 kb, has a lower dimer frequency than most of the genome, including those regions containing the β-actin, p53, β-globin and 754 loci. The insulin region is in an isochore which is 52-53% GC compared to 40% for the genome overall. Thus results are consistent with an expectation of reduced dimer content in relatively GC-rich DNA. However, results for the β-actin region, which resides in a different isochore with the same GC content as the insulin region, indicate that this conclusion may not be generally applicable to all GC-rich isochores and that factors other than GC content also affect dimer induction in the human genome.

C5-231 STRUCTURE/ACTIVITY ANALYSIS OF HAP1, THE MAJOR AP ENDONUCLEASE IN HUMAN CELLS, Gil Barzilay, Dominic Rothwell and Ian D. Hickson, Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.
The HAP1 protein is the human homologue of *E. coli* exonuclease III and is the major endonuclease in human cells which cleaves DNA specifically at apurinic/apyrimidinic (AP) sites. HAP1 also possesses the ability to alter the redox state of a conserved cysteine residue located in the DNA binding domain of the proto-oncogene products Jun and Fos. In addition to AP endonuclease activity, HAP1 shares with exonuclease III three other enzymatic activities; 3' phosphodiesterase, 3' phosphatase and RNase H activity, but lacks the 3' to 5' exonuclease activity characteristic of exonuclease III. We have cloned the HAP1 cDNA and a series of site-directed mutant forms of HAP1 into an *E.coli* expression vector and purified the proteins to homogeneity. Mutagenesis was targeted at residues conserved between HAP, exonuclease III and other AP endonucleases. Analysis of these mutants has verified key aspects of the catalytic mechanism of action of HAP1 predicted from the crystal structure of exonuclease III (generated by Dr John Tainer, the Scripps Research Institute, USA).
Functional dissection of the four exons within the HAP1 gene revealed three possible functional domains within the HAP1 protein; a nuclear localisation signal at the extreme N-terminus, a redox domain centred around cysteine-65 and a DNA repair domain at the C-terminus. We have also begun to study the physiological roles of HAP1 in human cells to assess whether the protein acts both as a DNA repair enzyme and as a redox regulator of transcription *in vivo*. Depletion of HAP1 from HeLa cells using antisense RNA constructs confers hypersensitivity to a variety of DNA damaging agents. An analysis of whether DNA repair or transcription factor functions are compromised in these cells is underway.

C5-232 ß-BENZOYLOXYACROLEIN, AN ACTIVATED FORM OF THE ENDOGENOUS DNA MUTAGEN MALONDIALDEHYDE, GENERATES DNA ADDUCTS THAT ENHANCE TOPOISOMERASE II-MEDIATED DNA CLEAVAGE. Kingma, P.S., Corbett, A.H., Burcham, P.C., Marnett, L.J. and Osheroff, N., Vanderbilt Univ., Nashville, TN 37232-0146.

Malondialdehyde (MDA), a naturally occurring product of human lipid peroxidation and prostaglandin biosynthesis, produces DNA adducts that induce point mutations as well as small insertions and deletions. Topoisomerase II-targeted drugs also induce DNA insertions and deletions in treated cells. Therefore, in order to study the potential role of topoisomerase II in MDA mutagenesis, pBR322 DNA was treated with ß-benzoyloxyacrolein (ßBA)(an activated form of MDA that yields a similar spectrum of DNA adducts) and the effect of ßBA adducts on topoisomerase II-mediated DNA cleavage was investigated. ßBA adducts enhanced DNA cleavage ~6-fold at a concentration of ~100 nM adduct (~1 per 400 bases). This corresponds to a potency that is ~1000-fold greater than that of the topoisomerase II-targeted drug, etoposide (the most widely prescribed antineoplastic drug in clinical use). Cleavage enhancement followed a sigmoidal time course, suggesting that the enzyme was scanning the DNA for adduct sites. ßBA had to be incorporated in the plasmid in order to stimulate DNA cleavage. The increase in DNA breaks by adducts was due primarily to a stimulation of the forward rate of enzyme-mediated DNA cleavage rather than an inhibition of religation. Despite the cleavage enhancing properties of ßBA adducts, no inhibition of enzyme-catalyzed DNA relaxation or ATP hydrolysis was observed. Competition assays indicate that ßBA adducts share a common interaction domain on topoisomerase II with several antineoplastic drugs. These data suggest that topoisomerase II may play a role in MDA-induced DNA mutagenesis. Supported by NIH Grant GM33944 and ACS Grants FRA-370 and NP-812A.

C5-233 INHIBITION OF HUMAN DNA TOPOISOMERASE I BY ULTRAVIOLET LIGHT-INDUCED DNA DAMAGE, Antonia M. Pedrini, Annalisa Lanza, Carlo Rodolfo and Silvia Tornaletti, Istituto di Gentica Biochimica ed Evoluzionistica del CNR, 27100 Pavia, Italy.

It has been proposed that DNA topoisomerases are the proteins involved in the formation of UV-induced DNA-protein crosslinks. This proposal is consistent with the formation during the DNA topoisomerase reaction of a DNA-protein intermediate, termed "cleavable complex". Since, it was shown that DNA topoisomerase II inhibition by UV-light was not due to the accumulation of the cleavable complex, we have undertaken a study aimed to establish whether UV-lesions were affecting DNA topoisomerase I activity.

We found that the presence of UV damage impaired the enzyme's ability to relax negatively supercoiled pAT153 DNA. Decreased relaxation activity correlated with the stimulation of the "cleavable complex". Low resolution mapping at the plasmid level of the sites where cleavable complexes were concentrated indicated UV-induced cleavage at discrete positions. All sites, except for one, corresponded to those stimulated by campthotecin (CPT), a specific inhibitor of eukaryotic DNA topoisomerase I. High resolution mapping at nucleotide level within the sequence encompassing the UV-specific cleavage region indicated that some sites corresponded to those induced by CPT, although at a different extent. Some sites were instead specific for UV damage. Interestingly one of the UV stimulated cleavage sites was localised within a sequence that did not contain dimerizable pyrimidines, suggesting long distance transmission of the distortion caused by photodamage to DNA. These results suggest that DNA structural alterations induced by the formation of UV-lesions can be sufficient stimulus to induce crosslinking of Topoisomerase I to DNA. (This work was supported by the PF Ingegneria Genetica, CNR)

C5-234 URACIL-DNA GLYCOSYLASE INHIBITOR PROTEIN STRUCTURE, Dale W. Mosbaugh*, Samuel Bennett*, Amy Lundquist*, Suganthi Balasubramanian^, Richard D. Beger^ and Philip H. Bolton^, *Departments of Agricultural Chemistry and Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331 and ^Chemistry Department, Wesleyen University, Middletown, CT 06450.

The *Bacillus subtilis* bacteriophage PBS2 uracil-DNA glycosylase inhibitor (Ugi) protein is a monomeric, heat-stable, acidic protein with a molecular weight of 9,477 daltons. Ugi inactivates *Escherichia coli* uracil-DNA glycosylase (Ung) by forming an Ung·Ugi protein complex with 1:1 stoichiometry. The secondary structure of Ugi has been determined by solution state multi-dimensional nuclear magnetic resonance utilizing isotopically labeled protein. Ugi was observed to adopt a single well defined structure with five anti-parallel β-strands which may explain the heat stability of the protein. This 84 amino acid protein contains a high percent of negatively charged residues (12 Glu + 6 Asp). Interestingly, about one half of the negative residues were found in the six loop or turn regions that link the β-strands to one another. Two α-helices (residues 5-13 and 27-35) are positioned near the amino terminus of Ugi. One α-helix (residues 27-35) has a distinctly charged side and a hydrophobic side; however, the other α-helix does not exhibit similar structure. The tertiary structure of the Ugi protein has been solved and the structure of Ugi in the Ung·Ugi complex is currently under investigation. Based on NMR results, Ugi appears to undergo a conformational change during complex formation. This observation is consistent with kinetic studies that indicate the Ugi association with Ung occurs by a two-step mechanism involving a rapid pre-equilibrium "docking" step, followed by a rearrangement or "locking" step that leads irreversibly to the final complex. (Supported by NIH grants GM32823 and ES00210 (DWM) and NSF grants DMB91-05003 and BIR93-03077 (PHB)).

C5-235 DIFFERENCES IN DNA REPAIR ACTIVITY AND SENSITIVITY TO UV-B LIGHT AMONG EGGS AND OOCYTES OF "DECLINING" AND STABLE AMPHIBIAN SPECIES, John Hays[1], Peter D. Hoffman[1], and Andrew R. Blaustein[2], Departments of Agricultural Chemistry[1] and Zoology[2], Oregon State University, Corvallis, OR 97331

The populations of many amphibian species, in widely scattered habitats, appear to be in severe decline, but other amphibians show no such declines. We have addressed the hypothesis that differential sensitivity to UV-B radiation contributes to these declines: by measuring specific activities of photolyase and other repair enzymes in eggs and oocytes from the wild, for a variety of species; and by measuring UVB-dependent mortality (hatching success) of eggs in sunlight, for a subset of anurans (frogs and toads), some in decline, some not. Photolyase levels varied > 80-fold among various species, and were generally correlated with expected exposure of eggs to sunlight. Among the three anurans, the eggs of the Pacific tree frog (*Hyla regilla*), whose populations appear stable, showed the highest photolyase levels and the greatest hatching success. The Western toad (*Bufo boreas*), and the Cascades frog (*Rana cascadae*), whose populations have declined markedly, showed significantly lower photolyase levels and hatching success. Interestingly, laboratory populations of the African frog *Xenopus laevis* showed very low photolyase levels. Current work is focused on extending these measurements to other species and other repair activities, and to determining the extent to which differences might reflect differential exposure of maturing females to UV-B radiation. (Supported by NSF grant BSR-9024880 and EPA grant R 821275-01-0.)

C5-236 ISOLATION AND CHARACTERIZATION OF *S. pombe* cDNAs THAT CONFER ALKYLATION RESISTANCE TO 3-METHYLADENINE DNA GLYCOSYLASE DEFICIENT *E. coli*,
Asli Memisoglu and Leona Samson, Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA 02115
Alkylating agents are an abundant class of toxicants that damage cellular proteins and nucleic acids. Thus far, two types of proteins, 3-methyladenine (3MeA) DNA glycosylases and DNA repair methyltransferases have been shown to specifically repair DNA damage caused by alkylating agents. In *E.coli*, the *alkA* and *tag* genes encode 3MeA DNA glycosylases and cleave methylated 3MeA as well as other modified bases from the DNA backbone. *AlkA⁻tag⁻ E.coli* are exquisitely sensitive to the lethal effects of the alkylating agent methyl methanesulfonate (MMS). Functional homologues to these *E. coli* genes have been identified in several eukaryotes. 3MeA DNA glycosylase deficient (*alkA⁻ tag⁻*) bacteria were used to identify *S. pombe* cDNAs that rescue cells from MMS induced killing. Five plasmids, designated pSP102 and pSP104 through pSP107, were found to consistently confer a drug resistance phenotype. Southern analysis revealed that at least three unique cDNAs have already been cloned. Sequence analysis of the pSP102 insert revealed that a large open reading frame is similar to 3MeA DNA glycosylases from three other organisms. It is important to note that it is not clear that 3MeA DNA glycosylases are the only genes that can reverse the phenotype of alkylation sensitive *E. coli*. *S. pombe* is a genetically powerful organism that has not previously been exploited as a model system to study alkylating agent specific DNA repair and resistance. An exhaustive search for *S. pombe* cDNAs that complement alkylation sensitive *E. coli* may uncover novel genes that confer alkylating agent resistance by an as yet unidentified mechanism. These studies shed light on the the response of *S. pombe* cells to alkylation damage and may very well shed light on the response of other organisms.

C5-237 DNA REPAIR OF ALKYLATION DAMAGE IN *SALMONELLA TYPHIMURIUM*,
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S. typhimurium possesses O^6-methylguanine DNA methyltransferases (MTs) activities but does not exhibit an adaptive response conferring detectable resistance to mutagenic methylating agents. In order to characterize the DNA repair system to alkylation damage in *S. typhimurium*, we have cloned the *ada*-like gene (ada_{ST}) and *ogt*-like gene (ogt_{ST}) from this bacterium and constructed deletion mutants. Unlike Δ*ada* mutants of *E coli*, which are sensitive to the mutagenicity of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), the Δada_{ST} mutant of *S. typhimurium* had a similar sensitivity to that of the parent strain. Instead, the Δogt_{ST} mutant exhibited a much higher sensitivity to the mutagenicity of MNNG than did the parent strain. The Δogt_{ST} mutant also displayed a 2 to 3 times higher spontaneous mutation frequency than the parent strain and the Δada_{ST} mutant. These results suggest that the Ogt_{ST} protein, but not Ada_{ST} protein, plays a major role in protecting *S. typhimurium* from the mutagenicity of endogenous as well as exogenous alkylating agents.

C5-238 **HUMAN LEUKOCYTIC ANTIGEN (HLA) CLASS I PRESENTATION OF DNA REPAIR GENES: DIAGNOSTIC AND THERAPEUTIC TARGETS,**
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The immune and DNA repair systems may be linked in a novel, functional manner. DNA damages can cause mutations which are the genetic basis for cancer. The biological consequences of DNA damages are minimized by DNA repair systems that are composed of enzymes that recognize and repair the damages. Ideally, the immune system eliminates cancer cells by a cytolytic T-cell response that is mediated by CD8+ cells. Tumor recognition, prior to cell destruction, occurs as a result of specific T-cell receptor binding to HLA class I molecules that are presenting antigenic peptides on the surface of target cells. A number of protein sources for antigenic peptides have been determined. As a result, recent evidence suggests that all cellular proteins, including both nuclear and cytoplasmic ones, are potentially degraded into fragments, some of which may be presented on the cell surface by HLA class I molecules. Since DNA repair enzymes play such a crucial role in keeping a cells mutation rate low, peptides derived from mutated forms of DNA repair proteins would be especially effective targets for preventing cancer. Until now, the ability of DNA repair genes to be displayed on the cell surface by HLA class I molecules has not been examined. We are looking at the ability of hMSH2 derived peptides to bind, and be presented by, class I molecules *in vivo* and *in vitro*. Peptides will be isolated from cells that are overproducing the human mismatch DNA repair enzyme hMSH2.The ability of homologous peptides that contain mutations isolated from tumors will be tested for the ability to form stable HLA complexes. These compexes will be used to develop reagents, fabs and/or constrained peptides, with phage display technologies to investigate HLA class I presentation of DNA repair enzyme peptides. These reagents should be useful diagnostic and therapeutic tools because of their ability to uniquely target cancer cells.

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C5-239 **POTENTIATION OF TEMOZOLOMIDE CYTOTOXICITY BY AN INHIBITOR OF ALKYLGUANINE-DNA ALKYLTRANSFERASE.**
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Temozolomide (TZ) is an imidazotetrazinone which has demonstrated clinical activity in the treatment of melanoma, mycosis fungoides and high grade glioma. The antitumour activity of TZ is attributed to formation of the reactive methylating species 5-(3-methyl)-1-triazenyl-1-yl)imidazole-4-carboxamide; the cytotoxicity of which is thought to derive from its ability to methylate DNA at the O^6 position of guanine. The clinical utility of TZ may therefore be limited by the DNA repair protein, O^6-alkylguanine-DNA alkyl transferase (AGT), which repairs O^6-alkylguanine adducts in a stoichiometric autoinactivating reaction. AGT has already been shown to confer resistance to the established chloroethylating agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), which also forms an O^6-guanine adduct. However, recent preclinical experimentation has indicated that pre-administration of O^6-benzylguanine (O^6-BG), a potent inhibitor of AGT, can significantly increase the therapeutic index of BCNU. Potentiation of TZ cytotoxicity by O^6-BG pretreatment (100μM; 1h) was therefore studied *in vitro*, against a panel of cell lines with different AGT expression. Potentiation of a single TZ treatment (3h) by O^6-BG correlated with AGT content, in that greater potentiation was observed in cells expressing most AGT (i.e. potentiation ranged from 1.2 to 1.8 fold within an AGT range of 250-1000 fmol/mg protein). When O^6-BG and TZ were administered by a multiple (daily x 5) dosing schedule to a human colorectal tumour cell line (Mawi), potentiation of TZ cytotoxicity was found to increase linearly with each dose (from 1.5 fold (day 1) to 2.2 fold (day 5)). These results suggest that O^6-BG may increase the clinical effectiveness of TZ, in the treatment of malignancies which exhibit AGT-mediated resistance to alkylation therapy.

C5-240 EXPRESSION CLONING OF A CANDIDATE HUMAN MutY HOMOLOG

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8-oxo-deoxyguanosine (GO) appears to be a quantitatively important spontaneous and induced oxidative damage. The likely significance of GO species as promutagens is indicated by detection of specific DNA repair and triphosphatase activities in the several organisms examined to date. In *Escherichia coli*, a DNA glycosylase active on adenine/GO mispairs is encoded by the MutYgene, and works in combination with a GO-specific DNA glycosylase (MutM gene product) for the avoidance of G:C->T:A transversion mutations (Michaels et al., PNAS 89:7022, 1992); human homolog(s) of MutY would be of interest as antimutator and possible tumor suppressor activities. Isolation of a cDNA clone encoding a candidate activity of this kind (via expression in *E. coli* and complementation) is described here. **METHODS**: *E. coli* host cells defective for a DNA repair enzyme can be complemented by plasmid expression constructs that encode the corresponding gene product. Following DNA damaging treatment of the population of cells comprising a cDNA expression library, complementing plasmids can mediate repair *in vivo* and render themselves lesion-free; in contrast, clones containing irrelevant cDNA insert sequences remain damaged. When isolated and treated with the cognate repair enzyme *in vitro*, complementing plasmids remain intact while other molecules suffer nicking; retransformation following denaturation enriches for complementing plasmids. The process is then repeated. **RESULTS**: Human cDNA expression libraries were manipulated in this way, using recombinant MutY protein (kindly supplied by M. Michaels). Candidate MutY-homolog clones were screened by complementation of MutY+ MutM and MutY+MutT phenotypes. Assays for recombinant MutY-like activities in extracts of these cells are underway, as is subcloning and DNA sequence analysis. **CONCLUSIONS**: Candidate cDNA clones for human MutY have been isolated. Current results of their characterization will be presented. [M. Michaels, C. Cupples, and J. Miller are thanked for materials and invaluable discussions. Supported by NCI K08-CA-1590]

C5-241 SEARCH FOR HUMAN CELL FACTORS PROCESSING RADIATION-INDUCED DNA DAMAGES

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WI-L2-NS and TK6 human lymphoblastoid cells, though syngeneic, were phenotypically different in the radiation response. Relative to WI-L2-NS cells, TK6 cells showed a reduced X(γ)-ray survival and a decreased frequency of TG-resistant mutation induction with the apparent lack of the dose-rate effect. Thus, it seemed very likely that TK6 cells were defective as to a function involved in the processing of radiation-induced DNA damages. Attempting to identify such a function, we searched for proteins whose contents differed between WI-L2-NS and TK6 cells. Several proteins with differential yield between the two cell lines were successfully detected in the crude nuclear extract. Among them, there was an 80 kDa polypeptide that was absent in TK6 cells. Following preparation of a large scale culture ($3x10^9$ cells), the furhter purification was achieved through the combined use of column chromatographies, including DNA cellulose, anion exchange, and gel filtration columns. We finally obtained the protein detectable as a single band with a SDS-polyacrylamide gel electrophoresis. The release of the protein from the nucleus in the high ionic strength suggested its tight association with DNA, chromosome, and/or nucleus structure. We have initiated the characterization of its biochemical properties using model DNA substrates.

C5-242 **PHENOTYPIC CHARACTERISATION AND CLONING OF MUTANTS OF *ASPERGILLUS NIDULANS* SHOWING HYPERSENSITIVITY TO A RANGE OF DNA DAMAGING AGENTS**; **Simon May and Peter Strike, Department of Genetics and Microbiology, The University of Liverpool, P.O.Box 147, Liverpool L693BX, England.**

Of over 200 mutants originally isolated as hypersensitive to the DNA damaging agents MNNG and 4NQO(designated *nuv*), 11 were selected for further characterisation. UV-induced mitotic recombination assays revealed a range of recombination phenotypes amongst the mutants. UV and γ-ray sensitivity profiles of the mutants were similarly diverse,the *nuv*334 mutant in particular showed extreme hypersensitivity to UV-irradiation. Mutation assays revealed the *nuv*8 mutant as having a high spontaneous mutation frequency which remained largely unaffected by UV-induction. Mitotic mapping analysis has been employed to assign a number of *nuv* mutations to linkage groups, while complementation analysis with previously characterised *uvs* and *mus* mutants is in progress. Initial attempts are being made to clone the *nuv*110 mutation using pools of cosmids from chromosome specific libraries.

C5-243 LARGE-SCALE ISOLATION OF *DROSOPHILA* DNA REPAIR GENES BY *PlacW* SINGLE INSERTIONAL MUTAGENESIS, Wenya Huang and P.Dennis Smith, Department of Biological Sciences, Wayne State University, Detroit, MI 48202

We have initiated a large-scale scheme for the isolation and molecular characterization of DNA repair genes in *Drosophila melanogaster*. Our strategy utilizes single P-element insertional mutagenesis to generate and tag mutagen-sensitive strains. Coupled with the plasmid rescue technique, single P-element mutagenesis greatly facilitates the identification and recovery of genes involved in DNA repair mechanisms. The engineered transposon,*PlacW*, contains the mini-white eye color gene to identify transposition events. In addition, the ampicillin-resistance gene combined with the bacterial origin of replication allow direct cloning of the sequence flanking *PlacW* via simple plasmid rescue procedure. This poster presents the genetic and molecular characterization of one such *PlacW*-tagged X-linked MMS-sensitive strain. The sequence flanking *PlacW* containing the putative repair gene has been cloned. Analysis of this gene including sequencing and cDNA isolation are in progress. Determination of the allelic relationship to previously described DNA repair genes is underway by complementation testing to existing repair-deficient mutants, along with *in situ* hybridization to polytene chromosomes. Using this large-scale *PlacW* single insertion mutagenesis scheme, DNA repair genes throughout the Drosophila genome can now be tagged and isolated.

C5-244 A NOVEL INTRACHROMOSOMAL HOMOLOGOUS RECOMBINATION ASSAY TO STUDY MISMATCH REPAIR IN MAMMALIAN CELLS,

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Defective mismatch repair can result in the expansion of simple repeated sequences and a high frequency of homologous recombination between divergent repeated sequences. These events lead to genome instability which is a common feature of tumor cells and several hereditary genetic disorders. To study mismatch repair in relation to the stability of the mammalian genome, we have developed a novel intrachromosomal homologous recombination assay. This assay involves the formation of DNA heteroduplexes in normal and cancerous cell lines proficient and deficient in mismatch repair functions. Since it does not require selectable marker genes as recombination sustrates, this assay has many advantages when compared to those used currently. First, any DNA sequences with various degrees of homology can be used as recombination substrates. Second, it allows the recovery of both precise and imprecise recombinational DNA repair events. Third, it can be used in a wide variety of mammalian cells. The assay was developed using a vector that contains repeats of LINE-1 elements flanking a marker gene. A Pol2 promoter in one repeat allows, when transfered by recombination to the other flanking repeat, the expression of a promoterless neo gene situated downstream of the recipient repeat. Because donor and recipient sequences are both recovered, this assay has the added advantage of of permitting analysis of reciprocal and nonreciprocal recombination events. Finally, nucleotide sequence analysis of the recombination products allows determination of the origin of the recombined sequences.

C5-245 A NOVEL DNA REPAIR ACTIVITY CORRECTS UNPAIRED BASES IN MISMATCH REPAIR+/− HUMAN CELL FREE EXTRACTS.

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A replication error (RER+) phenotype, characterized by somatic instability in simple repeated sequences, is associated with several types of cancer. The extracts from RER+ cell lines are defective in heteroduplex repair while extracts of RER− cell lines are not. Instability of simple repetitive DNA sequences may arise during replication by strand slippage to generate several consecutive unpaired bases, i.e., loops. Heteroduplex DNA molecules with unpaired bases are not reapired in *E.coli*. We decided to study repair of unpaired bases in human cell free extracts which are defective in mismatch repair. We describe here a previously unreported activity in human cell extracts, repair of DNA with loops of five or more unpaired bases. Repair is strand-specific and is directed not by the extra bases but by a nick located either 5' or 3' to them. This repair differs from mismatch repair in that it is observed in extracts of RER+ cell lines that are deficient in repair of mismatches or of 1 or 2 unpaired bases. Moreover, an extract of cells having homozygous mutations in *hMLH1* alleles is proficient in repair of loops of five or more bases. These data suggest that repair of DNA containing loops does not require all components of homologs of *E.coli* methyl directed mismatch repair. Defects in this novel repair activity may be relevant to the repetitive-sequence instability observed in certain cancers or hereditary diseases characterized by microsatellite expansion.

C5-246 MISMATCH CORRECTION DEFECTS AND RESISTANCE TO METHYLATING AGENTS IN HUMAN COLORECTAL TUMOR CELL LINES.

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Human cells sensitive to killing by DNA methylation damage frequently acquire resistance, methylation tolerance, after exposure to methylating agents. One hypothesis proposes that methylation tolerance arises through loss of DNA mismatch repair. Tolerant cell lines with mutator phenotypes associated with defective mismatch binding or with impaired mismatch correction in cell extracts have been described. Defective mismatch repair is also implicated in >85% of hereditary non-polyposis colorectal cancers (HNPCC) and up to 20% of sporadic tumors. We have identified three (of eleven) colorectal adenocarcinoma cell lines with defective DNA mismatch binding and a mutator phenotype at the HPRT locus. One of these (LoVo) has a known deletion in the hMSH2 gene. A fourth cell line, SW48, is also a mutator but has normal mismatch binding. SW48 cells exhibit an unequivocal methylation tolerant phenotype and resemble HCT116 cells which are known to be mutated in a second mismatch correction gene, hMLH1. Among the mismatch correction deficient colorectal lines, there is a strong association between mutator phenotype and loss, through mutation, of β 2 microglobulin expression. The mutator phenotype and methylation tolerance of SW48 together with the apparent selective advantage provided by loss of β 2 microglobulin expression indicate that acquisition of methylation tolerance and escape from immune surveillance may be important factors in the emergence and survival of mismatch repair-deficient tumors.

C5-247 GENERATION OF MICE MUTATED IN THE DNA MISMATCH REPAIR GENE HOMOLOGUE, *mPMS2*.

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Recently, mutations in four DNA mismatch repair gene homologues, the MutS homologue, *hMSH2*, and three MutL homologues *hMLH1*, *hPMS1* and *hPMS2*, have been implicated in the development of hereditary non-polyposis colorectal cancer (HNPCC). It has been estimated that HNPCC accounts for 4-13% of colorectal cancers in the Western World, making it the most common form of hereditary colon cancer. One characteristic of DNA mismatch repair deficiency in bacteria, yeast and mammalian cells, is instability of short tandem repeat or microsatellite sequences. Microsatellite instability has been demonstrated not only in tumours from HNPCC patients, but also in a subset of sporadic colorectal and other cancers.

To better understand DNA mismatch repair function in the whole organism we isolated mouse *PMS2* (*mPMS2*) genomic sequences and designed a targeting vector to delete an exon that encodes a region highly conserved in all known MutL like proteins. We disrupted one *mPMS2* allele in mouse Embryonic Stem (ES) cells and generated mice chimeric for the *mPMS2* targeted cells. One male animal showing germline chimerism has produced offspring with the Agouti coat colouring, including animals that are heterozygous for the *mPMS2* mutation. Breedings are in progress to produce a large cohort of heterozygous mice which will be followed for tumour development. In addition we are attempting to produce animals homozygous for the *mPMS2* mutation. These "*mPMS2*" animals (by themselves and when crossed to other "cancer prone" mice) should prove useful for determining the role of DNA mismatch repair genes in development and oncogenesis and provide an isogenic set of cell lines for *in vitro* studies.

C5-248 SEQUENCE SPECIFIC MISMATCH REPAIR BY *E. COLI* MUTY. Sheila S. David, Bruce. D. Meads, David P. Smith, Yinghui Wu, *Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064*

MutY plays an important role in the prevention of mutations caused by oxoguanine (OG) in *E. Coli.*[1] In particular, MutY recognizes G:A and OG:A mispairs *in vitro* and corrects this mismatch using N-glycosylase activity to remove the undamaged adenine. Our research is focused on delineating the factors involved in the recognition and repair of mismatches by MutY.

A variety of structures for G:A mismatches have been observed by NMR and X-ray crystallography which are dependent on the sequence environment and the pH. These structures differ in the conformation of the N-glycosidic bond at the G and the A of the mismatch and include G(anti)-A(anti), G(syn)-A(anti), G(anti)-A(syn). This observation suggested that MutY's G:A mismatch repair activity may be sequence specific. We have investigated four 16 base-pair sequences which are based on an 11 base-pair region of the K-*ras* oncogene which has been shown to be a "hot-spot" for mutations in mammalian cells. The 11-mer sequence, d(GCCACAAGCTC)-d(GAGCTGGTGGC) has been structurally characterized by NMR spectroscopy as a function of pH and exhibits a pH dependent conformation of the G:A mispair.[2] Surprisingly, the sequences tested show *considerable* variability as substrates for MutY. Indeed, a 500 fold range of activity was observed with sequences which differ only in the base-pair flanking the mismatch with the parent sequence as the least efficiently repaired substrate tested. These results clearly indicate a *substantial* sequence dependent cleavage with MutY which is influenced by the flanking sequence.

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C5-249 MUTANTS OF VSR MISMATCH ENDONUCLEASE

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The Vsr gene product of *E. coli* K-12 is a DNA mismatch endonuclease that recognizes T/G mismatches in a special sequence context and initiates their strand-specific repair by setting an endonucleolytic cut at the 5' side of the mismatched thymine residue (1). Action of the enzyme has a profound influence on the structure of the *E. coli* K-12 genome in that it catalyses events of mutation avoidance, active mutagenesis and recombination (2, 3). Though the enzyme has so far been characterized biochemically (1), nothing is known about the structure of the endonuclease. In order to obtain structural and functional information about the enzyme, we mutagenized the *vsr* gene randomly by error-prone PCR. Individual clones were screened for loss of endonuclease activity by an assay that allows to investigate in parallel large numbers of samples of crude cell extracts (4). We identified several different single amino acid substitutions that resulted in loss of endonuclease activity with unimpaired production of stable protein. Mutants are phenotypically characterized and divided into three different classes: (a) folding-deficient proteins, (b) folding-competent proteins with no DNA recognition activity and (c) DNA-binding competent, cleavage deficient proteins.

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C5-250 PROTEIN-PROTEIN-INTERACTIONS BETWEEN MUTH AND MUTL FROM *E. COLI*, Heidrun Hoffmann-Uhlig and Hans-Joachim Fritz, Institut für Molekulare Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, 37077 Göttingen, Federal Republic of Germany

In *E. coli*, replication errors are corrected by the methyl-directed MutHLS-repair system. For the initiation of this process, the proteins MutH, MutL and MutS are necessary (1). MutH is responsible for the incision of the newly synthesized DNA-strand containing the error (2,3). MutS binds to the mismatch (4) and MutL probably connects MutH and MutS (5). By means of a genetic system for protein interaction (6) a contact between MutH and MutL was detected. The detailed structure of the MutH contact sites to MutL are under further investigation. Preliminary results suggest an α-helical stretch near the C-terminus of the protein.

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C5-251 FRAMESHIFT MUTAGENESIS BY 9-AMINOACRIDINE: THE EFFECT OF MISMATCH REPAIR, George Kopsidas and D.G. MacPhee*, National Institute of Child Health and Development, National Institutes of Health, Bethesda, Maryland, 20892. *Department of Microbiology, La Trobe University, Melbourne, Australia, 3083

Intercalating compounds such as 9AA can induce frameshift mutations at runs of identical, adjacent base-pairs. Mismatch repair is postulated to be important in correcting acridine-provoked mismatches primarily at or near the replication fork, therefore there is thought to be a connection between acridine mutagenesis and the position of the replication fork. This correlation implies that the free polynucleotide end necessary for strand slippage and misalignment (required for mutagenesis) is supplied either by the replication fork or by gaps between Okazaki fragments on the lagging strand. Consequently, many of the 9AA-provoked mismatches occurring at such regions would be candidates for mismatch repair. This notion is supported by the following data. A mismatch defective (*mut*) genotype appeared to enhance 9AA mutagenesis in actively growing cells of *Salmonella typhimurium*. The *mutH101*, *mutS121* and *mutL111* alleles gave almost confluent levels of reversion. Clearly, the mismatch repair system accurately removes many of the 9AA-provoked mismatches in actively growing cells, however, the experiments presented here indicate that the situation with cells not actively engaged in DNA replication could be dramatically different. Stationary phase cells deficient in various alleles involved in mismatch repair, appeared to be less mutable by 9AA.

The newly synthesized DNA strand following replication is methylated some time after DNA synthesis by the product of the *dam* gene at GATC sequences. Such delayed methylation is thought to supply the recognition necessary for incision of the strand containing mismatched bases. Mutants defective in the *dam* gene generally demonstrate an enhanced mutation frequency as a consequence of indiscriminate mismatch repair. Such indiscriminate repair would certainly account for the enhanced mutation rate demonstrated by log phase cells of a *S. typhimurium dam-1* mutant. However, the *dam-1* mutation did not seem to influence the mutation frequency in stationary phase cells, in fact the Dam$^-$ phenotype appeared to decrease the mutation frequency. The data is consistent with the notion that the mismatch repair system in stationary phase cells may actually generate 9AA-induced mutations. This postulates a paradoxical situation where the mismatch repair system, generally associated with enhanced fidelity in replicating cells, becomes associated with the generation of repair errors in stationary phase cells.

C5-252 RECOGNITION OF DNA INSERTION/DELETION MISMATCHES IN YEAST, Robert Lahue, Juan Jose Miret and Breck Parker, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, MA 01655.
An activity in nuclear extracts of the yeast *S. cerevisiae* binds specifically to oligonucleotide heteroduplexes containing three to nine extra bases in one strand, as judged by a band shift assay. The specificity of this activity (IMP, for insertion mismatch binding protein) was confirmed by competition experiments; binding of radiolabelled heteroduplexes is abolished in the presence of excess unlabelled heteroduplex but not by excess homoduplex. IMP is biochemically and genetically distinct from the *MSH2* dependent, single base mismatch binding activity. The two types of mismatch binding complexes migrate differently during electrophoresis, they are differentially inhibitable in competition experiments and their spectra of mispair binding are distinct. Furthermore, IMP activity is observed in extracts from *msh2*⁻ and *msh3*⁻ mutant strains and also from an *msh2*⁻ *msh3*⁻ *msh4*⁻ triple mutant. Single-stranded binding activities are unlikely to be responsible for IMP activity since single-stranded probes yield complexes migrating at positions distinct from the IMP complex and excess unlabelled single-stranded oligonucleotides are unable to displace insertion/deletion heteroduplexes from the complex. IMP exhibits specificity for insertion/deletion mispairs in two different sequence contexts. Finally, IMP binding to insertion/deletion mismatches is influenced by the structure of the mismatch. An insertion mismatch with a hairpin configuration is not recognized by this activity. These findings are consistent with the idea that yeast contains a distinct mismatch recognition factor specific for insertion/deletion mismatches.

C5-253 TRANSCRIPTION OF *mutS* AND *mutL*-HOMOLOGOUS GENES IN YEAST DURING MITOSIS AND MEIOSIS, Wilfried Kramer, Eike C. Ringbeck and Berthold Fartmann, Institut für Molekulare Genetik, Universität Göttingen, Grisebachstraße 8, D-37077 Göttingen, Germany
We have analysed the transcription of the *MSH1*, *MSH2*, *MSH3*, *PMS1* and *MLH1* genes of *S. cerevisiae*, which are homologus to the DNA mismatch repair genes *mutS* and *mutL* from *E. coli*, respectively. We have developed an RT-PCR assay for determining the relative amounts of mRNA. cDNA fragments are produced by subjecting total RNA extracted from synchronized cells at various time points to a reverse transcription reaction in the presence of specific short cDNA primers. A fragment of each cDNA is amplified subsequently by a separate PCR using fluorescently labeled primers. PCR products are analysed and quantitated on an automated laser fluorescence sequencer (ALF). Transcription of the *PMS1* and *MSH2* gene was found to be cell cycle regulated during mitosis with a pattern identical to that of the *TMP1* gene, which is known to be regulated by MluI cell cycle boxes (MCBs). Transcription of *MSH1*, *MSH3* and *MLH1* was constitutive. Mutant analysis demonstrated that the regulation of *PMS1* is also dependent on MCBs. The *PMS1* promotor mutant displayed a mitotic mutator phenotype. The regulation pattern of *MSH2* and the presence of MCB-like sequences in the 5'-flanking region suggest that MSH2 is regulated by MCBs as well. During meiosis, *PMS1* and *MSH2* are induced prior to DNA synthesis just like the *TMP1* gene suggesting a regulatory function of MCBs in meiosis as well. This will be further studied by mutant analysis. Transcription of *MLH1* was constitutive. Furthermore, *MSH2* and probably *MSH3* are more strongly induced later in meiosis in a pattern coinciding with that of *SPO11*. Both *MSH2* and *MSH3* contain sequences in the 5'-flanking region, which are similar to known meiosis specific control elements. The regulation of *MSH2* suggests a function of this gene in meiosis that is independent of *PMS1* and *MLH1*.

C5-254 DOMINANT NEGATIVE MUTATOR MUTATIONS IN THE mutS GENE OF ESCHERICHIA COLI. Wu, T.-H. and Marinus, M.G. Dept. Pharmacology, Univ. Mass. Med. Sch., Worcester MA 01655
The MutS protein of E. coli is part of the dam-directed MutHLS-mismatch repair pathway which rectifies replication errors and which prevents recombination between related sequences. In order to more fully understand the role of MutS in these processes, dominant-negative mutS mutations on a multicopy plasmid were isolated by screening transformed wild type cells for a mutator phenotype using a Lac⁺ papillation assay. Thirty-eight hydroxylamine and 22 N-methyl-N-nitro-N-nitroso-guanidine indued dominant mutations were isolated. Nine of these mutations altered the P-loop motif of the ATP-binding site resulting in four amino acid substitutions. With one exception the remaining sequenced mutations all caused substitution of amino acids conserved during evolution. The dominant mutations in the P-loop consensus caused severely reduced repair of heteroduplex DNA in vivo in a mutS mutant host strain. In a wild type strain, the level of repair was decreased by the dominant mutations to between 12-90% of the control value consistent with interference of wild type MutS function by the mutant proteins. Increasing the wild type mutS gene dosage resulted in a reversal of the mutator phenotype in about 60% of the mutant strains, indicating that the mutant and wild type proteins compete. In addition, 20 mutant isolates showed phenotypic reversal by increasing the gene copies of either mutL or mutH. There was a direct correlation between the levels of recombination and mutagenesis in the mutant strains suggesting that these phenotypes are due to the same function of MutS.

C5-255 ANALYSIS OF HUMAN URACIL-DNA GLYCOSYLASE BY SITE-DIRECTED MUTAGENESIS Geir Slupphaug, Ingrun Alseth, Bodil Kavli, Terje Haug and Hans E. Krokan, UNIGEN Center for Molecular Biology, University of Trondheim, N-7005 Trondheim, Norway
We have previously cloned and characterized a cDNA (pUNG15) encoding human uracil-DNA glycosylase (UDG) which catalyses the first step in the excision repair pathway of uracil in DNA. The gene product of Mr=33,800 (304 amino acids) contains an N-terminal sequence of 77 amino acids not present in the presumed mature form of 25,800. Recent findings indicate that this presequence is involved in mitochondrial targeting, and that signals directing nuclear targeting reside in the mature region. Furthermore, deletion studies have shown that the first 93 residues of the preprotein (including 16 residues of the presumed mature region) are not necessary for enzyme activity, while the extreme C-terminal is essential. The mature form of the enzyme is highly conserved from bacteria to higher eukaryotes, and contains 6 conserved blocks in addition to several other conserved amino acids distributed along the peptide.

To identify regions of catalytic activity/DNA-binding in the mature enzyme, a series of amino acid substitutions were introduced in conserved regions and the mutant proteins expressed in *E. coli*. Until now, 17 of the mutants have been purified to apparent homogeneity, and they are presently being investigated with respect to catalytic activity, non-specific binding to DNA, specific uracil binding, salt dependency and various kinetic parameters. So far, mutation at 4 independent residues have demonstrated >99% loss of activity, and striking alterations in specific and non-specific substrate binding have been observed. The pattern of the latter alterations indicate that DNA-binding and uracil binding domains may be located at different sites at the enzyme surface. The experiments will provide valuable information in the mapping of functionally important regions of uracil-DNA glycosylase, and a summary of the results will be presented at the symposium.

Replication/Transcription

C5-300 USE OF YEAST ARTIFICIAL CHROMOSOMES TO STUDY DNA REPLICATION AND ITS REGULATION WITHIN THE CELL CYCLE, Christine Alfano and Ronald W. Davis, Department of Biochemistry, Stanford School of Medicine, Stanford, CA 94305

A system has been established in *S. cerevisiae* to study DNA replication and its regulation within the cell cycle. This system makes use of yeast artificial chromosomes (YACs) that have origins of replication only on the terminal arms, and a long central region devoid of origin function. Complete replication of the YAC is monitored using a colony color sectoring assay based on maintenance of the *ADE2* gene carried on one end of the YAC. A library of YACs of such a structure were constructed, and a subset were subsequently screened to identify a set of YACs of different lengths.

Characterization of these YACs has shown that both the rate of color sectoring and the quantitative rate of loss of the YACs increase in proportion to the length of the YAC, and are a simple function of the length of DNA that has to be replicated from each origin of replication on the YAC. Sectoring and loss are completely suppressed in YACs that have sufficient origins. The YACs are less stable in mutant backgrounds that affect DNA synthesis. Thus, at least a fraction of the cells in a population are able to enter mitosis with an incompletely replicated YAC. Characterization of the cell cycle distribution of strains propagating replicatively unstable YACs indicates that the cell does not detect ongoing replication on the YAC, and respond by delaying the cell cycle until replication is complete.

These replication-defective YACs are currently being used in a variety of genetic screens based on a change in the rate of color sectoring. These screens are expected to identify genes involved in initiation of DNA replication, regulation of the timing of initiation within the cell cycle, as well as factors required for DNA synthesis.

C5-301 BASE ANALOG 6-*N*-HYDROXYLAMINOPURINE AS A TOOL TO STUDY DNA REPLICATION MECHANISM IN YEAST. Polina V.Shcherbakova and Youri I. Pavlov. Dept. of Genetics, Sankt-Petersburg State University, Sankt-Petersburg, 199034, Russia

6-*N*-hydroxylaminopurine (HAP) in deoxytriphosphate form is incorporated into DNA in place of normal precursors during replication and can provoke mistakes due to its ambivalent coding potential (can mimic both "G" and "A"). HAP is highly mutagenic in yeast and induces both types of transitions. Yeast *pol2-4* and *pol3-01* mutations eliminating the 3'->5' exonuclease activity of DNA polymerases ε and δ respectively increase HAP-induced reversions of *URA3* gene missense mutations. HAP-revertible mutations fall into two classes: those which reversions were enhanced in *pol2* exonuclease deficient mutant but not in *pol3* one, and those which exhibited increased reversion frequency only in *pol3* mutant. This grouping coincided with the DNA strand in which presumed HAP misincorporation occurred. When the particular *ura3* allele was placed into chromosome III in two orientations near defined replication origin *ARS306* the effects of exonuclease mutations on HAP-induced reversion were orientation-dependent. The results support the current opinion that DNA polymerases II and III play different roles in replication of leading and lagging DNA strands.

C5-302 FIDELITY OF MMLV REVERSE TRANSCRIPTASE AND THERMUS THERMOPHILUS DNA POLYMERASE DURING REVERSE TRANSCRIPTION AND DNA AMPLIFICATION, Thomas W. Myers, Christopher L. Sigua, Frances C. Lawyer, and David H. Gelfand, Program in Core Research, Roche Molecular Systems, Alameda, California 94501.

The need for highly accurate cDNA for molecular cloning applications has raised issues about the high mutation frequency of the mesophilic viral reverse transcriptases and the detrimental effect of Mn^{2+} on the fidelity of cDNA synthesis by the *Thermus thermophilus* DNA polymerase (r*Tth* pol) when used as a reverse transcriptase. The relative mutation frequency of Moloney murine leukemia virus (MMLV) reverse transcriptase and r*Tth* pol was determined *in vitro* during reverse transcription (RT) and/or PCR. We utilized a forward mutation assay requiring the synthesis of a 2.1 kb cDNA from a synthetic RNA transcript of the plasmid pFC104. The resultant cDNA or precursor plasmid DNA was then amplified by PCR, ligated to form intramolecular monomer circles, and transformed into a bacterial host. The PCR product contained a reporter gene encoding *E. coli* Lac repressor and two copies of the *lac* operator. Mutations in the amplified *lac*I gene are scored as colonies with a blue phenotype. Under reaction conditions generally utilized for RT/PCR, the fidelity of the retroviral enzyme was determined to be significantly lower than the Mn^{2+}-activated r*Tth* pol during reverse transcription. While the fidelity of both enzymes was worse during RT/PCR than observed for just PCR, major improvements were observed for both enzymes upon altering the reaction conditions. Variables such as dNTP concentration, divalent metal ion type and concentration, and enzyme concentration were screened by this genetic fidelity assay to determine conditions favoring the lowest mutation frequency during reverse transcription and/or PCR amplification.

C5-303 BYPASS OF AN ABASIC SITE IN A DEFINED GAPPED DUPLEX OLIGONUCLEOTIDE, Tamar Elizur*, Masaru Takeshita** and Zvi Livneh*, Department of Biochemistry, Weizmann Institute of Science, Rehovot 76100, Israel*, and Dept. of Pharmacological Sciences, State University of New York, Stony Brook, NY 11794, U.S.A**.

Bypass of DNA lesions by a DNA polymerase is believed to be the key step in mutagenesis caused by chemicals and radiation. We have established an assay system for studying bypass synthesis on a defined gapped duplex oligonucleotide, carrying a single synthetic abasic site at a pre-determined location. The gapped duplex oligonucleotide was prepared by annealing two short oligonucleotides to a 40-mer template: A ^{32}P-labeled primer, and an unlabeled oligonucleotide, complementary to the 5' end of the template. A series of short oligonucleotides was used, to create gapped templates with varying gap length, and varying location of the 3'-terminus of the primer, and the 5'-terminus of the downstream oligonucleotide, relative to the location of the abasic site.

Both DNA polymerase I and its Klenow fragment were able to bypass the abasic site, although the intact polymerase performed slightly better. Bypass was accompanied by nick translation (by the intact polymerase) or strand displacement (by the Klenow fragment). A mutant polymerase, lacking both the 5'→3' and 3'→5' exonuclease activities, was at least 15-fold faster than the other forms, indicating that proofreading interferes with the bypass process. The effect of the parameters of the gap are currently under investigation. In addition, the individual kinetic steps of polymerization opposite the lesion, and polymerization past the lesion are being characterized.

C5-304 GENETIC ORGANIZATION OF HUMAN FEN-1,
Binghui Shen, Julia A. Gonzales, Babetta L. Marrone and Min S. Park, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM87545
Genetic defects in human DNA repair can cause an autosomal recessive disorder xeroderma pigmentosum (XP). Among seven XP complementation groups, XP-G gene encodes a structure specific DNA endonuclease and contains putative functional domains that are conserved among several proteins. These proteins include *S. cerevisiae* RAD2, *S. pombe* RAD13, frog and mouse homologs of XPG, and *S. cerevisiae* YKL510, *S. pombe* RAD2, and mouse FEN-1 (Flap ENdonuclease-1). In order to study the structure-function relationship to XPG protein, the human homolog of FEN-1 was cloned, sequenced, mapped to human chromosomes. By using a combination of degenerate PCR and library screening, we cloned both cDNA and genomic DNA of human FEN-1. The FEN-1 cDNA (1,143 bp) encodes a protein with the predicted*Mr* of 42.5 kDa and hybridizes to three different messenger RNAs on Northern blots (4.6 kb, 1.9 kb, and 1.0 kb). The major messenger RNA (1.9 kb) shows differential expression in various human tissues, which is high in testis and ovary, and low in thymus and spleen. Genomic Southern analysis with a full-length cDNA identified a BamHI/EcoRI fragment (~4 kb) in genomic DNA clones isolated from a pWE15 cosmid library. By using fluorescent in situ hybridization, one of the genomic cosmid clone was mapped to the centromeric region of chromosome 7. Another independent cosmid clone hybridized to the telomeric region of chromosome 13Q. We are currently analyzing the detailed genomic organization of the human FEN-1 gene at the nucleotide sequence level to elucidate the origin of these distinct signals on two different human chromosomes. This research is supported by the US DOE under contract #KP0204000.

C5-305 CHARACTERIZATION OF A *Saccharomyces cerevisiae* STRAIN DELETED FOR THE *RAD27* GENE; A STRUCTURAL HOMOLOG OF THE *RAD2* NUCLEOTIDE EXCISION REPAIR GENE, Michael S. Reagan, Wolfram Siede and Errol C. Friedberg, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75235
We have constructed a strain of the yeast *Saccharomyces cerevisiae* which is deleted of the YKL510 open reading frame, initially identified in chromosome XI as a homolog of the *RAD2* nucleotide excision repair gene (A. Jacquier, P. Legrain, and B. Dujon, Yeast 8:121-132, 1992). The mutant strain exhibits moderate sensitivity to ultraviolet (UV) light and severe sensitivity to the alkylating agents methylmethane sulfonate, methylnitrosourea, and N-methyl-N'-nitro-N-nitrosoguanidine, but is not sensitive to ionizing radiation. We have renamed the YKL510 open reading frame the *RAD27* gene, in keeping with the accepted nomenclature for radiation-sensitive yeast mutants. Epistasis analysis indicates that the gene is in the *RAD6* group of genes involved in DNA damage tolerance and mutagenesis. The mutant strain is temperature-sensitve, with the cells arresting uniformly as large-budded dumbell shaped cells containing a single nucleus with a 2N DNA content. The strain also exhibits increased plasmid loss and increased spontaneous mutagenesis, but is normal with respect to UV-induced mutagenesis. Transcript levels of the *RAD27* gene are cell cycle regulated in a manner similar to that of several other genes whose products are known to be involved in DNA replication. These data are consistent with the Rad27 protein having a role in both DNA replication and DNA repair.

C5-306 NORMAL HUMAN LYMPHOBLASTOID CELLS EXPOSED TO METHYL METHANESULFONATE CONTAIN BOTH DAMAGE-RESISTANT (S_{DR}) AND DAMAGE SENSITIVE (S_{DS}) REPLICATIVE DNA SYNTHESIS SUBSETS, W. Clark Lambert, Hon-Reen Kuo, and Nydia I. Ramos, Department of Pathology, UMDNJ-New Jersey Medical School, Newark NJ 07103
We have developed an autoradiographic system, based in part on newly developed image analysis technology (Leitz), which not only automates assessment of radio-label of individual cells but also quantitatively evaluates each cell for numerous additional markers. Application of this system to cells treated with DNA damaging agents produces an integrated response pattern showing unscheduled (UDS) and scheduled (SDS) DNA synthesis as well as other parameters and their covariant interactions simultaneously. Normal human lymphoblastoid cells exposed in culture to 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM methyl methanesulfonate (MMS) for one hour followed by radiolabeling with ^{3}H-thymidine, when analyzed in this way, showed a number of unexpected results, among the most striking of which is that a subset of cells in S phase (S_{DR}) showing high levels of replicative DNA synthesis failed to show any diminution in their rate of DNA synthesis even following super-lethal doses of MMS. Other S phase (S_{DS}) cells markedly reduced their rate of DNA synthesis following exposure to high doses of MMS, with many cells ceasing DNA synthesis altogether. Cells treated with lower doses of MMS uniformly showed radiolabel, but the quantitative range of label by cells undergoing UDS was much broader than expected. Additional DNA synthesis could be detected in cells in S phase treated with low doses of MMS, suggesting that it may be possible to detect repair related DNA synthesis (unscheduled scheduled DNA synthesis; USDS) as well as SDS in S phase cells using this approach. This system is now being used to delineate defects we and others have detected in the response of cells derived from patients with neurodegenerative and other diseases to treatment with agents which produce chemical changes in cellular DNA.

C5-307 **Ku PROTEIN COMPLEXES TO HeLa DNA POLYMERASE EPSILON** Pei-Ing Hwang, Gloria Chui, Sharleen Zhou and Stuart Linn, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202
DNA polymerase epsilon (pol ε) from cultured human cells was first isolated as a DNA repair factor for permeabilized human fibroblasts (Nishida et al. 1988 **JBC 263**, 501). Extensive purification of HeLa pol ε gave two major peptides: a >220 kDa catalytic peptide and a tightly-bound, 55-kDa peptide. When monoclonal antibodies were prepared against a purified preparation of HeLa pol ε, antibodies recognizing the catalytic subunit were obtained as expected. However, antibodies to a 85-kDa peptide, a 70-kDa peptide and to a 70- and 49-kDa peptide pair were also obtained. These peptides are found in side fractions of purified pol ε. Among the antibodies recognizing the >220-kDa subunit or the 70- and 49-kDa peptide pair are species which can neutralize- and/or immunodeplete the polymerase activity. The antibodies recognizing the 85-kDa peptide are currently being sub-cloned and characterized.

Microsequencing of the 85-kDa and 70-kDa peptides identified them as the subunits of Ku protein, a DNA binding protein and helicase implicated in recombination-, repair- and transcription functions. Indeed, the antibodies can recognize Ku subunits expressed in *E. coli* and antibodies to the Ku subunits can immunodeplete the catalytic pol ε subunit. We have not yet characterized the 49-kDa peptide.

We have also used the antibodies to show that the same >200-, 70-, and 49-kDa antigens are present in a mammalian protein complex (RC-1) that repairs double-strand breaks and deletions by recombination *in vitro* (Jessberger, et al. 1993 **JBC 268**, 10570). Moreover, the large form of a mammalian damage DNA binding protein (Keeney, et al. 1993 **JBC 268**, 21293) contains the >220-, 85-, 70-, and 49-kDa antigens. All of these observations suggest that Ku protein can form functional complexes with pol ε.

This work was done in collaboration with Drs. Rolf Jessberger (Basel Inst. of Immunology) and Westley H. Reeves (Univ. of North Carolina) and was supported by NIH Grants R01GM30415 and P30ES100896 and DOE grant 92ER61458.

C5-308 SMALL DNA POLYMERASES OF *Trypanosoma brucei*,
Phyllis R. Strauss, Department of Biology, Northeastern University, Boston, MA 02115

DNA polymerase β (pol-β) is involved in base excision repair and short gap repair synthesis and may play an important role in recombination. We are particularly interested in pol-β in African trypanosomes or its equivalent, because of its potential role in repair and recombination, necessary elements in DNA conversion likely to be associated with variable surface glycoprotein switching in African trypanosomes. Previously we reported the presence of a pol-β from *T. brucei* with extreme sensitivity to the trypanocide berenil (diminazene aceturate) (FASEB J. 7, A1291,1993). We have now identified three small DNA polymerases from *T. brucei* that differ on the basis of physical isolation properties, size, and sensitivity to dideoxynucleoside triphosphates, inorganic phosphate and berenil. Two of the three are markedly sensitive to berenil at concentrations as low as 5 nM, while one is only marginally sensitive at 500 nM. (See below). This sensitivity contrasts sharply with that of human DNA polymerase α or β, which regain full activity at 1 μM. Further purification and characterization are in progress.

Properties of Small DNA Polymerases from *Trypanosoma brucei*

Size	Elution Conditions (mM KCl)		Phosphate Sensitivity	Dideoxynucleotide Sensitivity
	P-cellulose	ssDNA	(50 mM)	(10:1 ddNTP:dNTP)
44	400	400	--	+
33	750	600	+	--
19	400	400	+	+

(Supported by funds from WHO and Northeastern University)

C5-309 CLOSELY-OPPOSED PYRIMIDINE DIMERS ARE RESOLVED DURING DNA REPLICATION IN CHO CELLS,
R.J. Reynolds, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

The induction of pyrimidine dimers at closely-opposed positions in the complementary strands of a single DNA double helix creates a complex lesion, the repair of which is complicated by the absence of an undamaged template strand. Successful replication past closely-opposed lesions during semiconservative DNA sysnthesis would resolve the two lesions into separate DNA helices and provide an intact template strand opposite each lesion. A bifilar enzyme-sensitive site (ESS) assay has been employed to examine the fate of closely-opposed dimers in CHO cells, which excise only a small proportion of DNA lesions before DNA synthesis. Unlike dimers induced at isolated positions, the frequency of bifilar ESS observed in UV irradiated CHO cells exhibits an abrubt and extensive decrease during postirradiation incubation. This decrease is independent of excision repair capacity, is coincident in time with semiconservative DNA synthesis and is inhibited by aphidicolin at concentrations that inhibit semiconservative synthesis but that do not inhibit nucleotide excision repair. Newly synthesized DNA was found to be significantly longer than the interdimer distance and replicated molecules appeared to be free of closely-opposed lesions and/or gaps opposite dimers. Thus the majority of closely-opposed lesions induced in CHO cells appears to be resolved during semiconservative DNA synthesis. Support for this research has been provided by research grants RO1 42390 and RO1 55019 from the U.S. National Institutes of Health. Research at Los Alamos National Laboratory is conducted under the auspices of the U.S. Dept. of Energy.

C5-310 **CHARACTERIZATION OF E.COLI DNA POLYMERASE II MUTANTS WITH ALTERED POLYMERASE-EXONUCLEASE SWITCHING.**
Neda Mashhoon and Myron F. Goodman, Dept. of Molecular Biology, University of Southern California, Los Angeles, CA 90089.

Bacterial DNA Polymerase II (pol II), 89.9 KD, contains both polymerase and 3'->5' exonuclease activities on a single polypeptide chain. Genetic evidence suggests possible roles for pol II in response to oxidative damage, spontaneous and adaptive mutation (Escarceller et al (1994) *J. Bacteriol.* 10, 6221-28). Pol II activity is stimulated by DNA polymerase III accessory proteins β, γ complex by increasing the processivity of pol II.

We have made single amino acid substitutions, L423M and I428V, in one of the most conserved regions of pol II called motif A with the sequence **DXXSLYPSII**. Analogous mutations, L412M and I417V, in T4 DNA polymerase result in a polymerase with higher or lower processivity, respectively (Reha-Krantz et al (1994) *J. Biol. Chem.* 269, 5635-43).

A comparison of the processivity of L423M and I428V mutants with the wild type protein will be presented and our data will be discussed in the context of how the interplay between polymerase and exonuclease domains govern accuracy of DNA replication.

C5-311 PROCESSING OF DNA TERMINI: MAMMALIAN DNA KINASES AND DNA LIGASE I.
Dana D. Lasko. Carolyn Slack, Ramani Varanasi, Panagiotis Prinos, Sharon L. Barker, Jehangir Appoo, and Colin H.-P. Ong. Departments of Medicine and Human Genetics, Division of Medical Genetics, McGill University, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1

By fractionation of whole cell extracts of fresh or frozen calf thymus glands using Polymin P, we have purified and characterized an enzymatic activity in the Polymin P pellet fraction (PP-PNK) that is capable of 5' phosphorylation of certain nucleic acid substrates. We have also identified three peaks of DNA kinase activity in the Polymin P supernatant fraction following batch chromatography on phosphocellulose and gradient chromatography on Q-sepharose. These activities were designated SNQI, SNQII, and SNQIII. One (SNQI) has an acidic pH optimum and may represent the DNA kinases previously described and extensively purified from rat liver and calf thymus(1,2). There is genetic and biochemical evidence that DNA ligase I functions in DNA replication and DNA repair in mammalian cells. In immunoblotting experiments, we have observed and increase in the DNA ligase I protein signal following exposure of MDBK cells to ultraviolet light (254 nm), in agreement with results in other cell lines(3). We have expressed the carboxyl-terminal domain of human *LIG1* gene product and the full length human *LIG1* and *S. cerevisiae cdc9* gene products as active GST fusion proteins in *E. coli*.

(1) Zimmerman, S.B., Pheiffer, B.H. 1981. The Enzymes, Vol. XIV. pp 315-329.
(2) Tamura, S., Teraoka, H., Tsukada, K. 1981. Eur J Biochem. **115:** 449-453.
(3) Montecucco, A., Biamonti, G., Savini, E., Focher, F., Spadari, S., and Ciarrocchi, G. 1992. Nucleic Acids. Res. **20**: 6209-6214.

C5-312 THE DNA REPAIR GENE *mus308* OF DROSOPHILA ENCODES A PROTEIN WITH EXCEPTIONAL SEQUENCE SIMILARITY TO POLI-LIKE DNA POLYMERASES AND ALSO TO THE SUPERFAMILY OF DNA/RNA HELICASES, Paul V. Harris, James B. Boyd, and Kenneth C. Burtis, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616

We previously characterized the *mus308* gene, which confers resistance to DNA crosslinking agents but not to monofunctional alkylating agents, a characteristic shared by the Fanconi anemia genes in man. The 7 kb *mus308* transcript encodes a hypothetical protein of 229 kDa. The N-terminal domain contains the seven conserved motifs characteristic of RNA/DNA helicases. The C-terminal domain shares over 50% sequence similiarity to the polymerase domains of bacterial group A (polI-like) DNA polymerases. All amino acid residues which others have identified as critical to polymerase function are 100% conserved. Various lines of evidence indicate that this is not Drosophila polymerase gamma, and therefore may represent a new class of eukaryotic DNA polymerase/helicase specifically involved in the repair of DNA crosslinks. We are currently overexpressing portions of the gene in order to evaluate and characterize enzymatic activities.

C5-313 YEAST DNA POLYMERASES REQUIRED FOR REPAIR SYNTHESIS: Judith L. Campbell, and Martin E. Budd, Division of Biology, California Institute of technology, Pasadena, CA 91125

We have used analysis of postirradiation molecular weight changes in cellular DNA to study the ability of yeast DNA polymerase mutant strains to carry out repair synthesis after UV irradiation. Neither DNA polymerase α, δ, ε nor Rev3 single mutants evidence a defect in repair. A mutant defective in all four of these DNA polymerases, however, showed a similar accumulation of single-strand breaks as is seen in a ligase mutant. Pairwise combination of polymerase mutations revealed a repair defect only in DNA polymerase δ and ε double mutants. The extent of repair in the double mutant was no greater than that in the quadruple mutant, suggesting that DNA polymerase α and Rev3p play very minor, if any, roles. Taken together, the data suggest that DNA polymerases δ and ε are both potentially able to perform repair synthesis and that in the absence of one, the other can efficiently substitute. Thus, two of the DNA polymerases involved in DNA replication are also involved in DNA repair, adding to the accumulating evidence that the two processes may be linked.

Despite considerable biochemical effort, a homolog of mammalian DNA polymerase β had not been found in yeast until recently. By expressing an open reading frame on *Saccharomyces cerevisiae* chromosome III that has 26% identity to rat DNA polymerase β, we demonstrated a new yeast DNA polymerase activity similar to mammalian DNA polymerase β and designate the ORF as *POL4*. Deletion mutants are viable and show normal resistance to ultraviolet irradiation, X-rays, and methyl methanesulfonate during vegetative growth. However, an important function in meiosis is suggested since *POL4* mRNA is very rare in mitosis but is induced at least 30 fold during meiosis. *pol4Δ/pol4Δ* strains show a significant drop in spore viability. Thus, DNA polymerases δ and ε seem to be more important for repair in mitosis, but DNA polymerase β may play an important role in meiotic repair.

C5-314 MUTAGENESIS OF HIGHLY CONSERVED AMINO ACID RESIDUES IN MOTIF II OF A DNA HELICASE, Robert M. Brosh, Jr. and Steven W. Matson, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280

Site-directed mutagenesis has been employed to address the functional significance of the highly conserved aspartic and glutamic acid residues present in the Walker B (also called motif II) sequence in *Escherichia coli* DNA helicase II. Two mutant proteins, UvrDE231Q and UvrDD230NE231Q, were expressed and purified to apparent homogeneity. Biochemical characterization of the DNA-dependent ATPase activity of each mutant protein demonstrated a k_{cat} <0.5% that of the wild type protein with no significant change in the apparent K_m for ATP. The E231Q mutant protein exhibited no detectable unwinding of either partial duplex or blunt duplex DNA substrates. The D230NE231Q mutant, however, catalyzed unwinding of both partial duplex and blunt duplex substrates, but at a greatly reduced rate as compared to the wild type enzyme. Both mutants were able to bind DNA. Thus the motif II mutants, E231Q and D230NE231Q, were able to bind ATP and DNA to the same extent as wild type helicase II, but demonstrate a significant reduction in ATP hydrolysis and helicase functions.

The *uvrDE231Q* and *uvrDD230NE231Q* alleles were also characterized by examining their abilities to complement the mutator and ultraviolet light sensitive phenotypes of a *uvrD* deletion mutant. Neither mutant *uvrD* allele, supplied on a plasmid, was able to complement either phenotype. Further genetic characterization of the mutant *uvrD* alleles demonstrated that *uvrDE231Q* confers a dominant negative growth phenotype; the *uvrDD230NE231Q* allele does not exhibit this effect. The observed difference in effect on viability may reflect the gene products' dissimilar kinetics for unwinding duplex DNA substrates *in vitro*.

C5-315 DNA POLYMERASE δ IS REQUIRED FOR BASE EXCISION REPAIR OF DNA METHYLATION DAMAGE IN *SACCHAROMYCES CEREVISIAE*. A. Blank, B. Kim & L.A. Loeb, Dept. of Pathology, University of Washington, Seattle, WA 98195

We present evidence that DNA polymerase δ of *Saccharomyces cerevisiae*, an enzyme which is essential for viability and chromosomal replication, is also required for base excision repair of exogenous DNA methylation damage. The large catalytic subunit of DNA polymerase δ is encoded by the *CDC2 (POL3)* gene. We find that the mutant allele *cdc2-2* confers sensitivity to killing by methyl methanesulfonate (MMS) but allows wild type levels of UV survival. Survival of haploid *cdc2-2* strains is lower than wild type when stationary phase cells are treated with MMS at the permissive growth temperature of 20°C. Survival is further decreased relative to wild type by treatment with MMS at 36°C, a non-permissive temperature for growth of mutant cells. Thus, the methyl excision repair deficit in *cdc2-2* cells is temperature sensitive, as is growth, DNA replication, and the catalytic activity of the mutant DNA polymerase δ *in vitro*. A second DNA polymerase δ allele, *cdc2-1*, also confers a temperature sensitive defect in MMS survival while allowing nearly wild type levels of UV survival. These observations provide an *in vivo* genetic demonstration that a specific eukaryotic DNA polymerase is required for survival of exogenous methylation damage. MMS sensitivity of a *cdc2-2* mutant at 20°C is complemented by expression of mammalian DNA polymerase β, an enzyme which fills single-strand gaps in duplex DNA *in vitro* and whose only known catalytic activity is polymerization of deoxyribonucleotides. The simplest explanation for our results is that the MMS survival deficit in *cdc2-2* cells is caused by failure of the mutant DNA polymerase δ to fill single-strand gaps arising in base excision repair of methylation damage. We discuss our results in light of current concepts of the physiologic roles of DNA polymerases δ and ε in DNA replication and repair.

C5-316 *E.COLI* HELICASE II UNWINDS DNA BY AN ACTIVE MECHANISM, Janid A. Ali and Timothy M. Lohman, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, MO 63110-1093

E.coli Helicase II, the product of the *uvrD* gene is a DNA helicase which functions in uvrABC excision repair and methyl directed mismatch repair and has been implicated in DNA replication.

We have used rapid quench-flow methods to investigate the mechanism of Helicase II-catalyzed DNA unwinding of a series of short DNA duplex substrates at low DNA concentrations (1 nM) to prevent re-annealing of the substrate during the time course of the reaction. Helicase II was pre-incubated with DNA substrate and unwinding was initiated by rapid mixing with ATP. Excess $(dT)_{16}$ was also included with the ATP to trap any free Helicase II or non-productively bound helicase II that rapidly dissociates from the DNA, enabling us to study DNA unwinding by "productive" Helicase II complexes under single turnover conditions ([Helicase II] >[DNA]). Under these conditions, a 3' ss-DNA covalently attached to the duplex region is required for rapid unwinding by Helicase II; a 5' ss-DNA tail is neither sufficient for unwinding nor does it enhance unwinding of a DNA with a 3' ss-DNA flanking region. A 16 nucleotide region is the minimum length required for the 3' ss-DNA tail to support unwinding in the presence of the $(dT)_{16}$ trap.

To determine if Helicase II unwinds DNA by a "passive" mechanism in which the helicase interacts only with the 3' ss-DNA tail and translocates into the duplex region at the ss-/ds-DNA junction only during a transient fraying of the duplex end due to thermal fluctuations, we constructed a series of non-natural DNA substrates in which the 3' ss-DNA tail contains a stretch of nucleotides where the backbone polarity of each alternating nucleotide is reversed. Helicase II unwinds these substrates efficiently ruling out a passive mechanism of unwinding. These results indicate that Helicase II unwinds DNA by an "active" mechanism. Since the active form of Helicase II is at least dimeric, it is likely that the helicase binds simultaneously to both the duplex and ss-DNA regions during an intermediate stage of the unwinding reaction as appears to be the case for the dimeric *E. coli* Rep helicase. (supported by NIH GM45948).

C5-317 CHARACTERIZATION OF THE 5'→3' EXONUCLEASE ACTIVITY OF A *THERMUS THERMOPHILUS* DNA POLYMERASE, Tatiana Auer, Phoebe A. Landre, and Thomas W. Myers, Program in Core Research, Roche Molecular Systems, Alameda, California 94501.

Eubacterial single-subunit DNA polymerases such as *Escherichia coli* DNA pol I typically possess a 5'→ 3' exonuclease activity. This nucleolytic activity is known to be responsible for removing DNA lesions during excision repair and for removing the RNA primers formed during lagging-strand DNA synthesis. A recombinant 94 kDa *Thermus thermophilus* DNA polymerase (r*Tth* pol) was found to release $[^{33}P]$UMP when incubated with a RNA•DNA hybrid containing a $[^{33}P]$UTP-labeled RNA strand. The RNase H-type activity was optimally active in the presence of low monovalent salt concentrations and when Mn^{2+} was used as the divalent cation activator. RNase H activity was also observed when Mg^{2+} replaced the Mn^{2+}, but to a much lesser extent. A 60-nucleotide long, 5'- or 3'-labeled, RNA or DNA oligomer hybridized to a complementary DNA oligomer was used to determine the mode of enzymatic digestion. The 5'-labeled $[^{32}P]$-RNA•DNA hybrid and $[^{32}P]$-DNA•DNA duplex were incubated with r*Tth* pol using various metal ion conditions and different incubation times. The $[^{32}P]$-DNA•DNA duplex showed very little enzymatic cleavage regardless of the Mn^{2+} or Mg^{2+} concentration. However, nearly complete digestion of the $[^{32}P]$-RNA•DNA hybrid was observed over a wide Mn^{2+} concentration range, thus demonstrating a preferential degradation of RNA•DNA hybrids. Time course reactions of the enzymatic digestion of the 3'-labeled RNA•DNA hybrid and DNA•DNA duplex by r*Tth* pol indicated that digestion of the substrates occurred exonucleolytically in the 5'→3' direction.

C5-318 *DNA2*, A NEW YEAST GENE REQUIRED FOR DNA REPLICATION ENCODES A DNA HELICASE: Martin E. Budd and Judith L. Campbell, Division of Biology, California Institute of Technology, Pasadena, CA 91125

A new yeast gene has been identified by screening for DNA replication mutants using a permeabilized cell in vitro replication assay. The mutant is temperature sensitive for growth and shows a cell cycle phenotype typical of DNA replication mutants. RNA synthesis is normal in the mutant but DNA synthesis is inhibited upon shift to the nonpermissive temperature indicating a defect in the elongation stage of DNA replication. The DNA sequence of the gene reveals that it encodes a protein with characteristic DNA helicase motifs. Purification of the protein encoded by the gene and assay of the protein reveals a DNA-dependent ATPase and a DNA helicase activity specific for substrates that mimic DNA replication forks. The helicase has a 3' to 5' directionality on the strand to which it binds, suggesting that it, like the SV40 large T antigen, is associated with the leading strand at the replication fork.

C5-319 REQUIREMENT OF AN INTACT ACTIVATION DOMAIN OF THE BPV TRANSACTIVATOR E2 FOR REPLICATION AND TRANSCRIPTION, Mary K. Ferguson and Michael R. Botchan, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94702

The bovine papilloma virus protein E2 is a 48 kDa. transcriptional activator that also plays a direct role in the initiation of viral replication. E2, which binds DNA as a dimer, interacts with the viral helicase E1, and can cooperatively stimulate the ability of E1 to bind the BPV replication origin. E2 has a modular structure consisting of an amino terminal activation domain and a DNA binding/dimerization domain at the carboxyl end, separated by a relatively unstructured hinge region. The activation domain is necessary for both the transcriptional activation and replication functions of the protein. The E2 genes from ten different serotypes have been compared and show about 30% homology in their activation domain, and the function is clearly conserved as E2 proteins from different species can complement each other. This would indicate that analysis targetting the conserved residues would provide insight into a functional and structural map of the domain. In order to determine which regions of the domain are necessary for these two functions we have constructed a series of 30 single amino acid substitutions, changing conserved residues throughout the activation domain. These mutants were analyzed in transient transfection assays to determine the effect of the substitutions on replication and on transcription. Our analysis reveals that more than half of these mutations affect the accumulation of E2 in transient assays, indicating that the structure of the domain may be easily destabilized. These destabilizing changes span the entire domain from residue 7 to 179. Furthermore, many mutants which affect either folding kinetics or protein stability show no phenotype when intracellular levels reach a critical threshold. Apparent separation of function in certain instances may thus reflect a different requirement for protein levels in these two roles of the protein. Most mutations that we analyzed affect both replication and transcription equally, implying that a single structure is involved in replication and transcriptional activation. Only changes at one residue separate transcription from replication; changes of isoleucine at position 73 to asparagine or alanine both severly reduce transcriptional activation, but leave the replication functions relatively intact. Interestingly, IA73 has a temperature sensitive phenotype for transcription. In assays done at 33.5 °C, transactivation is mostly restored. We are in the process of structurally characterizing this domain, and conclude that the actvation domain cannot be divided into discrete subdomains of function.

C5-320 GENETIC FACTORS AFFECTING REPLICATION SLIPPAGE BETWEEN DISTANT SHORT REPEATS IN YEAST. Dmitry Gordenin[1,2], Hiep Tran[1], Natalya Degtyareva[2], Nadejda Koloteva[2] and Michael Resnick[1] [1]Laboratory of Molecular Genetics, Nat. Inst. of Environmental Health Sciences, Research Triangle Park, NC 27709 (FAX: 919 541-7593), [2]St. Petersburg State Univ., Russia.

Since small direct repeats are common in all genomes, deletions arising by slippage between them could be an important source of chromosomal instability. A temperature-sensitive DNA polymerase III mutant (*pol3-t=tex1*) exhibits frequent deletion between small (4-9 nt) direct repeats when separated by large inverted repeats. We proposed that this was due to slippage during replication of the inverted repeat region[1]. Intrinsic to our model is the idea that a large inverted repeat could bring distant small direct repeats into close proximity, providing an opportunity for slippage during replication. Since deletions between distant short repeats can be mediated by LIRs, we wanted to know if the mutant polymerase III could also "jump" between closely spaced repeat sequences. Deletions were examined in the *LYS2* using a set of 31-156 bp inserts. The set included two quasipalindromes and two inserts with no potential for secondary structure. This enabled us to compare deletions of sequences containing various inverted repeats *vs* deletion of random sequences. All inserts were flanked by 6-9 bp direct repeats of the *LYS2* sequence providing an opportunity for LYS^+ reversion *via* precise excision. Reversion could also occur by "in-frame" imprecise deletion. Breakpoints of both types of deletions were always associated with short (3-9 bp) direct repeats in agreement with the model of replication slippage. For the nonpalindromic deletions selected in the DNA polymerase δ mutant (*pol3-t*) some of the deletions involved repeats with a one base pair mismatch. The repeat that was retained depended upon the direction of replication through *LYS2*. The deletions in our system thus appear to be generated by replication slippage primarily in one strand (leading or lagging). The *pol3-t* mutation increased the rates and expanded the deletion spectrum. The elevated rates were reduced by *rad50* and *rad52* mutations, suggesting an interaction between the double-strand break repair and the replication systems.

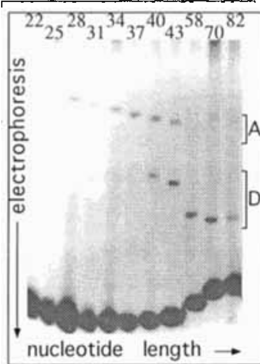
[1]Gordenin *et al.*, PNAS 89 (1992) 3785; MCB 13 (1993) 5315.

C5-321 DNA POLYMERASE β BYPASSES A SINGLE d(GpG)-CISPLATIN ADDUCT, Jean-Sébastien Hoffmann[1], Marie-Jeanne Pillaire[1], Giovanni Maga[2], Vladimir Podust[2], Ulrich Hübscher[2], and Giuseppe Villani[1], [1]Laboratoire de Pharmacologie et Toxicologie Fondamenntales du CNRS, 205 route de Narbonne, 31077 Toulouse cedex, France and [2]Dept of Veterinary Biochemistry, Winterthurerstrasse 190, University of Zürich-Irchel, CH-8057 Zürich, Switzerland.

We have recently reported that a single-stranded vector containing a unique d(GpG)-cis-diamminedichloroplatinum adduct placed on codon 13 (GGT) of the human *H-ras* proto-oncogene was efficiently replicated in Monkey cells (M.J. Pillaire, A. Margot, G. Villani, A. Sarasin, M. Defais, and A. Gentil (1994) *Nucleic Acids Res.* 23, 2519-2524). However, the steps leading to replication past the Pt-d(GpG) adduct remain to be established. We therefore undertook the present investigation to determine whether direct biochemical evidence for translesion past the adduct could be obtained with purified mammalian DNA polymerases. For this purpose, we have constructed an oligonucleotide substrate bearing a single Pt-d(GpG) lesion on the codon 13 of the same *H-ras* sequence used in the *in vivo* study. We have examined the capacity of calf thymus DNA polymerases α, β, δ, and ε, to replicate the platinated substrate, either separately or in combination with calf thymus RP-A and PCNA. We report that only DNA polymerase β is capable of translesional synthesis, suggesting a role for this enzyme in replication of platinated single-stranded vector observed *in vivo*. Furthermore, we show that pol β is able to initiate DNA replication opposite the cisplatin adduct, in contrast to what observed with pol α, δ, and ε. We propose that initiation of DNA synthesis from the position of the lesion could be a key step for d(GpG)-cisplatin adduct bypass.

C5-322 "A MAMMALIAN PROTEIN FORMS SALT-STABLE COMPLEXES WITH SINGLE-STRANDED DNA FRAGMENTS THAT CONTAIN A MINIMUM OF 40 RESIDUES" Michael Mitas,[1]Jeffrey Dill,[1] Adong Yu,[1]Timothy J. Kamp,[2]Johanna Y. Chock,[3] and Wenjun Huang.[1] [1]Department of Biochemistry and Molecular Biology, 246 Noble Research Center, Oklahoma State University, Stillwater, OK 74078. [2]Department of Medicine, Division of Cardiology, Johns Hopkins University School of Medicine, 844 Ross Avenue, Baltimore, MD 21205. [3]NHLBI, NIH, Bethesda, MD 20892.

To identify mammalian single-stranded DNA binding proteins (SSBs) potentially involved in DNA replication or DNA repair, electrophoretic mobility shift assays (EMSAs) were performed with DNAs of varying length and bacteriophage T4 gene 32 protein, *E. coli* SSB, human or rodent cell extracts, or with human recombinant replication protein A (RPA). The electrophoretic mobilities of two protein-DNA complexes (A and D) formed with the extracts increased as a function (~directly proportional) of the length of the DNAs, thus revealing two descending staircase patterns (see figure). The minimum nucleotide lengths required for formation of complexes A and D were 28 and 40, respectively. Complex A (but not complex D) formed with human extract was supershifted with antibodies to the 34 or 70 kDa subunits of human RPA. Complex D was stable in 2 M NaCl and its maximal electrophoretic mobility occurred with DNA fragments 70 nts in length. The rodent protein responsible for formation of complex D (named SSB-D), was not eluted from a DNA-cellulose column with buffer containing 600 mM $(NH_4)_2SO_4$, but was eluted with buffer containing ss DNA. SDS-PAGE analysis of the eluted fraction revealed a protein purified to near homogeneity of 43 kDa, a size not corresponding to the subunits of RPA. We suspect that SSB-D is expressed in all mammalian cells and plays an important role in DNA replication or in DNA repair.



C5-323 DIFFERENTIAL BLOCKAGE AND BYPASS OF THYMINE DIMERS IN LEADING AND LAGGING STRANDS OF A HUMAN REPLICATION FORK, Daniel L. Svoboda* and Jean-Michel H. Vos*†, *Lineberger Comprehensive Cancer Center and †Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

SV40 mini-replicons containing a uniquely placed *cis,syn*-thymine dimer (T[]T) were constructed and assayed for replication using HeLa cytoplasmic extract *in vitro*. Three constructs were prepared; one with T[]T in the leading strand, another with T[]T in the lagging strand, and a third with unmodified TT. Labeled replication products from a time course ranging from 10-60 min. were treated with T4 UV endonuclease and analyzed on agarose gels. The level of nicking by T4 UV endonuclease was <50% indicating that there was significant synthesis of daughter molecules from the undamaged template strand while synthesis from the T[]T containing strand was blocked. This would result from uncoupling of the concerted synthesis of the two strands of the replication fork at the site of blockage or the formation of a gapped duplex structure from the T[]T containing template strand. Form II DNA was produced from the lagging strand T[]T template consistent with the production of daughter molecules with small gaps. Form II was not produced from the leading strand T[]T template suggesting that uncoupling is responsible for the excess form I synthesized from the undamaged lagging template strand. Quantitative analysis of restriction fragments run on sequencing gels suggests that when T[]T is in the leading strand template, there are replicative intermediates where elongation of both the leading and lagging strands is blocked representing a stalled replication fork. The proportion of form I daughter molecules nicked by T4 UV endonuclease yields a rate of synthesis of T[]T containing daughter molecules relative to the rate of synthesis from unmodified template of 20% for lagging strand T[]T and 30% for leading strand T[]T. Synthesis opposite T[]T as well as the blockage site was directly observed from sequencing gel analysis of restriction enzyme digests of replication products.

C5-324 CHARACTERIZATION OF DNA POLYMERASE β MUTANTS USING A HETEROLOGOUS SYSTEM, **Joann B. Sweasy, Margaret S. Yoon, Jessica L. Kosa, Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510.**

Vertebrate DNA polymerase ß is a 39 kD protein that has only one known catalytic activity, namely polymerization of deoxyribonucleotides. We previously described the ability of DNA polymerase β to substitute for DNA polymerase I of *E. coli* in DNA replication and repair (1). Here, we make use of this quantitative complementation assay to characterize individual DNA polymerase ß mutants with regard to their abilities to function in DNA replication and DNA repair. We also describe a screen for the detection of mutator activity of DNA polymerase ß mutants. By using these bioassays, together with DNA polymerase activity gels, we characterize 15 new DNA polymerase ß mutants that display a wide spectrum of phenotypes. Our work affords a novel perspective on the functional properties of DNA polymerase ß mutants *in vivo*. It also provides a model for the use of complementation assays, in a heterologous host, to analyze mutant mammalian enzymes whose phenotypes *in vivo* are otherwise difficult to assess.

1. Sweasy, J.B. & Loeb, L.A. 1993. Detection and characterization of mammalian DNA polymerase β mutants by functional complementation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90**: 4626-4630.

C5-325 EXPRESSION OF HUMAN DNA POLYMERASE EPSILON Juhani E. Syväoja, Lahja Uitto, Tapio Kesti and Jussi Tuusa, Biocenter Oulu and Department of Biochemistry, University of Oulu, FIN-90570 Oulu, Finland

Steady state mRNA levels of human DNA polymerase ε are elevated prior to the peak of DNA replication in a similar manner with DNA polymerase α when quiescent cells are activated to proliferate. The enzyme protein is also much more abundant in extracts from proliferating tissues than in those from nonproliferating or slowly proliferating tissues. In actively cycling cells the message levels remain relatively constant throughout the cell cycle suggesting constitutive expression in proliferating cells. With the exception of UV light, treatment of density inhibited cell cultures with various DNA damaging agents, do not significantly affect steady state levels of the mRNA. In addition to repair of UV damage, mammalian DNA polymerase ε is clearly involved in replication of chromosomal DNA or in a process closely associated with replication. This is in contradiction to the results obtained earlier from studies on model system SV40 DNA replication in vitro. A model on the role of DNA polymerase ε will be presented.

C5-326 CLONING OF A CANDIDATE GENE FOR COCKAYNE SYNDROME GROUP A, Karla A. Henning, Prakash Bhatia, Randy Legerski[1] and Errol C. Friedberg, Laboratory of Molecular Pathology, Department of Pathology, The University of Texas Southwestern Medical Center, Dallas, TX 75235, and [1]Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Using an episomal vector-based cDNA library, we have cloned a novel human cDNA which complements the UV sensitivity of Cockayne syndrome group A cells. CS-A cells carrying this cDNA were also complemented for their ability to reactivate a UV-irradiated plasmid expressing the chloramphenicol acetyl transferase (*cat*) gene. The cDNA had no effect on the UV sensitivity of Cockayne syndrome group B cells, or on the *cat* reactivation ability of CS-B cells. The CS group A-complementing cDNA is 2.2 kb in length, with an open reading frame which could encode a 396 amino acid peptide. Analysis of the protein sequence places this protein in the WD-repeat family of regulatory proteins. WD-repeat proteins are evolutionarily conserved throughout eukaryotic systems, and we are in the process of identifying homologs from Drosophila and yeast. In order to establish whether the complementing cDNA is in fact the *CSA* gene, we are presently sequencing RT-PCR products from CS-A cell lines to search for mutations in the coding region.

C5-327 CLINICAL ASPECTS AND FUNCTIONAL ANALYSIS OF TRANSCRIPTION/REPAIR UNIT TFIIH. Wim Vermeulen, Hanneke J. van Vuuren, Geert Weeda, Dirk Bootsma, Jean-Marc Egly and Jan H.J. Hoeijmakers. MGC Dept. of Genetics, Erasmus University, PO Box 1738, 3000DR Rotterdam, The Netherlands.

Deficient nucleotide excision repair (NER) is observed in three clinically distinct hereditary syndromes: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). All three disorders are phenotypically and genetically heterogenous (respectively 7, 5 and 3 complementation groups). However, a significant overlap between these diseases has recently become evident. A subgroup of NER-deficient patients exists, exhibiting features of 2 of these syndromes (combined XP/CS and XP/TTD), predominantly in XP groups B and D. *XPB* and *XPD* encode for helicases, with opposite directionality. It has been shown that these repair helicases are associated with basal transcription factor TFIIH. Using *in vivo* and *in vitro* NER-assays we demonstrate that a third repair factor (TTDA) is connected with TFIIH. The repair correcting activity of TFIIH, for these three complementation groups, is superimposable on the intrinsic transcriptional and helicase activities, in elution profiles, of TFIIH. Together with antibody depletion experiments it is further shown that probably the entire TFIIH, as a functional unit, is involved. Patients belonging to TFIIH-associated groups (XP-B,-D and TTD-A) exhibit features (such as: neurodysmyelination, retarded growth and brittle hair) which are difficult to explain on the basis of a defective DNA repair. Several of these peculiar symptoms might be the result of an slightly impaired functioning of the transcription role of TFIIH. We propose to categorize these combined XP/CS and XP/TTD individuals as members of a new clinical entity: "repair/transcription syndrome".

The differential effect of mutated TFIIH components (XPB, D and p44), after overexpression in human cells, on repair and transcription is investigated. With the aid of alternative purification protocols (tagged components), the association of TFIIH (under native conditions) with, either other basal transcription factors, or other NER proteins will be presented.

C5-328 MOUSE MODELS OF HUMAN NUCLEOTIDE EXCISION REPAIR/TRANSCRIPTION DEFICIENCY SYNDROMES,

[1]David L. Cheo, [2]Robert Hammer, [1]Luis Nieves, [1]Kathleen Wilson and [1]Errol C. Friedberg, [1]Department of Pathology and [2]Department of Biochemistry, Howard Hughes Medical Research Institute, University of Texas Southwestern Medical School, Dallas, TX 75235

Deficiencies in human nucleotide excision repair genes can result in three distinct genetic diseases, xeroderma pigmentosum (XP), Cockayne's syndrome (CS) and trichothiodystrophy (TTD). Patients with these diseases demonstrate a diverse and complex spectrum of clinical phenotypes. To investigate the molecular pathology of these diseases we are developing mutant mouse models. Mutations within several mouse XP genes have been generated and targeted to their normal loci by homologous recombination in embryonic stem (ES) cells. Injection of blastocyst-stage embryos with ES cells carrying a knockout of the mouse *XPC* gene produced several germline chimeric mice. The *XPC* knockout mutation has been bred to homozygosity. Characterization of the complete phenotype of these mice is in progress. We have also constructed and introduced into mouse ES cells a knockout of the mouse *XPG* gene and subtle mutations in the mouse *XPD* gene. Our goal is to precisely model mutations identified in humans that result in XP, XP complicated by CS, and TTD. These mouse models are expected to serve as important tools to examine the molecular pathology of these diseases.

C5-329 **Generation of mouse models for human DNA repair/transcription syndromes**

Geert Weeda, Ingrid Donker, Jan de Wit, Jan de Boer, Hans Morreau*, Harry van Steeg#, Dirk Bootsma and Jan H.J. Hoeijmakers., MGC-Dept. of Cell Biology and Genetics, Erasmus University. P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. * Department of Pathology, Erasmus University, #RIVM, Bilthoven.

Patients suffering from the rare autosomal recessive repair disorders xeroderma pigmentosum (XP) and Cockayne syndrome (CS) exhibit severe sun (UV)-sensitivity, frequently neurological dysfunction and -in the case of XP- cutaneous abnormalities and predisposition to cancer. The molecular defect in these genetically heterogeneous diseases resides in the nucleotide excision repair pathway (NER). Using excision-deficient (UV-sensitive) rodent mutant cell lines we have isolated several complementing human repair genes.

The *ERCC1* gene specifically corrects rodent group 1 (mutant sensitive for UV and DNA crosslinking agents) and appears to be not involved in any of the known XP and CS complementation groups.

The *ERCC3* gene encodes a DNA helicase. The gene was initially cloned by virtue of its ability to correct rodent group 3 mutants and underlies the genetic defect in the rare combined XP/CS group B. Recently it was shown, both *in vivo* and *in vitro*, that the *ERCC3* gene (and its yeast homolog *RAD25*) is an indispensable component of the basal transcription initiation factor TFIIH, and is essential for viability.

To gain more insight in the function of both genes and their contribution in preventing mutagenesis and carcinogenesis, we decided to utilize gene targeting in ES-cells for generation of corresponding mouse mutants.

For *ERCC1* homozygous "knockout" and more subtle pointmutations proved viable, although in both cases the frequency of -/- offspring was below Mendelian expectation, the mice exhibit severe growth defects and have a reduced lifespan (-/- knock-out die before 3 weeks, subtle mutant females reach 10 weeks, males 4 weeks). Pathological findings include absence of subcutaneous fat, striking polyploidy and chromatin abnormalities in liver and kidney as well as ferritin deposition in the spleen. No overt abnormalities were seen in other tissues like CNS, ovaria, testis and the eyes. These mice probably die as a consequence of liver failure. *ERCC1*-deficient mice are very sensitive to DMBA/TPA application and -/- cells are completely repair defective. Heterozygous mice show delayed clinical symptoms. We attribute these features which in some respects are reminiscent of premature ageing to a combination of repair and recombination defects.

A ERCC3 deficiency aimed of mimicking a mutation in a XP/CS patient proved embryonal lethal for mice in homozygous state, consistent with the essential role of the gene in basal transcription.

We are currently crossing these mice with a LacZ reporter gene-containing mouse to monitor mutation induction in different organs and tissues.

C5-330 ANALYSIS OF REPAIR OF CYCLOBUTANE PYRIMIDINE DIMERS AND PYRIMIDINE 6-4 PP PYRIMIDONE PHOTOPRODUCTS IN TRANSCRIPTIONALLY ACTIVE AND INACTIVE GENES IN CHINESE HAMSTER CELLS,

Maaike P.G. Vreeswijk, Anneke van Hoffen, Birgit E. Westland, Harry Vrieling, Albert A. van Zeeland, and Leon H.F. Mullenders, MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, 2333 AL Leiden, The Netherlands.

Irradiation of cells with short-wave ultraviolet light induces both cyclobutane pyrimidine dimers (CPD) as well as pyrimidine 6-4 PP pyrimidone photoproducts (6-4 PP). We have focussed on the removal of both types of DNA photolesions from the transcriptionally active adenine phosphoribosyl transferase (APRT) and hypoxanthine-guanine phosphoribosyl transferase (HPRT) genes and the inactive c-mos gene. Unlike the HPRT gene, in which CPD were selectively removed from the transcribed strand, both strands of the APRT gene were efficiently repaired, suggesting the existence of a transcript running in the opposite direction of the APRT gene. Only a marginal part of the CPD was removed from the inactive c-mos gene after 24 h. In all three genes investigated, 6-4 PP were repaired more rapidly than CPD and, as demonstrated for the HPRT and APRT genes, without strand specificity. The difference in the repair phenotype of CPD between the HPRT gene and the APRT gene coincides with differences between both genes with regard to the DNA strand distribution of previously published UV-induced mutations.

C5-331 GENE-SPECIFIC REPAIR OF N-ACETOXY-ACETYLAMINOFLUORENE INDUCED DNA ADDUCTS.

Michiel F. van Oosterwijk, Leon H.F. Mullenders and Albert A. van Zeeland. MGC-Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands.

Excision repair of UV-induced DNA photolesions (cyclobutane pyrimidine dimers and 6-4 photoproducts) in transcriptionally active genes is mediated by two pathways : (i) transcription coupled repair (TCR,dependent on transcription) and (ii) global repair (GR). To investigate the contribution of both repair pathways to repair of structurally different lesions we examined the repair of N-Acetoxy-AcetylAminoFluorene (NA-AAF) induced DNA adducts in active and inactive genes in normal human (TCR and GR), xeroderma pigmentosum group C (TCR) and Cockayne's syndrome (GR) cells. We employed the UvrABC excinuclease to determine adduct frequencies in restriction fragments of genes. The most frequently induced adduct i.e. the deacetylated dG-C8-AF, is capable to block transcription. Repair experiments indicated that in all cell lines the removal of DNA adducts from the active ADA gene is faster than from the inactive 754 locus. Comparison of the various cell lines revealed that removal of NA-AAF induced lesions from active genes in normal human and Cockayne's syndrome (CS) cells is more rapidly than in xeroderma pigmentosum group C (XP-C) cells which only performed slow repair of the ADA gene. This is in contrast to repair of UV-induced cyclobutane pyrimidine dimers in active genes which is more rapidly in XP-C than in CS cells. In spite of the limited repair XP-C cells are only mildly sensitive to NA-AAF and able to recover RNA synthesis. On the contrary CS cells are sensitive to NA-AAF and unable to recover RNA synthesis. The results indicate that NA-AAF induced DNA adducts are removed by TCR and GR and that the defect in CS is not simply related to a defect in preferential repair of active genes

C5-332 TRANSCRIPTION-COUPLED REPAIR REMOVES BOTH CYCLOBUTANE PYRIMIDINE DIMERS AND 6-4 PHOTOPRODUCTS WITH EQUAL EFFICIENCY AND IN A PROCESSIVE WAY FROM TRANSCRIBED DNA IN XERODERMA PIGMENTOSUM GROUP C FIBROBLASTS. Anneke van Hoffen, Jaap Venema, Roberta Meschini, Albert A. van Zeeland and Leon H.F. Mullenders.
We investigated whether transcription-coupled nucleotide excision repair can act on structurally different lesions with equal efficiency and in a processive way. The repair kinetics of UV-induced cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP) were determined in active genes in xeroderma pigmentosum group C (XP-C) fibroblasts and in normal human fibroblasts. In a previous study it was shown that in normal human cells exposed to a UV-dose of 10 J/m^2 repair of CPD takes place via two pathways: global repair and transcription coupled repair, the latter being responsible for accelerated repair of the transcribed strand of active genes. In XP-C cells only transcription coupled repair is active. Here we show that in XP-C cells exposed to 30 J/m^2, CPD as well as 6-4PP appear to be removed selectively and with similar kinetics from the transcribed strand of the adenosine deaminase (ADA) gene. The nontranscribed strand of the ADA gene and the inactive 754 gene were hardly repaired. The similarity of the rate and the much lower efficiency of repair of both 6-4PP and CPD in the transcribed strand at 30 J/m^2 as compared to 10 J/m^2 suggests that transcription-coupled repair of photolesions takes place in a processive way. In normal cells exposed to 30 J/m^2, global repair appears to overrule transcription-coupled repair resulting in lack of strand specific repair of both CPD and 6-4PP. However, 6-4PP are removed faster from the active ADA gene than from the inactive 754 gene, suggesting a role for chromatin structure in the mechanism of preferential repair of active genes. Moreover, 6-4PP are repaired much faster than CPD, propably due to the higher affinity for 6-4PP of repair enzymes which specifically are involved in the global repair pathway. Our results strongly suggest that the significance of transcription-coupled repair for removal of lesions depends on the type of lesion and on the dose employed.

C5-333 STRAND-SPECIFIC AND SITE-SPECIFIC RATES OF REPAIR OF BENZO(a)PYRENE DIOL EPOXIDE ADDUCTS IN THE *HPRT* GENE OF HUMAN FIBROBLASTS, Veronica M. Maher, Ruey Hwa Chen, Dong Wei, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.
To investigate the effect of excision on the spectrum of mutations induced by BPDE, we exposed synchronized cells in early S or early G at least 12 h prior to replication. The frequency of mutants was 300 X 10^{-6} for S cells and 100 X 10^{-6} for G_1 cells. 98% of the base substitutions involved G·C, mainly G·C to T·A, but the strand distribution of the guanine differed significantly. For S cells 24% were in the transcr. strand; for G1 cells, none were. No such cell cycle-dependent difference was seen in repair-deficient XP cells, suggesting strand-specific repair of BPDE adducts. To test this we used UvrABC to excise the adducts and analyzed the DNA with strand-specific *HPRT* probes. Within 7 h after treatment with BPDE, 55% of the adducts had been removed from the transcr. strand, but only 25% from the nontranscr. strand; after 20 h, these values were 87% and 58%. Only 38% had been removed from the genome overall. For S cells 5% of the substitutions were at nt 212 and 5% at nt 229. However, in G_1 cells these values were 21% and 10%. No such difference was seen in XP cells, suggesting site-specific repair had occurred. To test this, we adapted LM-PCR to measure the rate of removal of adducts from individual sites in *HPRT*. Cells were treated in G_1 (0.5 μM), harvested at 0, 10, 20, 30 h, and analyzed for adducts using UvrABC. A gene-specific primer was annealed and extended to generate a blunt end at the site of each cut. A linker was ligated to the blunt end, and the desired fragments were isolated, amplified, and analyzed. Rates of repair at individual sites varied and were slowest at nt 212 and 229, indicating that inefficient repair is involved in mutation hot spots (NCI Grants CA21253 & CA56796).

C5-334 DEFECTS IN TRANSCRIPTION-COUPLED REPAIR OF IONIZING RADIATION-INDUCED DNA DAMAGE IN XP GROUP G AND COCKAYNE SYNDROME, Priscilla K. Cooper and Steven A. Leadon, Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720 and Department of Radiation Oncology, University of North Carolina, Chapel Hill, NC 27599-7512

Consistent with the fact that there is little overlap in the spectrum of lesions induced, very few mammalian cell mutants are hypersensitive to both UV and ionizing radiation. However, two different human genetic syndromes that confer defects in UV repair include representatives with cellular radiosensitivity. One is Cockayne syndrome (CS), in which the rapid repair of UV damage in actively transcribing DNA is absent. At least two genes, *CSA* and *CSB*, are required for this transcription coupling of repair. While mutants of each have equal UV sensitivity, we find that all four CS-B strains tested are significantly hypersensitive to γ-rays, while two CS-A strains are intermediate in sensitivity. We recently showed that both normal human cells and UV-sensitive xeroderma pigmentosum (XP) group A cells preferentially repair radiation damage in the transcribed strand of active genes. We find that all CS-B strains tested are completely defective in this preferential repair of γ-ray damage in transcribed strands of the active metallothionein (MT) IA and IIA genes, repairing them at the same rate as either strand of the inactive *MTIIB* gene or as the genome overall. Correlating with its intermediate sensitivity, the CS-A strain CS3BE repairs γ-ray damage in the transcribed strand at an intermediate rate. This result implies a differential role for CSA and CSB in coupling repair to transcription. We have also identified cross-sensitivity to both UV and ionizing radiation in a number of xeroderma pigmentosum group G strains. The *XPG* gene, which is identical to *ERCC5* and homologous to *RAD2* of yeast, appears to encode one of two endonuclease activities required in the incision step of NER. We find that radiosensitive XP-G strains have a substantial defect in overall repair synthesis after X-irradiation and also deficient removal of thymine glycols, while rejoining of double strand breaks occurs normally. Because Cockayne syndrome is frequently associated with XP-G, we examined transcription-coupled repair in the active MTIIA gene of several XP-G strains after X-irradiation. We find a complete absence of strand-selective repair of ionizing radiation damage in two XP-G strains with associated CS and greatly reduced rates in other XP-G strains.

C5-335 A NEW SENSITIVE PCR METHOD TO QUANTIFY REPAIR OF IN VIVO INDUCED DNA DAMAGE IN TRANSCRIPTIONAL (IN)ACTIVE GENES, Sandra A.M. Bol, Michiel, F. van Oosterwijk, Rob, A. Baan*, Harry Vrieling, Leon H.M. Mullenders and Albert A. van Zeeland, MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, Sylvius Laboratory, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands, *TNO Nutrition and Food Research Institute, Department of Genetic Toxicology, PO Box 5815, 2280 Rijswijk, The Netherlands,
The repair in the genome overall has been studied in the liver of mice after intraperitoneal injection of N-hydroxy-acetylaminofluorene (N-OH-AAF). In the liver DNA no deoxyguanosine-C8-acetylaminofluorene adducts could be detected by high performance liquid chromatography analysis. The frequency of deoxyguanosine-C8-aminofluorene adducts at 1 day after injection was 6.4 adducts / 1000 kB ($\pm$ 20 pmole/mg) and its removal was 64% after 7 days. Since the adduct level is low, the standard uvrABC method to measure adduct frequencies in defined sequences up to 25 kB could not be used. Therefore, a sensitive PCR method is being developed for the quantification of in vivo induced DNA damage in transcriptional active and inactive genes. This technique involves the immunological extraction of only the adducted liver DNA fragments, using an adduct specific antibody acting on single stranded DNA. Subsequently quantitative PCR of the, in the liver transcriptional active Hypoxanthine Phosphorybosyl Transferase- and passive Duchenne Muscular Dystrophy genes, is performed on the extracted DNA. Quantitative PCR on immunological extracted DNA will be used to study preferential repair in the liver of mice treated with N-OH-AAF. In future we aim to study the role of transcription in gene specific repair using excision repair deficient mice.

C5-336 DIFFERENTIAL REPAIR IN YEAST AFTER THE INDUCTION OF DNA MONO- AND BIADDUCTS

Meniel V., Magaña-Schwencke N., Waters R*. and Averbeck D., Institut Curie, Section Biologie (URA 1292, CNRS), Paris (France),*University College, Swansea (Wales : UK). In genomic DNA, repair is heterogeneous depending on transcriptional activity and chromatin structure. We investigate the differential repair of the active *MATα* locus compared to the inactive *HMLα* locus in *Saccharomyces cerevisiae*. So far, most studies were performed on UV induced pyrimidine dimers which require nucleotide excision repair (NER), but little is known about the repair of other types of lesions. In previous studies, it was shown that in the active *MATα* locus, UV-induced pyrimidine dimers were faster repaired than in the inactive *HMLα* locus. We report here on the repair of psoralen plus UVA induced monoadducts (MA) and interstrand crosslinks (ICL) in both loci. In contrast to the repair of MA, that of ICL involves the NER as well as the recombination repair pathways. For both types of lesions, we demonstrate that *MATα* (active) is faster repaired than *HMLα* (inactive). No difference is seen in a *sir-3* mutant in which both loci are active. Thus, DNA lesions necessitating either NER or NER and recombinational repair are subject to preferential repair. In addition, for the preferential repair of ICL, the contribution of each repair pathway (NER and recombination) is analysed using different *rad* mutants : *rad1*, *rad2Δ*, *rad16* which belong to the NER pathway and *rad52* which belongs to the recombination repair pathway. Futhermore, preliminary data suggest that specific stages in the cell cycle affect the preferential repair of ICL.

C5-337 PROTEIN-PROTEIN INTERACTIONS IN THE HUMAN RNA POLYMERASE II BASAL TRANSCRIPTION FACTOR TFIIH.

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The human basal transcription factor TFIIH plays a role in two distinct processes. It is an obligatory component of the initiation complex for RNA polymerase II-mediated transcription. It also forms the core structure around which the components of the nucleotide excision repair machinery assemble to constitute the human nucleotide excision 'repairosome'. At least two of the gene products (XPB and XPD proteins) responsible for the disease xeroderma pigmentosum are subunits of TFIIH. We have exploited the availability of the cloned *XPB*, *XPD*, *p62*, *p44* and *p34* genes to examine specific interactions between *in vitro*-translated polypeptides by co-immunoprecipitation. Our analysis demonstrates that the XPB, XPD and p62 proteins interact strongly with each other. The p44 and p34 proteins interact with each other and with XPB and XPD proteins, but not with p62. The N-terminal 65 amino acids of p62 protein are sufficient to support its interactions with other proteins in TFIIH. Multiple domains of XPB and XPD proteins are required for their interactions. A comparison of all these interactions with those in the homologous proteins in TFIIH (factor b) of the yeast *S. cerevisiae*, indicate multiple differences. These results suggest that the yeast and human TFIIH complexes, and consequently the repairosome into which they assemble, are architecturally distinct. We propose an architectural model for the human TFIIH complex.

C5-338 GENE-SPECIFIC DNA REPAIR AND STEADY STATE TRANSCRIPTION OF THE *MDR*1 GENE IN HUMAN TUMOR CELL LINES

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Efficient gene-specific DNA repair has been linked to transcription. It is not clear however, how changes in the level of mRNA expression in vivo significantly affect the efficiency of repair at the gene level. We have employed a gene-specific repair assay, to study removal of UV-induced pyrimidine dimers in the *MDR*1 gene at different levels of *MDR*1 mRNA expression. The parental human carcinoma cell line, KB-3-1, has very low levels of *MDR*1 mRNA expression, but its multi-drug resistant derivatives KB-8-5 and KB-C1 have 42-fold and 3,800-fold increases in *MDR*1 mRNA expression, respectively. In the KB-3-1 cell line, we find a low level of *MDR*1 gene-specific repair and inefficient repair of the transcribed strand of the gene. In the KB-8-5 cell line, we find only a minor increase in dimer repair in the *MDR*1 gene. Here, the repair in the transcribed strand is not significantly higher than that in the KB-3-1 cell line. However, in the KB-C1 derivative, we find a substantially increased repair in the *MDR*1 gene. In addition, the *MDR*1 transcribed strand repair is markedly more efficient than the repair in the nontranscribed strand. Our data suggest that the rate of transcription in the *MDR*1 gene must be substantially increased before there is any measurable effect on DNA repair. Repair in the dihydrofolate reductase gene, was similar and preferential in all three tumor cell lines. However, it was markedly lower than previously reported in normal human fibroblasts. We suspect that these tumor cell lines have deficient gene-specific DNA repair.

C5-339 MUTATION FREQUENCY DECLINE (MFD) AND TRANSCRIPTION-COUPLED EXCISION REPAIR (TCR) IN *E. coli*,

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Historically MFD is a part of UV-mutagenesis studies using reversion of nonsense defective auxotrophic *E. coli* as an assay for mutation. Specifically the frequency of glutamine tRNA suppressor mutations assayed on semi-enriched defined agar rapidly decreases as a function of certain types of intervening incubation between UV and plating. Recently Selby and Sancar have suggested that MFD is a particular example of TCR (J. Bact. **175**: 7509-7514). In support of this model, we have examined MFD to (1) find effects by genetic defects in mismatch repair, a possible *in vivo* hallmark of TCR just discovered by Mellon and Champe, and (2) demonstrate rapid repair of the targeting photoproducts *during* the post-UV incubation required in this process:— The kinetics of MFD are slower when the B/r strain WU3610 contains the defect *mutS201*::Tn5 or *mutL*::Tn*10*. Similar slow kinetics are produced by *mutH34* but not by *mutH471*::Tn5 which has no apparent effect. The strain WU3610-45 (*mfd-1*) produces the slower kinetics if transcription is inhibited during the post-UV incubation, although it produces no decline in normal circumstances. The slower kinetics therefore are attributed to bulk excision repair remaining when rapid TCR is inactivated by certain defects in mismatch repair. Overt repair of a mutation-targeting cyclobutane pyrimidine dimer, -T<>C-, during the post-UV incubation is demonstrated with a correlation between the mutation frequency normally associated with this lesion and the rate of mutation production via spontaneous deamination of cytosine in this lesion. Rediscovery of the neglected fact that MFD can be stimulated by post-UV incubation in buffer alone is a part of the deamination analyses and suggests a relation with *liquid holding recovery*. The results support the simple model: effective repair of photoproducts in the transcribed strand targeting glutamine tRNA suppressor mutations occurs during the appropriate intervening incubation and is responsible for MFD. Research supported by N.I.H. grant GM21788.

C5-340 NUCLEOTIDE EXCISION REPAIR PATHWAYS,

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We have taken different approaches to study nucleotide excision repair. In an in vitro system, we can monitor transcription coupling. The repair activity is stimulated by increased promotor activity, and there is a strand bias. This transcription coupling is being investigated in repair deficient syndromes and in syndromes of premature aging such as Cockayne's. We are improving the in vitro system with the use of single lesion vectors containing pyrimidine dimers or cisplatin adducts, and we are using antibodies towards components to identify proteins involved in the repair process. Using human cell lines that are mutated in mismatch repair genes, we are investigating whether there are common components between nucleotide excision and mismatch repair in mammalian cells.

C5-341 MUTATIONS IN BASAL TRANSCRIPTION FACTORS SUPPRESS THE *hpr1* HYPERREC MUTANT OF YEAST,

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hpr1 was originally isolated as a hyperrecombination mutant that specifically increased intrachromosomal recombination between direct repeats 500-1000 fold over wild type. The hyperrec phenotype is partially dependent on *RAD52* and *RAD1* repair genes. In addition, the *hpr1* null strain is temperature sensitive for growth. To further understand the function of the Hpr1 protein, extragenic suppressors were isolated using the ts phenotype. The 14 *soh* (suppressor of *hpr*) mutants recovered represent eight complementation groups and suppress the phenotypes of *hpr1* to different extents. Some of the *soh* mutants suppress the hyperrec of *hpr1*, but differ from the *rad* mutants that suppress *hpr1* hyperrec phenotype. Three interesting interactions are observed among the *soh* mutants. 1) some double *soh* mutants show very poor growth at 37°C regardless of the genotype of the *HPR1* gene; 2) other double *soh* mutants grow very poorly at 37°C in a *hpr1* background; 3) some combinations of double *soh* mutants suppress *hpr1* hyperrec more than the product of two single *soh* mutants. *SOH1* has been cloned and sequenced. The putative Soh1 protein shows slightly homology to DNA and RNA polymerases. The *soh1* null mutant is hyperrec. *SOH1* shows genetic and physical interaction with *RAD5*, which is involved in the post-replication DNA repair pathway. *SOH2* is *RPB2*, which encodes the RNA polymerase II beta subunit in yeast. *SOH4* is *SUA7*, which is the hTF-IIB homolog. The finding that mutations in the basal transcriptional apparatus suppress a hyperrec mutant suggests a link between transcription and recombination.

C5-342 PREFERENTIAL REPAIR OF ACETYLAMINOFLUORENE DNA ADDUCTS IN TRANSCRIBED DNA BY HUMAN CELL EXTRACTS,

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We have developed an *in vitro* assay for the preferential repair of transcribed genes by human cell extracts. This assay is based on the estimation of DNA repair synthesis performed by transcription-competent extracts prepared by the method of Manley *et al.* on an acetylaminofluorene (AAF)-damaged plasmid carrying the human CMV promoter. After extended periods of incubation (up to 2 h) there is no significant difference in the extent of repair of the entire plasmid carrying the promoter with or without transcriptional activation, or in the extent of repair of the entire plasmid with or without the promoter. There is a small (1.3 fold) increase in the repair of a 650 bp region located immediately downstream of the promoter after transcriptional activation. There is a 2.0 fold increase in the preferential repair of this region when compared to the same fragment without the promoter. Stimulation of repair by transcriptional activation is more dramatic during shorter incubation periods (1.8-2.0 fold). Cells from Cockayne syndrome are defective in this preferential repair *in vitro*. This data is in good agreement with *in vivo* data on the preferential repair of transcribed genes in human cells.

C5-343 ESTABLISHMENT OF CELL-FREE DNA REPAIR-RNA TRANSCRIPTION ASSAY WITH MAMMALIAN NUCLEAR EXTRACTS,

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The preferential removal of certain types of DNA damage from the transcribed DNA strand relative to the non-transcribed strand in expressed genes implicates the arrest of RNA polymerase II in the recruitment of repair enzymes. The molecular basis for such a mechanism in mammalian cells, however, has not yet been studied because we have lacked a cell-free system that carries out both efficient repair and transcription under the same assay conditions.

Previously, whole cell extracts (Manley et. al., Meth. Enzym., 101, 568, 1983) were tested for this purpose (Spivak et.al., Environ. Mol. Mut., Suppl. 23, 64, 1994). In the present study, we have established a cell-free DNA repair/RNA transcription system employing nuclear as well as cytosolic extracts prepared from HeLa cells. The typical repair/transcription reaction mixture contains dNTPs, NTPs, ATP regenerating system and two different types of plasmid, such as UV irradiated-endonuclease III treated pBluscript KS$^+$ for repair and pAdBam for transcription. RNA synthesis is monitored by the incorporation of [^{32}P]UMP into RNA, and efficient initiation of RNA transcription from the adenovirus late promoter has been shown. The reaction reaches plateau after 60 min incubation, and is suppressed by addition of 1 μg/ml α-amanitin. In contrast, with whole cell extracts, only weak transcription activity was obtained under the assay conditions used in this study. The repair of UV-induced damage is monitored by incorporation of [^{32}P]dAMP into substrate plasmid DNA. This extract carries out repair of UV-induced damage to a similar extent as the whole cell extracts which have been used for assay of nucleotide excision repair (Wood et.al., Cell, 53, 97, 1988). In addition, only a negligible amount of [^{32}P]dAMP incorporation occurred when non-UV treated pBluscript KS$^+$ was employed. Thus this new cell-free system which carries out both efficient repair and transcription, should be useful for analysis of the coupling of nucleotide excision repair to RNA polymerase II arrest.

C5-344 TRANSCRIPTION OF TEMPLATES CONTAINING A SPECIFIC DNA LESION AT A DEFINED SITE, Brian A. Donahue, Daniel Reines* and Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305 and *Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322

Some types of DNA damage are removed by nucleotide excision repair much more rapidly from the transcribed strand of expressed genes, than from the nontranscribed strand or from unexpressed genomic domains. Although the mechanism of this transcription coupled repair (TCR) has not been elucidated, our current model suggests that the arrested RNA polymerase II directs the repair complex to the transcribed strand of active genes. The stalled polymerase may hinder access of repair enzymes to the lesion and prevent DNA strands from reannealing so that repair can begin. If the polymerase were translocated upstream from the lesion on the template, the DNA might be repaired and transcription could resume. An example of transcriptional readthrough past sites of polymerase arrest is provided by the transcription elongation factor SII. SII facilitates the transcription of RNA pol II through a variety of pause sites by a mechanism in which nascent transcripts are cleaved before the polymerase can elongate, presumably by restoring the polymerase to an elongation competent conformation.

In order to study the possible role of the arrested RNA pol II and transcript cleavage in TCR we have constructed DNA templates in which a cyclobutane pyrimidine dimer (CPD) is located downstream from the major late promoter of adenovirus at specific sites on either the transcribed or nontranscribed strand of all templates. We have shown that a CPD on the transcribed strand is a strong block to RNA pol II whereas a CPD on the nontranscribed strand has no effect on transcription. Furthermore the arrested transcription complex blocks the CPD from recognition by a repair protein, photolyase. SII induces transcript cleavage by the arrested polymerase but does not facilitate bypass of the lesion. Shortened RNAs can be re-elongated up to the site of the CPD; thus the polymerase remains competent throughout this process. TFIIH, a protein complex involved in both transcription and repair has no affect on the arrested polymerase. We will also present data in which other lesions such as acetylaminofluorene and aminofluorene have been studied. A model for the role of arrested RNA pol II and transcript cleavage in TCR will be presented.

C5-345 DIFFERENTIAL EXPRESSION OF THE NUCLEOTIDE EXCISION REPAIR / GENERAL TRANSCRIPTION FACTOR GENE, ERCC2(XPD), IN HUMAN TISSUES.

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Persons afflicted with Cockayne's Syndrome[CS] present with marked skin sensitivity to UV light, profound defects in growth and development, and progressive neurological degeneration. Likewise, subgroups of persons afflicted with Trichothiodystrophy [TTD] and/or xeroderma pigmentosum group D [XP-D] have skin sensitivity to UV and progressive neurological degeneration. As DNA repair syndromes these diseases affect not only the integument but many other organs systems including the central and peripheral nervous systems, the musculo-skeletal system, and possibly the immune system.

The human nucleotide excision repair [NER] gene ERCC2(XPD) has been identified as the defective gene in the XP group D and mutations in this gene are being identified in cDNAs from XP-D, CS, and TTD patients. The localization of the ERCC2 gene product to the general transcription complex TFIIH has implicated this gene in transcription-dependent NER and defects in the ERCC2 gene product may have an impact on general class II transcription leading to some of the complex manifestations of the DNA repair syndromes.

In an effort to examine the role of ERCC2 in the pathobiology of XP-D, CS, and TTD we have examined the expression of ERCC2 in human tissues by Northern blots and RNase protection assays. We report that this general transcription factor gene is differentially expressed in cells from different tissues. The relative expression appears to be lowest in those tissues affected the most (e.g. the brain).

This work supported by D.O.E. Contract No. DE-AC03-76-SF01012

C5-346 THE EFFECTS OF DIHYDROURACIL BASE DAMAGE ON SP6 AND T7 RNA POLYMERASES. J. Liu and P.W. Doetsch, Departments of Biochemistry and Radiation Oncology, Emory University School of Medicine, Atlanta, GA 30322.

Cellular DNA is subject to many chemical insults by different kinds of DNA damaging agents in a reactive environment. In order to maintain the genetic integrity, damaged DNA has to be repaired. The biological consequence of DNA damage if unrepaired is determined primarily by the interaction of damage with the DNA replication and RNA transcription machinery. Dihydrouracil (DHU) is a type of DNA base damage caused by ionization radiation under anoxic conditions. We have built this lesion into synthetic oligonucleotides with DHU placed at single locations downstream from SP6 and T7 promoters and have determined its interaction with these two prokaryotic RNA polymerases during transcription elongation. Both SP6 and T7 RNA polymerases pause at the damaged site transiently with subsequent bypass and generation of full length transcripts at high efficiency. Direct RNA sequencing shows both polymerases insert an adenine opposite of the dihydrouracil. These results suggest that dihydrouracil could be mutagenic at the level of transcription if these events happen *in vivo*. Supported by NIH grant CA 55896.

C5-347 *RAD26*, THE FUNCTIONAL *S.CEREVISIAE* HOMOLOG OF THE COCKAYNE SYNDROME B GENE *ERCC6*.
Alain J. van Gool, Richard Verhage, Sigrid M.A. Swagemakers, Pieter van de Putte, Jaap Brouwer, Christine Troelstra, Dirk Bootsma and Jan H.J. Hoeijmakers.
MGC Dept. of Cell Biology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR, Rotterdam. and Laboratory of Molecular Genetics, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA, Leiden. The Netherlands

Transcription-coupled repair (TCR) is a subpathway of the nucleotide excision repair (NER) system that is limited to the transcribed strand of active genes. It accomplishes the preferential elimination of transcription-blocking DNA lesions and permits rapid resumption of the vital process of transcription. A defect in TCR is responsible for the rare, hereditary disorder Cockayne syndrome (CS). Recently we found that mutations in the *ERCC6* repair gene, encoding a putative helicase, underly the repair defect of CS complementation group B. Here we report the cloning and characterization of the *Saccharomyces cerevisiae* homolog of *CSB/ERCC6*, that we designate *RAD26*. A *rad26* disruption mutant appears viable and grows normally, indicating that the gene does not have an essential function. In analogy with CS, preferential repair of UV-induced cyclobutane pyrimidine dimers in the transcribed strand of the active *RBP2* gene is severely impaired. Surprisingly, in contrast to the human CS mutant, yeast *RAD26* disruption does not induce any UV-, cisPt- or X-ray sensitivity, explaining why it was not isolated as a mutant before. Recovery of growth after UV-exposure was somewhat delayed in *rad26*. These findings suggest that TCR in lower eukaryotes is not very important for cell survival and that the global genome repair pathway of NER is the major determinant of cellular resistance to genotoxicity.

C5-348 RECOMBINATION IN RETROVIRAL PACKAGING CELLS DEPENDS ON TRANSCRIPTIONALLY ACTIVE CHROMATIN, Dagmar Wirth, Carsten Münk, Renate Grannemann, and Hansjörg Hauser, Gesellschaft für biotechnologische Forschung mbH, Braunschweig, F.R.G.

Recombination between retroviral vector and helper sequences can result in the formation of replication competent retroviruses (RCRs), a process which limits the use of retroviral gene transfer. In order to create safer packaging cells we intended to determine the relevance and conditions of recombination events leading to RCR formation .

In retroviral packaging cells recombination may happen on the level of DNA within the packaging cell and on RNA level during reverse transcription. In order to distinguish these two pathways, we analyzed DNA recombination using cells carrying a transcriptionally inactive helper locus. Only 1 of 6 independently obtained clone mixtures and none of the 17 cell clones gave rise to replication competent viruses during 37 weeks of cultivation indicating that chromosomal DNA recombination is a rare event in transcriptionally inactive chromatin.

In contrast, replication competent viruses could be frequently detected in cells carrying transcriptionally active helper functions. As expected, a high virus titer is a prerequisite for this recombination pathway. In 7 of 7 clone mixtures and 7 of 28 cell clones recombined virus was detected. RCRs had usually formed right after transfection of the vector and persisted during cultivation. However, some populations released replication competent viruses only temporarily which is compatible with the view that these recombination events took place in the infected cells on the level of RNA.

C5-349 TRANSCRIPTION BYPASS OR BLOCKAGE AT SINGLE-STRAND BREAKS AND GAPS ON THE DNA TEMPLATE STRAND BY T7 RNA POLYMERASE, Wei Zhou and Paul W. Doetsch, Departments of Biochemistry and Radiation Oncology, Emory University School of Medicine, Atlanta, GA 30322.
Cellular DNA is constantly modified by a variety of agents *in vivo*. These DNA damages, if left unrepaired, can interfere with both the replication and transcription process which may lead to cell death, mutation or neoplastic transformation. Single-strand breaks are frequently occurring DNA lesions which can be produced by ionizing radiation, a variety of chemical agents, or as repair intermediates of DNA base damages. To study the interaction between DNA strand breaks and RNA polymerases during transcription elongation, we have constructed synthetic DNA templates for T7 RNA polymerase transcription which contain nucleotide gaps of different sizes on the template strand. Our *in vitro* transcription experiments indicate that T7 RNA polymerase can bypass a gap on the template strand up to 24 nucleotides in length during transcription elongation. Compared to the full length transcript generated from a DNA template with an intact template strand, the full length transcripts generated the gapped templates are shortened by the same number of nucleotides as are missing in the template gap. RNA sequencing of the transcripts indicated that sequence content of these transcripts is completely DNA template-dependent. Hence, the continuity of the DNA template strand is not a necessary requirement for DNA-dependent RNA polymerase transcription elongation. Our experimental evidence also suggests that the 3' and 5' DNA termini at the single-strand break site play important roles in RNA polymerase transcription termination at strand breaks on the template strand. This work was supported by research grant CA55896 from the National Cancer Institute.

C5-350 DNA DAMAGE-INDUCED MODIFICATION OF RNA POLYMERASE II LARGE SUBUNIT
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The genetic defects in Xeroderma pigmentosum (XP) complementation groups B and D indicate that transcription factor TFII H, which interacts functionally with RNA polymerase II (Pol II), also helps recognize and repair DNA damage. The large subunit of Pol II, which appears to bind directly to DNA, may provide an additional step in the molecular pathway linking DNA damage to its recognition and repair.

Two forms of the large subunit of Pol II (IIa and IIo) with distinct roles in transcriptional initiation and elongation are distinguished by C-terminal domains (CTDs) which are hypo-(IIa) or hyper-(IIo) phosphorylated. Using immunoblot analysis with mAbs specifically recognizing the Pol II large subunit, we have identified novel, slow migrating forms of Pol II (II c and II c') which are induced after subjecting mammalian cells to DNA damaging conditions including UVB or UVC irradiation or the chemotherapeutic agents actinomycin D or cisplatin. IIc and IIc' appear in UVB or UVC irradiated cells only after 10-15 minutes of incubation at 37°C and fail to appear in cells maintained at 4°C after UV irradiation suggesting that an energy requiring process leads to the formation of IIc and IIc'. IIc and IIc' will appear when UV treated cells held at 4°C for 2 hours are shifted to 37°C for 15 minutes. Interestingly, when XP-D cells were analyzed after UV irradiation Pol IIc and IIc' were found to persist significantly longer than in identically treated control cells. These results suggest that IIc and IIc' appear in response to damaged DNA and remain if the damage is not corrected.

Molecular characterization of Pol IIc and IIc' is in progress. Because IIc and IIc' persist after SDS-PAGE run under denaturing conditions they are likely to result from covalent modification of Pol IIa or IIo. Preliminary results indicate that IIc and IIc' may result from changes in the phosphorylation state of the CTD.

C5-351 HIGH LEVELS OF TRANSCRIPTION ARE ASSOCIATED WITH INCREASED MUTATION RATES IN YEAST,
Abhijit Datta and Sue Jinks-Robertson, Department of Biology, Emory University, Atlanta, GA 30322
Complex processes such as transcription, replication, repair and recombination require changes in chromatin structure and interactions with numerous trans-acting factors with DNA sequences, raising the possibility that the efficiencies of these processes may be interrelated. The present study examines the effect of transcription on the rate of spontaneous mutation in the yeast Saccharomyces cerevisiae. A reverting frameshift allele (lys2ΔBgl) was placed under the inducible GAL1-10 promoter in two identical strains except for a GAL80 disruption in one, enabling constitutive expression from the GAL1-10 promoter. The pGAL-lys2ΔBgl construct is thus differentially expressed in the two strains. We find that the rate of spontaneous reversion of the lys2 allele is increased dramatically when the mutant gene is highly transcribed. In addition, forward mutation rate to lysine auxotrophy is elevated under induced transcription conditions. Our results demonstrate an association between transcriptionally active DNA and enhanced spontaneous mutation rates in a model eukaryotic system.

C5-352 THE EFFECTS OF GENE AMPLIFICATION AND TRANSCRIPTION ON THE FORMATION AND REPAIR OF ANTITUMOR ANTIBIOTIC CC-1065 INDUCED DNA ADDUCTS. M.-s. Tang, M. Qian, and A. Pao, University of Texas M. D. Anderson Cancer Center, Science Park, Smithville, Texas 78957
CC-1065 is a potent antitumor antibiotic which specifically bonds to duplex DNA, and the biological effects of the drug are presumably the consequences of its DNA interactions. In order to investigate the factors which may affect drug-DNA bonding in cells, we have developed a method using a thermal-alkaline treatment to induce phosphodiester bond breakage at the drug-DNA bonding sites coupled with Southern DNA transfer-hybridization to quantify drug-DNA binding at defined sequences in drug-treated cultured mammalian cells. We have found that ERCC1 and ERCC2 mutant cells are sensitive to CC-1065 induced cytotoxicity and are deficient in the repair of drug-DNA adducts. In vivo, CC-1065 bonds twice as efficiently in the highly amplified dihydrofolate reductase (DHFR) gene domain as in the nonamplified adenine phosphoribosyltransferase (APRT) gene domain. However, in vitro, CC-1065 bonds equally to both DHFR and APRT genes in purified CHO cellular DNA. Although we observed significant degrees of "gene-specific" and "transcribed-strand specific" preferential repair for drug-DNA adducts in the amplified DHFR gene domain, repair appeared to be faster and more efficient in the APRT gene than in the amplified DHFR genes. These results suggest that DNA amplification and transcription may affect both drug-DNA adduct formation and repair.

C5-353 XPA GENE EXPRESSION IS INVOLVED IN TRANSCRIPTION AT TWO LEVELS, J.E. Cleaver, M. McDowell, W.C. Charles, G.H. Thomas, S. Layher, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750
The XPA DNA binding protein has a high specificity for binding to certain UV-induced and other damaged sites in DNA. Its more obvious role in repair is to highlight damaged sites and act as a nucleation center for other excision repair gene products (helicases and nucleases). Several repair gene products are known to regulate repair in transcriptionally active regions: Cockayne syndrome A & B gene products are specifically required for repair of actively transcribed genes; XPB, D and other components of the basal transcription factor TFIIH are required for repair of both transcriptionally active and inactive regions of the genome. To investigate the role of XPA expression in repair and cell survival, we have constructed an expression vector in which the XPA cDNA is placed downstream from an inducible promoter (LacSwitch™, Stratagene). Low levels of expression of XPA appeared to direct repair to the [6-4] photoproducts in the whole genome and to photoproducts in active genes; very high levels of expression were required before repair was observed in non-transcribed regions of the genome. High survival after UV irradiation was associated with [6-4] repair but not with extensive pyrimidine dimer excision. Consistent with these observations, mouse cells express less XPA mRNA than human cells, and consequently have limited ability to repair pyrimidine dimers in the whole genome. The major influence of XPA expression on cell survival is exerted within the first 2-4 hours after irradiation and subtle changes in rates of repair over this time period appear to have disproportionate effects on survival. A role for XPA in its own expression may be seen in heterozygous cells: when only one allele produces a normal gene product, transcription from the other allele is reduced. Other transcriptionally active loci are unaffected(e.g. actin). This suggests the XPA gene product acts as an allelic suppressor of its own transcription. The XPA gene product therefore, exercises a number of unexpected regulatory and repair properties associated with gene expression.
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C5-354 REGULATION OF DAMAGE-RESPONSIVE GENES: IDENTIFICATION OF SEQUENCES REQUIRED FOR EXPRESSION OF PHR1, Gwendolyn B. Sancar, Robert Ferris, Frances W. Smith, Brian Vandeberg and Lubica Cernakova, Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
The PHR1 gene of Saccharomyces cerevisiae encodes a DNA photolyase that catalyzes photoreactivation of pyrimidine dimers and stimulates nucleotide excision repair in the absence of light. Transcription of PHR1 is induced following treatment of cells with a variety of DNA damaging agents. Induction is controlled by at least 3 cis-acting elements which lie in the order (promoter distal to promoter proximal) Upstream Essential Sequence (UES), Upstream Activating Sequence (UAS), and Upstream Repressing Sequence (URS). The URS is bound by a damage-responsive repressor called Prp; Prp binding activity is present in nondamaged cells and disappears rapidly following UV irradiation. The results of mutagenesis experiments indicate that a 22 bp palindrome within the Prp footprinted region is involved in repression. The PHR1 UAS begins 16 bp upstream of the Prp-protected region and consists of a 20 bp interrupted palindrome; each half of the palindrome is similar to sequences found upstream of the RNR2, RAD2, RAD7, RAD10, RAD23, RAD51, SPK1 and MAG genes suggesting that it is a common regulatory element. Alone the palindrome functions as an efficient UAS in the context of a heterologous promoter but does not confer damage-responsiveness. This is consistent with the observation that deletion or mutation of the UAS reduces basal level and induced expression but has no effect on the induction ratio. Specific sequences upstream of the palindrome are required for UAS function in the context of the PHR1 promoter, but not in the context of a heterologous promoter, indicating the presence of a UES. The UES is comprised of multiple elements, some of which are contained within sequences similar to those found in the 5' flanking regions of the damage-inducible genes RAD6 and RAD18. The role of DUN1, a protein kinase recently implicated in the damage-responsiveness of RNR2, in the function of each of these elements is under investigation.

C5-355 EFFECTS OF DOSE, ADDUCT DISTRIBUTION AND ADDUCT TYPE ON THE REPAIR OF 4-NITROQUINOLINE-1-OXIDE DAMAGE IN MAMMALIAN CELLS, Sharlyn J. Mazur, Arlene Hankinson and Vilhelm Bohr, Laboratory of Molecular Genetics, Gerontology Research Center, National Institute on Aging, National Institutes of Health, 4940 Eastern Ave. Baltimore MD 21220.

In mammalian cells, cyclobutane pyrimidine dimers in the transcribed strand of active genes are preferentially repaired. Several other bulky DNA lesions, including 6/4 photoproducts, benzo[c]phenanthrene adducts and cisplatin or nitrogen mustard monoadducts, are also preferentially repaired in active genes, although the effect is less pronounced. In contrast, 4-nitroquinoline-1-oxide (4NQO) lesions are not preferentially repaired in active genes. The pattern of 4NQO-induced mutations, however, suggests that the repair of at least one class of 4NQO lesions is biased toward the transcribed strand of active genes under some circumstances. Several lines of evidence suggest that the two main adducts produced by 4NQO, the N2-dG adduct and the C8-dG adduct, are repaired differently. We are investigating factors that may influence the degree of preference in the repair of 4NQO damage in human and hamster cells, including analyzing the repair of N2 and C8 adducts separately, the induction of DNA damage and its relation to cell survival, the partitioning of lesions between active and inactive chromatin and the specificity and efficiency of various repair subpathways.

C5-356 DEFECTIVE REPAIR OF UV-INDUCED DAMAGE IN TRANSCRIPTIONALLY ACTIVE GENES IN COCKAYNE'S SYNDROME CELLS: A CONSEQUENCE OF THE LACK OF RECOVERY OF UV-INHIBITED RNA SYNTHESIS?, Leon H.F. Mullenders, A.S. Balajee, A. van Hoffen and A.A. van Zeeland, MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, Leiden, The Netherlands

Cockayne's syndrome (CS) is an autosomal recessive disorder associated with severe photosensitivity. At the cellular level, CS is characterized by an increased sensitivity to lethal effects of UV-irradiation and a lack of preferential and transcription coupled repair of UV-induced cyclobutane pyrimidine dimers (CPD) in transcriptionally active genes. The finding of a reduced ability to perform fast repair of active genes provided an explanation for the lack of recovery of transcription and is consistent with increased levels of cell killing and mutagenesis in UV-irradiated CS cells. However, since transcription is an essential condition for preferential repair of active genes, an alternative explanation would be, that the reduced repair of active genes in CS is due to lack of transcription recovery after UV-irradiation. A number of observations are in favour of this hypothesis: (i) sensitivity of CS cells to N-AAAF correlates with the lack of RNA synthesis recovery, but is inconsistent with normal level of active gene repair; (ii) lack of preferential repair in UV-resistant CS transfectants containing the ERCC6 gene; (iii) the XP-D/CS phenotype is observed in the complete absence of CPD repair in active genes in these cells.

Our results indicate, that the genetic defect in CS cells relates to the inability to recover RNA synthesis following DNA damage and consequently to the lack of preferential repair of active genes. The lack of transcription recovery may affect the chromatin structure of active genes, thereby reducing the repair of CPD in active genes in CS cells beyond the level of the nontranscribed strand in normal human cells. Moreover, such alterations in chromatin structure may also underlie the reduced repair of 6-4 PP (which are not repaired predominantly by transcription coupled repair) in CS cells. At the molecular level the CS gene product may be involved in removing repair factors from the BTF2 transcription complex, thus allowing initiation of transcription.

Recombination/Strand Joining

C5-400 PROTEIN-DIRECTED BRANCH MIGRATION OF HOLLIDAY JUNCTIONS, Alison H. Mitchell[1], Kevin Hiom[1] Carol A. Parsons[1], Andrzej Stasiak[2] and Stephen C. West[1], [1]Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K., [2]University of Lausanne, CH-1015 Lausanne, Switzerland.

The RuvA and RuvB proteins of *Escherichia coli* mediate the branch migration of Holliday junctions during homologous genetic recombination and DNA repair. The *ruvA* and *ruvB* genes form an operon that is controlled by the LexA repressor and is part of the SOS-inducible DNA repair response. *In vitro*, the RuvA protein binds specifically to Holliday junctions, to which it directs RuvB (a DNA-dependent ATPase). RuvB protein forms double hexameric rings on duplex DNA in the presence of $MgCl_2$ and ATPγS, as visualised by electron microscopy, with the DNA passing through the hollow core of each ring. To determine whether the rings are biologically active, the conditions required for their formation and activity have been analysed. We have observed the condition-dependent assembly of two discrete forms of RuvB; dodecamers, which are functionally inactive, and hexamers, which appear to be the active form of the protein. The RuvB hexamers interact with tetramers of RuvA to generate stable complexes in the absence of DNA. Complexes formed between RuvA, RuvB and Holliday junctions have been analysed by DNAse I footprinting, and visualised by electron microscopy. RuvA protein binds and protects a region of 28 nucleotides on all four strands of DNA at the crossover point. In contrast, RuvB binds the DNA on only two of the four strands, covering a region of approximately 25 nucleotides. The polarity of branch migration may therefore be determined by the asymmetric assembly of RuvB with respect to RuvA. The asymmetric arrangement of the proteins on the junction point has been confirmed by electron microscopy. Further data showing that RuvB-dependent ATP hydrolysis is greater on circular duplex than linear DNA indicates that RuvB protein is able to translocate along duplex DNA. Given that RuvA and RuvB together exhibit DNA helicase activity, these results lead us to propose that RuvB may drive branch migration by helical rotation coupled with DNA unwinding as the DNA passes through the RuvB ring structure.

C5-401 **RESOLUTION OF HOLLIDAY JUNCTIONS BY *E. coli* Rus PROTEIN,** Gary J. Sharples, Sau N. Chan, Akeel A. Mahdi, Matthew C. Whitby, and Robert G. Lloyd, Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, United Kingdom

E. coli RuvABC proteins function in processing Holliday junction recombination intermediates. RuvAB work to drive the junction away from the point of strand exchange initiated by RecA. RuvC acts as an endonuclease that resolves the Holliday junction into repairable products. The recombination and repair defect of *ruv* mutations can be alleviated by extragenic suppressor mutations known as *rus*. These mutations consist of insertion elements 5' of the *rus* gene that improve transcription. *rus* is located in a multigene operon of short open-reading frames. The organisation of genes in the *rus* locus is reminiscent of bacteriophage genomes and *rus* does indeed appear to be of lambdoid origin. The search for a *rus* analogue in lambda is presented.

The 14 kDa Rus protein has been purified and shown to behave very like RuvC. It preferrentially binds to Holliday junction-containing DNA and in the presence of magnesium ions resolves the junction by symmetrical cleavage. The endonuclease cut sites can be repaired *in vitro* by *E. coli* DNA ligase. Unlike the phage T7 and T4 general structure resolvases, Rus and RuvC do not cut Y-DNA or at base mismatches or bulges. The suppression of the DNA repair and recombination defects of all *ruv* mutants by a Holliday junction resolvase suggests a close relationship between the three Ruv proteins and junction resolution. We discuss pathways of Holliday junction processing that involve RuvAB, RuvC, RecG and Rus.

C5-402 CONSERVATION OF *ruv* GENES AND FUNCTION BETWEEN DISTANTLY-RELATED EUBACTERIA

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The products of the *Escherichia coli ruvC, B* and *A* genes are involved in recombinational repair. The genes are clustered both in the Gram-negative bacterium *E. coli* (GenBank Nos. X07091 and X59551, R.G. Lloyd, Nottingham, UK) and in the only other previously available example, *Mycobacterium leprae* (GenBank No. U00011, unanotated, K. Robison, Harvard), an organism phylogenetically related to Gram-positive organisms. The amino acid sequences of the RuvC resolvases, RuvA proteins and RuvB helicases demonstrate 36, 32 and 53% amino acid identity, respectively, including the 4 (of 174) amino acids identified to be in the active site in the *E. coli* RuvC crystal structure [M. Aryoshi *et al.* (1994) *Cell* **78**:106-72]. The *M. leprae ruv* genes are contiguous, with a potential LexA binding sequence (SOS regulation) upstream from *ruvC*. In *E. coli*, an orf separates *ruvC* from an SOS-regulated *ruvAB* operon. The RuvB helicase was cloned from *Thermus thermophilus*. *E. coli* and *T. thermophilus* RuvB helicases demonstrated 54% amino acid identity. Surprisingly, the *T. thermophilus ruvB* gene was not part of a *ruv* operon. The *ruvC* genes were cloned from *E. coli* and from *M. leprae* cosmid B1177 (kindly supplied by J.-i Mao, Collaborative Research) into an expression vector. Expression of either *E. coli* or *M. leprae* recombinant RuvC resolvase restored UV resistance to *recG ruvC E. coli.*

C5-403 BINDING OF THE JUNCTION-RESOLVING ENZYME T7 ENDONUCLEASE I TO DNA: SEPARATION OF BINDING AND CATALYSIS BY MUTATION. Derek R Duckett and David M.J. Lilley. CRC Nucleic Acid Structure Group, Biochemistry Dept., The University, Dundee. U.K.

T7 endonuclease I is a resolving enzyme that selectively cleaves four-way junctions and related branch species. We have isolated a series of mutants of this protein that retain full structural-selectivity of binding, but which are completely inactive as nucleases. This is consistent with a divisibility of structure-selective binding and catalysis. The mutations that inactive endonuclease I as a nuclease are clustered into the second quarter of the primary sequence; a region that is homologous with the related junction-resolving enzyme endonuclease VII from phage T4. This suggests that these residues may form the active site of these enzymes. The configuration of the helical arms of the junction bound to mutant endonuclease I has been investigated by gel electrophoretic methods. We find that the junction is bound in the presence or absence of magnesium ions, and that the global structure of the bound form is apparently identical with or without cations. The patterns of mobilities suggests that the structure of the junction becomes perturbed by the binding of the protein.

C5-404 **PARAMETERS THAT INFLUENCE DNA BRANCH MIGRATION.**

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In genetic recombination, branch migration of the four-way Holliday junction is a key step that determines the extent of transfer of information between two homologous DNA duplexes. Despite the importance of branch migration in genetic recombination and in cruciform formation, relatively little is known about the mechanics of branch migration. We previously developed an assay to study parameters that influence spontaneous branch migration and determined that the rate of branch migration varies some four orders of magnitude depending on the presence of different metal ions. In light of the fact that metal ions greatly influence the conformation of the four-way junction, we have embarked on experiments examining the affect of Holliday junction structure on the rate of branch migration. Our results suggest that magnesium-induced base stacking at the crossover point constitutes a kinetic barrier to branch migration. Disruption of base stacking at the crossover point circumvents this slow step. We are also interested in describing the effect of sequence heterology such as base mismatches on branch migration. A single base pair heterology is sufficient to block spontaneous branch migration. We are currently exploiting this feature of sequence heterology to estimate the number of base pairs that are transiently opened during the migratory step. Our findings concerning these fundamental aspects of base pairing and opening may have more general implications for the dynamics of short repeated sequences such as dinucleotide repeats.

C5-405 A SELECTION SYSTEM FOR SOMATIC RECOMBINATION EVENTS IN *DROSOPHILA MELANOGASTER:* CONSTRUCTION OF THE TEST SYSTEM AND INITIAL STUDIES IN SCHNEIDER LINE 2 CELLS, Bärtsch, S.,Sengstag, C. and Würgler, F., Institute of Toxicology, Dept. of Genetics, ETH and University of Zurich, 8603 Schwerzenbach, Switzerland

Our primary target is to get insights into molecular mechanisms of mitotic DNA recombination in *Drosophila* cells. To study inter- and intrachromosomal recombination involving tandemly repeated homologous DNA sequences, Schneider line 2 cells (SL2) were stably transfected with an extrachromosomal DNA element providing a putative recombination substrate. The DNA substrate consisted of two truncated fragments of the bacterial neomycin resistance gene (neo) interrupted by a bacterial hygromycin resistance gene (hyg). The hyg-gene was flanked by direct repeated homologous 352bp fragments of neo-gene. *D. melanogaster* promoters were inserted upstream to both bacterial genes. Restoration of a functional neo-gene after recombination between the direct repeated sequences should allow us to select for mitotic homologous recombination events. Transgenic cell lines were cloned from transfected cells. Clonal SL2 44CD4 cell line showed the lowest spontanous recombination frequency and was therefore used for further studies. Preliminary results concerning induction of genetic rearrangements after UV light, mitomycin-C (MMC) and ethyl-methane-sulphonate (EMS) treatment of 44CD4 cells will be shown. In order to establish the role of UV, MMC and EMS in the intact *Drosophila* organism we also constructed a transgenic strain carrying the described recombination-reporter neo-hyg-neo cassette in the genome.

C5-406 EXPRESSION STUDIES OF *Escherichia coli recO* AND *recR* GENES.
Tzu-Chien V. Wang, Chi-Ling Yang, Yi-Cheng Su, and Ying-Hsiu Liu. Department of Molecular Biology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan.
The structural portion of *Escherichia coli recO* and *recR* genes was synthesized by polymerase chain reaction and cloned onto the ATG-fusion cloning site of several expression vectors. The ability of the constructed *recO* and *recR* plasmids to complement chromosomal *recO* and *recR* mutations and to overexpress RecO and RecR proteins was examined. A mutation at the second codon of *recR* from CAA to GAA had little effect on the *in vivo* function of RecR. Fusion of the His·Tag oligohistidine to RecR at the N-terminus produced a fusion protein which is fully functional *in vivo*. With regards to the ability to overexpress RecO and RecR, we observed that the plasmids carrying a T7 Ø10 promoter overexpressed RecO and RecR protein in large quantity while the plasmids carrying a P_{tac} promoter overexpressed RecO and RecR poorly. Overexpresseon of RecO or RecR had little effect on cell viability but did affect DNA repair in several *uvr*+ cells. The effect of overexpression on DNA repair was not observed in *uvrA* or *uvrB* backgrounds, however. We suggest that the overexpression of RecO or RecR may allow certain *uvr*+ cells to perform more excision repair.

C5-407 DETECTION OF V(D)J REARRANGEMENTS AND DELETIONS USING RECOMBINATION SUBSTRATES IN FANCONI ANEMIA CELLS,
Dora Papadopoulo, Jean-Christophe Andrau, Noëlle Doyen, Sacha Kallenbach, Agnès Laquerbe, François Rougeon and Ethel Moustacchi, URA 1292 du CNRS, Institut Curie-Biologie, Paris, France

Fanconi anemia (FA) belongs to the group of inherited disorders which associate anomalies in the processing of specific lesions with predisposition to malignancy. An important feature of FA pathology is an increased genomic instability. This is reflected at endogenous loci by a substantial increase of spontaneous and induced (by cross-linking agents) deletions. The analysis of sequences at the breakpoint sites of HPRT deletions derived from FA cells revealed the presence of motifs identical or similar to the conserved heptamer which plays an important role in the V(D)J recombination.

To address the question of whether and how an aberrant V(D)J recombination may contribute to deletions in FA cells, *in vitro* experiments using V(D)J recombination constructs have been performed. These substrates, contain the recognition signal sequences (RSS) which direct the recombinational activity of the V(D)J machinery. The substrates are transfected into normal human and FA complementation group "D" lymphoblasts, simultaneously with RAG1 and RAG2, two recombination activating genes necessary and sufficient to induce normal V(D)J activity. In these conditions almost all recombinant plasmids (98%) recovered in normal lymphoblasts have correct coding joints. In contrast to the normal cells, only a fraction of rearranged substrates (40%) recovered from FA cells have junctions consistent with normal V(D)J activity. When the V(D)J constructs are transfected in the absence of RAG1 and RAG 2, no rearrangements are detected in normal cells, whereas in FA cells abnormally large deletions are observed. The analysis of the breakpoint junctions of rearranged substrates should provide further information on the mechanism of deletions involved in FA cells.

C5-408 HIGH FREQUENCY ERROR-PRONE DNA RECOMBINATION IN ATAXIA-TELANGIECTASIA (A-T) CELL LINES.
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The DNA repair defect in A-T cells which gives rise to ionizing radiation sensitivity and cancer predisposition remains unclear. Genetic instability manifest by hyper-recombination and misrepair of DNA termini have been described. To investigate recombination in A-T cells, we used two types of plasmid repair probes. Intra-molecular recombination was measured by a plasmid (pTPSN) containing two mutant copies of the gene coding hygromycin resistance, plus a reporter gene (*neo*). The rate of spontaneous intra-molecular recombination was 100x higher in SV40-transformed A-T cells compared with transformed normal cells, NF (both fibroblasts). The relative frequencies were the same, whether hygromycin resistance was tested directly on *neo* containing cells or hygromycin resistance develops in expansion of a single *neo*+ colony which was initially hygromycin sensitive. Southern analysis of *neo*+ single colonies revealed that 1/8 had a functional hygromycin gene, but that 5/8 had altered restriction fragment sizes and sensitivity to hygromycin, suggesting low fidelity recombination. By contrast, *neo*+ normal fibroblast colonies showed 5/7 clones with an unaltered integrated plasmid. Hygromycin-resistant A-T cells showed exchange events in 8/11 colonies, whereas 5/7 NF colonies showed gene conversion. Inter-molecular recombination was measured by the co-transfection of linearized derivatives of pSV2*gpt*, which have partially-overlapping deletions within the *gpt* gene. To make a functional *gpt* gene, the two molecules must recombine with fidelity. The co-transfection frequency using plasmids cleaved distant from the *gpt* gene was 44% of the transfection frequency of linear pSV2gpt in A-T cells, but only 4% for normal cells. When cleavage was within the *gpt* gene, the co-transfection frequency was reduced 3x in A-T, but was increased 12% in normal cells. We conclude that A-T cells show a high frequency of recombination events, but the presence of DNA termini makes recombination error-prone.
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C5-409 SEARCH FOR GENES INVOLVED IN THE ATAXIA-TELANGIECTASIA PATHWAY,
Małgorzata Z. Zdzienicka[1], Wim Jongmans[1], Gerald W.C.T. Verhaegh[1], Nicolaas G.J. Jaspers[2], Peter Demant[3], Mitsuo Oshimura[4] and Paul H.M. Lohman[1], [1]MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, Leiden, [2]MGC-Department of Cell Biology and Genetics, Erasmus University, Rotterdam, [3]National Dutch Cancer Institute, Rotterdam, The Netherlands, [4]Department of Molecular and Cell Genetics, Tottori University, Yanago, Japan.

In order to establish the number of genes involved in the pathway defective in ataxia-telangiectasia (A-T) cells and to examine their mechanism of action, we studied complementation of the A-T phenotype in A-T and X-ray-sensitive hamster cells closely resembling A-T human cells, by using microcell-mediated chromosome transfer (MMCT). It has been shown that several cDNAs isolated *via* expression cloning and localized on different chromosomes (eg. chromosome 6, 17 or 18) complement the X-ray sensitivity of AT-D cells (Meyn et. al. 1993). However, using MMCT, we found that only human chromosome 11 complements the X-ray sensitivity as well as the radioresistant DNA synthesis (RDS) of AT-D and AT-C cells after ionizing radiation. These results indicate that these cDNA clones unspecifically complement the radiosensitivity of AT-D cells, and that MMCT may serve as an essential control for the identification of the A-T genes.

In order to localize the AT-like hamster gene on the human genome, hybrids between the AT-like mutant cells and single human chromosomes (except chromosomes 19, 21 and 22) were constructed by MMCT but all of them remained X-ray sensitive. Recently, we found that a single mouse chromosome 9 complements the X-ray sensitivity (but not RDS) of the AT-like hamster cells. As the ATA, ATC and ATD genes are localized on chromosome 11, our results indicate that the hamster mutants could be defective in another gene involved in the A-T pathway. Therefore, the identification of this gene is of great interest to unravel the complex etiology of the A-T syndrome.

C5-410 DEVELOPMENT OF A MODEL SYSTEM FOR THE INVESTIGATION OF GENE TARGETING IN MAMMALIAN CELLS, Ciaran Morrison and Ernst Wagner. Forschungsinstitut für Molekulare Pathologie/ Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030, Vienna, Austria.

Using frameshift mutations of reporter genes (beta-galactosidase, luciferase) which result in defective expression from these genes, we sought to recover expression in a number of different situations. The recovery of this expression was mediated by transfection of [non-expressing] repair fragments of the genes of interest, which contained the correct reporter gene sequence. The ability of cell lines (human A-549, murine NIH 3T3) to regenerate expression was investigated in an episomal context, by co-transfection of the defective reporter together with the repair fragments. Similarly defective reporter gene constructs were stably cloned into cell line hosts using G-418 selection for a neomycin resistance cassette on the reporter plasmid. Various approaches were used to improve the levels of reporter gene expression: factors such as strandedness of the DNA (also inclusion of RecA protein in complex formation), the size of the repair fragment (homology length) and the effect of co-transfection of expression constructs for putative repair enzymes were examined in this regard. Different systems (cationic lipids, receptor-mediated transfection) were used for transfection of these cell lines; results deriving from such work are presented. These experiments present a model system by which requirements for non-selectable gene targeting may be analysed. The development of advanced receptor-mediated gene transfer vehicles has meant that gene delivery to EBV-transformed B lymphocytes has become possible; this system is being used to address the possibility of transfection-mediated repair of an extant genetic dysfunction, namely mutation in the *hprt* locus. Such a mutation presents a well-characterized and selectable target for repair. We hope to be able to use this empirical approach to repair (i.e., targeting) to garner information about what happens to DNA transfected into mammalian cells.

C5-411 **DNA REPAIR & V(D)J RECOMBINATION,** Guillermo E. Taccioli, #Tanya M. Gottlieb, *Tracy Blunt, #Nicholas J. Finnie, Jocelyne Demengeot, *Anne Priestley, Ryushin Mizuta, *Penny Jeggo, #Stephen P. Jackson, Frederick W. Alt. Howard Hughes Medical Institute, Children's Hospital, and Department of Genetics, Harvard University Medical School, Boston. #Wellcome/CRC Institute and Department of Zoology, Cambridge University, UK. *Medical Research Council, Cell Mutation Unit, University of Sussex, Brighton, UK.

A large panel of cell lines deficient in DNA repair have been tested for the ability to perform V(D)J recombination after introduction of the RAG-1 and RAG-2 genes. Three radiosensitive CHO cell mutants, *xrs-6*, XR-1 and V-3, failed to generate normal V(D)J. These mutants are all defective in dsb repair but represent three different complementation groups. Genetic and biochemical evidence will be presented demonstrating that the gene defective in the *xrs* mutants (XRCC5) is the 80 kDa subunit of the Ku complex. Ku binds to free double stranded DNA ends and is a component of the DNA-dependent protein kinase (p350). Data also will presented showing that another component of the DNA-dependent protein kinase is defective in the V-3 cell line. By somatic cell complementation group we have demonstrated that V-3 is in the same complementation group as the murine *scid* cell line and our results support the conclusion that the *scid* cell line is defective in the same component.

C5-412 FUNCTIONAL COMPLEMENTATION OF THE MURINE SCID DEFECT, Cordula U. Kirchgessner, Christopher K. Patil, James W. Evans and J. Martin Brown, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA94305

Severe combined immonodeficient (scid) C.B-17 mice are deficient in V(D)J recombination, the process of assembling the immunoglobulin and T-cell receptor genes from gene segments. Scid mice are also sensitive to ionizing radiation, as a result of their deficiency in double-strand break repair. By transferring human chromosome 8 (tagged with a neo marker) into scid cells (scid/hu8 cell line) we have restored radioresistance. In an effort to clone the gene on the basis of its chromosomal localization we have constructed a series of irradiation reduced hybrids. Our panel of radiation hybrids consists of 24 independently derived cell lines, 7 hybrids are sensitive and 17 hybrids are resistant to ionizing radiation. The human DNA content of these cell lines has been characterized by fluorescence in situ hybridization and Alu-PCR and presence or absence of chromosome 8 markers markers (anonymous loci in the form of dinucleotide repeats and gene loci). The minimal region containing the scid gene product has been identified and spans one centiMorgan. YACs of this region have been identified from the CEPH-Genethon physical map (Cohen et al. 1993, Nature 366:698-701). Individual YACs of the contig were analyzed by FISH in order to map them to the region of chromosome 8 containing the scid gene and to ensure that they were non-chimeric. For functional complementation of the radiosensitivity in scid cells YACs were tagged with a selective neo-marker (obtained from Srivastava and Schlessinger, Gene 103: 53-59) and introduced into scid cells. Hybrid cell lines obtained from the fusion of one of these YACs with scid cells showed partial to full complementation of the radiosensitive phenotype.

Searching for candidate genes in this region of chromosome 8, we have mapped DNA dependent protein kinase (p350) to this region on chromosome 8. p350 a serine/threonine kinase forms a protein komplex with ku p70 and p80, which have been shown to bind to DNA double-strand breaks (p70) and to complement the radiosensitivity in another radiosensitive cell line xrs5 (p80). We are currently studying the involvement of p350 in double-strand break repair and its role in the scid mutation.

C5-413 DIFFERENTIAL EXPRESSION OF THE *rhp51*$^+$ GENE, A *recA* HOMOLOG OF THE FISSION YEAST, Yeun K. Jang[1], Yong H. Jin[1], Kyungjae Myung[1], Seung H. Hong[1,2] and Sang D. Park[1], [1]Department of Molecular Biology; [2]Institute for Molecular Biology and Genetics, Seoul National University, Seoul 151-742, South Korea.

We have mapped multiple transcripts of the *rhp51*$^+$ gene known as a *recA* homolog and a *RAD51* homolog in *Schizosaccharomyces pombe*. The *rhp51*$^+$ gene encodes three transcripts of 1.9, 1.6, and 1.3 kilobases which have different 3' termini. Northern blot analyses show that the three transcripts of *rhp51*$^+$ gene are expressed differently in response to a variety of DNA damages. During the mitotic cell cycle, only the large transcript of 1.9 kb shows a periodic expression, reaching maximum level faster than *cdc22*$^+$ transcript which peaks at the G1/S boundary. Unexpectedly, the steady-state levels of the three transcripts were differently regulated during the growth cycle. Both large 1.9-kb and small 1.3-kb transcript were highly accumulated at the diauxic shift and during the entry into stationary phase, respectively. In contrast, the middle 1.6-kb transcript levels remained constant during the growth cycle. To localize the regions responsible for these differential expression of *rhp51*$^+$ gene, we constructed the *rhp51-ura4* and *ura4-rhp51* hybrid genes and analyzed their expression patterns in response to MMS-induced DNA damage and during the growth cycle. Unexpectedly, we observed that *ura4*$^+$ gene based on a multicopy plasmid encodes three transcript species containing one major 0.8-kb and two minor 1.8-kb and 2.5-kb transcripts. We show that the 3' terminal region of *rhp51*$^+$ gene can regulate three transcript species from the *ura4-rhp51* hybrid but does not confer MMS-inducibility on the expression of the chimeric transcripts. We also show that the *rhp51*$^+$ promoter can confer MMS-inducibility to only 0.75-kb one of three transcripts produced from the *rhp51-ura4* hybrid. During the postdiauxic phase, the *rhp51*$^+$ promoter could increase the large 2.2-kb mRNA level of three chimeric transcripts derived from the *rhp51-ura4* hybrid. From these results, we conclude that this novel one gene-three products relationship is regulated by the cooperation of both promoter and 3' terminal regions.

C5-414 NONHOMOLOGOUS RECOMBINATION AT SELECTIVE SEQUENCES BETWEEN ADENOVIRAL DNA AND CELLULAR DNA IN A MAMMALIAN SYSTEM - *IN VITRO* STUDIES Katja Fechteler and Walter Doerfler; Institute of Genetics, University of Cologne, Cologne 50931, Germany

The molecular and biochemical mechanisms underlying nonhomologous recombination in mammalian cells are largely unknown. We have explored the mechanism of adenovirus type 12 (Ad12) DNA *integration because of its importance for viral oncogenesis and as an* example for a nonrandom, nonhomologous type of recombination. We established a cell-free recombination system from nuclear extracts of uninfected BHK21 hamster cells for monitoring the mechanism of adenoviral DNA integration and enzyme purification. We have purified the components of the postulated cellular 'recombination machinery' to five major protein bands (fraction V) retaining activity in cell-free recombination. By using standard chromatographic procedures about a 470fold purification was achieved. The main protein bands range in size between 90 and 40kDa. The first tests for enzymatic activities revealed a helicase activity in this recombinationally active fraction, no topoisomerase I activity could be detected. Furthermore, protein(s) of fraction V bind specifically to a doublestranded, 50bp oligodeoxyribonucleotide comprising a preferred target sequence. In contrast, we could observe an unspecific binding to singlestranded oligodeoxyribonucleotides. Protein-protein interactions seem to be also involved in complex formation. The sequence characteristics of the *in vitro* generated recombinants are patchy homologies at the junction sites between cellular and viral DNA. Moreover, though data from *in vivo* studies did not suggest a common or specific nucleotide sequence, at which viral DNA can insert into the mammalian genome, the sequence motif 5'-CCTCTCCG-3' has served repeatedly as preferred target sequence for Ad12 DNA integration. Assuming that the cell-free system resembles the natural integration event, the 'recombination machinery' could be supplied by the affected host cell. This finding does not necessarily exclude a contribution of virus-encoded proteins and preliminary data suggest an increased frequency of recombination in vitro when using extracts from Ad12-infected BHK21 cells.

C5-415 TARGETED RECOMBINATION AT THE *APRT* LOCUS IN DNA REPAIR-DEFICIENT, *ERCC1* MUTANT CHINESE HAMSTER CELLS. Gerald M. Adair, Debra G. Smith, Robin Schneider-Broussard, Marina Zabelshansky, and Chaline Brown. The University of Texas M. D. Anderson Cancer Center, Science Park-Research Division, Smithville, Texas 78957.

The yeast *RAD10* gene product is known to be involved inrecombination as well as in nucleotide excision repair. A Rad10/Rad1 complex ssDNA endonuclease activity appears to be required for removal of nonhomologous DNA from the 3' ends of recombining DNA. The *ERCC1* gene is the mammalian equivalent of the yeast *RAD10* gene. To examine the effects of an *ERCC1* mutant phenotype on recombination in mammalian cells, we have carried out a series of *APRT* gene targeting experiments employing two matched *ERCC1* mutant (U9S50tg) and repair-proficient ATS49tg) cell lines carrying identical *APRT* target gene deletions. *ERCC1* mutant U9S50tg cells consistently show lower frequencies of targeted homologous recombination and nontargeted integration of transfected sequences than their repair-proficient ATS49tg counterparts. Southern blot analysis of APRT+ recombinants has revealed differences in the types and distributions of targeted recombination events obtained from *ERCC1* mutant versus normal cell lines, with the mutant cells (like *rad10* mutants in yeast) showing lower frequencies of targeted insertions and other recombination events that require the removal of nonhomologous DNA from the 3' ends of recombining DNA. Thus, the *RAD10* and *ERCC1* repair genes appear to play similar roles in both the nucleotide excision repair and recombinational pathways of yeast and mammalian cells. (Supported by NIH grant CA-28711).

C5-416 MUTATIONS ARISING FROM RECOMBINATION: IDENTIFYING THE GENES INVOLVED, Susan L. Holbeck, Jeffrey N. Strathern, ABL-Basic Research Program, Laboratory of Eukaryotic Gene Expression, P.O. Box B, Frederick, MD 21702-1201

Double-strand breaks (DSBs) are well established as initiators of recombination. When HO endonuclease was used to initiate recombination at a defined chromosomal location in *S. cerevisiae*, mutation in a nearby interval was found to increase, indicating that the DNA synthesis associated with DSB repair is error-prone (McGill and Strathern, unpublished). *REV3*, the proposed error-prone DNA polymerase, is required for DSB-associated mutation, although *rev3* mutants are recombination proficient. We have begun a mutant search to identify other genes whose products are involved in this DNA synthesis event. Several mutants have been isolated which decrease DSB-associated mutation. Another class of mutants show increased levels of both DSB-associated and spontaneous mutation. Further analysis of these mutants is underway. Research sponsored by the National Cancer Institute, DHHS, under contract No. N01-CO-46000 with ABL.

C5-417 NOVEL PROPERTIES OF RecA FROM THE RADIATION RESISTANT BACTERIUM DEINOCOCCUS RADIODURANS, Kenneth W. Minton and J. David Carroll, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814-4799

The bacterium *Deinococcus* (formerly *Micrococcus*) *radiodurans* is the most ionizing- and UV_{254}-radiation resistant organism discovered to date. We have recently identified a RecA homologue in *D. radiodurans* and shown that the extreme DNA damage resistance of *D. radiodurans* is exquisitely dependent on the functional integrity of this RecA homologue. It appears that this protein acts by way of a novel deinococcal interchromosomal recombinational repair pathway that centers on deinococcal RecA (see Daly and coworkers poster). Based on *in vivo* cross-complementation studies using the *recA*$^+$-gene of *Shigella flexneri* (amino acid sequence identical to *E. coli* RecA) we have found some evidence of novel properties of deinococcal RecA:

We have expressed various genes in *D. radiodurans* mutants, including *polA* from *E. coli* (DNA Pol I). For example, while *D. radiodurans pol* mutants are very sensitive to DNA damage, expression of the *E. coli polA* gene results in full restoration of wild-type deinococcal resistance. However, this observation does not extend to include the deinococcal *recA* gene. Expression of the *S. flexneri recA*$^+$ gene in *recA*$^-$ *D. radiodurans* or expression of the *D. radiodurans recA*$^+$ gene in *recA*$^-$ *E. coli* is ineffective in restoring any aspect of the wild type *recA*$^+$ phenotype to either organism, even when the *recA*$^+$ gene was expressed at high levels, as determined by Western blotting. This finding contrasts with the fact that the vast majority of bacterial *recA*'s complement *recA*$^-$ *E. coli* phenotypic properties to at least some extent; and similarly, unlike the current case, the great majority of *recA*-defective organisms are complemented to some extent by the *E. coli recA*$^+$-gene. The failure of cross-complementation between these two *recA*$^+$ genes poses a puzzle that may be central to RecA-mediated radioresistance of *D. radiodurans*.

C5-418 INVOLVEMENT OF THE DNA-PK COMPLEX IN DNA DOUBLE-STRAND BREAK REPAIR, Fanqing Chen, Scott R. Peterson, David J. Chen, LS-1, MS M888, Los Alamos National Laboratory, Los Alamos, NM87545.

CHO mutants xrs-6 and XRV15B are highly sensitive to radiation and deficient in DNA double-strand break (DSB) repair. Several recent reports identified Ku80 as the gene responsible for DSB repair deficiency in these mutants. Ku80 is a subunit of Ku protein, which possesses a DNA end-binding activity and can form a complex with the p350 kinase subunit of DNA-PK. In this report, the role of DNA-PK complex in DNA DSB repair was examined. By Northern analysis, it was found that: (1) Ku80 gene in the mutants was disrupted, which led to the lack of functional Ku80 protein; (2) Ku70 mRNA expression in the mutants was normal. However, the Ku70 protein was undetectable by monoclonal antibody in the mutants, supporting several recent observations that the mutants lacked a Ku70-like DNA end-binding activity. Introduction of human Ku80 gene into the mutant led to an increase in detectable Ku70 protein, suggesting that mutation in Ku80 gene affected the formation of Ku70/Ku80 dimers and the stability of the Ku70 protein. The association of Ku70 and p350 with DNA ends was disrupted in the mutants, but restored after reintroduction of Ku80. Therefore, Ku80 mutation compromises the formation of Ku70/Ku80 dimer on DNA ends and the capability for Ku to recruit p350. Current studies include experiments on whether kinase activity of p350 is compromised in the mutants.

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C5-419 A NON-PROCESSIVE MECHANISM FOR CATALYSIS OF BRANCH MIGRATION BY RecG, Matthew C. Whitby and Robert G. Lloyd, Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham, NG7 2UH, UK.

The 76 kDa RecG protein is required for normal levels of recombination and DNA repair in *Escherichia coli*. The purified protein catalyses the branch migration of model Holliday junctions (1). However, it blocks strand exchange mediated by RecA and drives junction intermediates back to starting substrates, from which it has been suggested that RecG removes recombination intermediates *in vivo* by catalysing reverse branch migration (2). We have investigated the mechanism by which RecG promotes branch migration. RecG displays classical helicase activity on partial duplex DNA, unwinding with a 3' to 5' polarity and very low processivity. Improved unwinding, although with similar low processivity, is observed with branched DNA substrates, and this is attributed to RecG's particular affinity for junction DNA. Both branch migration and DNA unwinding exhibit the same reaction requirements that include a dependence on the hydrolysis of ATP. As with other helicases, the hydrolysis of ATP cycles RecG through changes in conformation that affect it's DNA binding activities. Upon hydrolysing ATP, junction binding is reduced and linear duplex DNA binding is increased. These changes are presumably an intrinsic part of the unwinding mechanism and suggest that the junction does not simply act as a high affinity site for RecG to load onto the DNA. Collectively these data indicate that the catalysis of branch migration by RecG depends on DNA unwinding. However, since RecG unwinds with low processivity, how can it maintain a directionality of branch migration? We show that within regions of homology, RecG can readily form two complexes simultaneously on junction DNA. This we propose provides a means of maintaining directionality by a kind of 'hand-on' mechanism. A model for the mechanism of RecG-mediated branch migration derived from our observations is presented.

1. Lloyd RG, Sharples GJ (1993) *EMBO J* **12:** 17-22.
2. Whitby MC, Ryder L, Lloyd RG (1993) *Cell* **75:** 341-350.

C5-420 CHARACTERIZATION OF RESIDUES IN THE LOOP 1 REGION OF THE RECA PROTEIN WHICH ARE CRITICAL FOR RECOMBINATIONAL DNA REPAIR AND LEXA CLEAVAGE, Horacio G. Nastri, Craig Lange and Kendall L. Knight, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, MA 01655

The RecA protein of *E. coli* plays a central role in recombinational DNA repair and regulation of the cellular SOS response. The active protein filament, formed by polymerization of subunits onto gapped or single stranded DNA, can bind a second DNA substrate (double stranded DNA) for the catalysis of strand exchange and also serves as a coprotease in the cleavage of LexA repressor.

Using a combinatorial cassette mutagenesis procedure we have introduced a large number of substitutions into an area of the RecA protein that overlaps the disordered loop 1 region (L1), previously hypothesized to be involved in DNA binding. Assays for recombinational DNA repair and LexA coprotease activity identify Glu^{154} as the most critical residue in this region. Gly^{157} is a "hot-spot" for the occurrence of constitutive coprotease mutations as 13 of 14 substitutions give rise to such mutants. In addition, the high frequency of second-site suppressor mutations in this area suggests a functional interaction among many of the residues, perhaps in the formation of a DNA and/or LexA binding site.

C5-421 **STRAND TRANSFER PROTEIN GP*uvsX* MEDIATES STRAND EXCHANGE ACROSS ASYMMETRIC INHOMOLOGIES (LOOPS) IN SHORT OLIGONUCLEOTIDES *IN VITRO*.** Kemper,B. & Birkenkamp,K., Institut für Genetik der Universität zu Köln, Zülpicher Straße 47, 50674 Köln, FRG.

Protein gp*uvsX* is a component of the recombination system of bacteriophage T4. It functions as a strand transfer protein (STP) *in vitro* and catalyzes the translocation of homologous single-strands from linear double stranded DNA (donor molecules) to circular single-stranded DNA (recipient molecules).

We have studied the strand transfer reaction using synthetic double-stranded donor DNA (93nt) and circular single-stranded M13 recipient DNA. Reactions contained homogeneously purified protein gp*uvsX* (Hinton & Nossal (1986), J.Biol.Chem. 261:5663ff), gp32 and an ATP-regenerating system. The products of the reactions were identified on native 1% agarose gels and quantitated by phosphorimaging. The donor DNA was systematically modified by adding internally or terminally located inhomologies.

Blocks of inhomologies with the same number of complementary nt in both strands and inhomologies forming loops of 10nt or 24nt in either strand of a heteroduplex donor were made. The basic sequence of the donor remained the same in all substrates.

Our results show that:1.) gp*uvsX* can use short ds-oligonucleotides with less then 100bp with the same efficiency as linear ds-M13 DNA of 7.2kb. Oligos with 4nt protruding 3'-ends were most effective; blunt ends and 4nt protruding 5'-ends caused a two-fold reduction of the relative efficiency of transfer. 2.) An internal block of 24 basepairs impairs the transfer reaction almost completely and reduces the relative efficiency below 20%. The same block placed at the distal end of the donor shows 47% relative transfer efficiency. 3.) A single mismatch reduces the relative transfer efficiency to about 50%. Loops of 10 or 24nts located in either of the two strands have little or no effect on the relative transfer efficiency. In summary, these results show that gp*uvsX* can drive strand exchange reactions across large inhomologies if they are located in one strand only.

C5-422 RANDOM INTEGRATION OF DNA IN MAMMALIAN CELLS, Raymond Merrihew, Kathleen Marburger, David Roth, and John H. Wilson, Department of Biocemistry, Baylor College of Medicine, Houston, TX 77030
Foreign DNA that enters a mammalian cell is readily integrated randomly throughout the genome. Integration is almost always associated with a genomic rearrangement. Although free DNA ends stimulate random integration, very little is known about how the ends of the input DNA are joined to the cellular DNA. We report here a mammalian cell system that selects for integrants in a small region of the genome by requiring events in an intron of the APRT gene. Around 100 cell-lines have been generated in which the 5' half of APRT randomly integrated in close enough proximity to its 3' half to reconstruct a functional APRT gene. By directly sequencing individual PCR products, 25 input DNA/cellular DNA junction sequences have been obtained. Of these, 1 has zero nucleotides of homology at the junction, 10 have one to five nucleotides of homology, and 14 have insert DNAs of usually unknown origin at the junction, ranging in size from 1 bp to 581 bp. The data suggest that mammalian cells can make use of a small degree of terminal homology in integrating foreign DNA. Still, the distribution of junction classes implies that a mechanism other than a simple end-to-end joining may be in effect.

C5-423 ABNORMAL RESPONSES TO UV RADIATION IN ATAXIA TELANGIECTASIA, Kathleen Dixon, Lise Loberg, Susan McGrath, and Michael P. Carty Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267
Ataxia telangiectasia (AT) is a human genetic disorder characterized by increased sensitivity to ionizing radiation, progressive degenerative neurological symptoms, and progeric changes, particularly in sun-exposed areas of the skin. Cultured skin fibroblasts from AT patients exhibit enhanced sensitivity to cell killing following exposure to ionizing radiation, but not 254 nm UV light. Here we show that AT cells are defective in the cell cycle arrest that normally occurs after UV irradiation as well as ionizing radiation. By fluorescence activated cell sorting (FACS) analysis, we showed that normal skin fibroblasts arrest DNA synthesis and accumulate in G1 after either ionizing radiation treatment (1 Gy) or UV irradiation (10-30 J/m^2, 254 nm), but AT fibroblasts do not. We have used an in vitro DNA replication system to examine the loss of DNA replication capacity on the biochemical level. We found that the loss of DNA replication capacity observed in cell extracts from either ionizing or UV radiation-treated normal cells is not observed in extracts from similarly treated AT cells. These results demonstrate that AT cells have abnormal responses to both ionizing radiation and UV radiation. The abnormal response to UV radiation might explain the progeric skin changes observed in AT patients.
This work was supported by the AT Children's Project and by ES05400 from NIEHS.

C5-424 Mouse Homolog of the Yeast RAD52 Gene: cDNA Cloning and mRNA Expression in Mouse Tissues. ***Zhiyuan Shen, Rebecca Lobb, Joe Gatewood, and David J. Chen***, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA.
The yeast RAD52 gene is involved in yeast DNA double strand break repair, and mitotic/meiotic recombination. Yeast and chicken RAD52 homologs have highly conservative N-terminus amino acid sequences. Based on the above conserved amino acid sequence, three inosine-containing degenerate primers were designed and used in PCR amplification of cDNA of C3H mouse embryo fibroblast 10T1/2 cells. Two PCR amplified cDNA fragments were identified as mouse RAD52 cDNA homologs by sequencing. These PCR products were used to screen a mouse λ-ZAP cDNA library. A full-length (~1.7kb) and a partial length (~1.0kb) cDNA clones were isolated. Analysis of cDNA-derived amino acid sequence revealed a highly homologous N-terminal sequence to yeast RAD52. One interesting finding was the 1.0 kb cDNA has two places that differ from the 1.7 kb clone in nucleotide sequence. One results in a single amino acid change, the other results in an extra amino acid. This may represent polymorphorisms in the C3H10T1/2 cells. A common mRNA species of 1.9-2.0 kb is expressed in mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. No significant difference of the 1.9-2.0 kb mRNA level was found among these tissues. However, an extra band of 0.8-0.9 kb is expressed in mouse testis. This result is different from what has been reported in chicken RAD52, and mouse RAD51. *(Studies supported by Department of Energy under contract KP0400/005181 to Los Alamos National Laboratory, and NIH grant CA50519)*

C5-425 MANY HUMAN EXPRESSED GENES CAN BE CATEGORIZED ACCORDING TO PHENOTYPIC CONSEQUENCES IN THE YEAST *RAD52* MUTANT, COMPROMISED FOR CHROMOSOME METABOLISM, Edward L. Perkins, Vera I. Hashem and Michael A. Resnick, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 (FAX: 919 541-7593)
Attempts to identify human genes involved in DNA repair and chromosome metabolism have generally been limited to methods based on complementation or relatedness to genes from other species. We have developed a new approach for the isolation of cDNAs based on dominant-negative consequences. This method provides for the rapid isolation and characterization of expressed human cDNAs according to their effects on phenotypic endpoints in "genetically sensitized yeast." Using this approach over 1000 cDNAs have been isolated from a human cerebellum cDNA library that specifically prevent growth of the "genetically-sensitized" *rad52* mutant that is altered in DNA double-strand break repair, recombination, mutagenesis, replication and chromosome segregation. Many of the isolated cDNAs are uniquely represented and several correspond to newly identified genes as well as previously described ESTs. Some of the cDNAs result in enhanced recombination. We propose that the products of a portion of the cDNAs can lead to negative interactions with components of chromosome metabolism, including repair, or directly cause chromosomal damage. Given the functional similarities that often exist between yeast and human proteins, there may be cognate roles in humans that relate to the phenotypes imparted by the expressed cDNAs in yeast. Included among the genes identified are those coding for a pancreatic tumor-associated protein, a potential single-stranded binding protein, an interferon regulated transcription factor (ISGF-3), cytochrome c oxidase subunit III and the SET gene implicated in some leukemias. We propose that expressed sequences of the human genome can be subcategorized into groups according to their effects when expressed in various sensitized yeast mutants including those affecting DNA repair. Furthermore, once isolated, the sensitized and wild-type strains provide an opportunity to study *in vivo* the biological activity of the isolated genes. Many of the genes isolated in this screen are currently being investigated for mode of action as well as role in DNA repair.

C5-426 FUNCTIONS OF Rad51 AND Rad52 PROTEINS INVOLVED IN RECOMBINATIONAL REPAIR,

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RAD51 and *RAD52* genes of *Saccharomyces cerevisiae* is required for repair of double strand breaks (DSBs) as well as mitotic and meiotic recombination. Products of the genes play important roles in a step after resection of the ends of DSBs: homology search and/or strand exchange. *In vivo* and *in vitro* studies show that both gene products interact with each other. Rad51 shows a weak homology to bacterial RecA proteins, while Rad52 does no homology to other proteins. Recently, it is shown that both genes are conserved from yeast to man.
To characterize activities of Rad51 and Rad52, we overproduced the proteins in *E. coli* and *S. cerevisiae* and purified them near homogenity. Rad51 is an ATP-depndent DNA binding protein which forms a helical nucleofilament on DNA. The strurcutre is very similar to that formed by RecA protein. It can catalyze a strand exchange reaction in vitro. To dissect mechanism of the recombination reaction by Rad51, we have employed D-loop and triplex assays.
Purifed Rad52 protein can bind to single-stranded DNA. Also has a strong annealing activity which hybridizes complementary DNA strands. The reaction is independent upon the presence of a high energy cofactor, such as ATP. To know the relationship between Rad51 and Rad52, we have been examining the effect of Rad 52 protein on strand exchange activity of Rad51 and Rad51 on annealing activity of Rad52.

C5-427 ANALYSIS OF THE *RAD55* AND *RAD57* GENES OF *SACCHAROMYCES CEREVISIAE* SUGGESTS PARTICIPATION IN A MULTI-PROTEIN COMPLEX INVOLVED IN DNA REPAIR AND RECOMBINATION.

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In *Saccharomyces cerevisiae* the genes comprising the *RAD52* epistasis group have been shown to be required for DNA double-strand break repair and recombination, however; the mechanism with which these proteins catalyze these reactions is unknown. We have used the two-hybrid system developed by Song and Fields to identify protein-protein interactions between members of the *RAD52* epistasis group and have shown that Rad55 interacts with Rad51 and Rad57. These data, in addition to the previously reported interaction between Rad51 and Rad52, suggest that at least four of the members of the *RAD52* epistasis group form a multi-protein complex. Other lines of evidence support the idea that these proteins form a multi-protein complex. It is known that *rad55* and *rad57* mutants are more sensitive to radiation at cold temperatures than at high temperatures. Cold sensitive mutants are most commonly seen for proteins comprised of multiple subunits. Additionally, we have shown that overexpression of Rad51 partially suppresses the γ-ray sensitive phenotype of *rad55* and *rad57* mutants.

In order to determine the nature and significance of these interactions we are purifying both Rad55 and Rad57. We constructed Rad55 and Rad57 fusion proteins with contain six additional histidine residues at the N-terminus of the proteins to facilitate purification from *E. coli* and yeast. Once purified we hope to be able to confirm the interactions between these proteins and delineate any biochemical activities they may have.

C5-428 WHICH ROLE HAS THE REV2 GENE PRODUCT FOR DSB REPAIR IN YEAST?

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Mutations in the *REV2* (≡ *RAD5*) gene of *Saccharomyces cerevisiae* cause intermediate sensitivity to ionizing radiation, however, the mutant phenotype differs from mutants of the *RAD52* group of genes. *rev2* mutants are hyperrecombinagenic and they are capable to repair γ-ray-induced DNA double strand breaks (DSB) and to recover under liquid holding conditions. For better understanding the *REV2* function in DSB repair we followed the time course of DSB repair applying pulsed-field gel electrophoresis(1). During the first 10 h little repair occurs in the *rev2* mutant diploid, but after 24 hrs the repair level of the wild type strain is reached. Possibly, the γ-ray sensitivity of the *rev2* diploid is due to a reduced fidelity of a DSB repair process rather than to its reduced efficieny. Therefore we studied the fidelity of repair of *ApaI*-caused DSB (sticky ends) and *ApaI-NcoI*-caused double strand gaps (DSG; 169 bp) in the URA3 gene on a YCp50-derived plasmid(2) being transformed into suitable *rev2-0* and *REV2* strains. Error-free repair is indicated by restoration of the *URA3* gene function. Our results indicate that the rev*2-0* mutant repairs DSB with an efficieny and fidelity comparable to the *REV2* wild type strain. In contrast, only 20 % of the DSG are correctly repaired in the mutant as compared to the wild type strain. Whereas DSG repair requires recombination of the plasmid with chromsomal DNA, *ApaI*-caused DSB could be restored by ligation indicating a role of the Rev2 protein for the fidelity of recombination. From DNA sequence analysis of the misrepaired plasmids we hope to gain some further insight.

(1) Friedl, A.A. et al. Int.J.Radiat.Biol. 63, 173-181, 1993; (2) Jha, B. et al., Curr. Genet. 23, 402-407, 1993.

C5-429 CHARACTERISATION OF TWO NEW *S. pombe rad* MUTANTS, *rad31* AND *rad31*.

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Two new *S. pombe rad* mutants, *rad31* and *rad32*, have been isolated in a screen to identify new mutants defective in recombination repair. The mutants are sensitive to both ionising radiation and UV radiation, with radiation sensitivities similar to other recombination repair mutants.
The *rad32* mutant is defective in meiotic recombination and has a high frequency of mini-chromosome loss. PFGE shows that the mutant has reduced ability to repair double strand breaks.
The *rad31* mutant displays an abnormal cell morphology and a 'cut' phenotype even in the absence of exposure to radiation. The mutant is defective in conjugation and loses viability in stationary phase.
The *rad31* and *rad32* genes have both been cloned and sequenced. Data will be presented on current work aimed at identifying the role of these genes.

C5-430 SUPPRESSION OF A NEW ALLELE OF THE YEAST *RAD52* GENE BY OVEREXPRESSION OF *RAD51*, MUTATIONS IN *SRS2* AND *CCR4*, OR MATING-TYPE HETEROZYGOSITY. David Schild, Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720; (510) 486-6013.

The *RAD52* gene of *Saccharomyces cerevisiae* is involved both in the recombinational repair of DNA damage and in mitotic and meiotic recombination. A new allele of *rad52* has been isolated which has unusual properties. Unlike other alleles of *rad52*, this allele (*rad52-20*) is partially suppressed by a *srs2* deletion; *srs2* mutations normally act to suppress only *rad6* and *rad18* mutations. In addition, while haploid *rad52-20* strains are very x-ray sensitive, diploids homozygous for this allele are only slightly x-ray sensitive and undergo normal meiosis and meiotic recombination. Since *rad52-20* diploids which are also homozygous for mating type are very x-ray sensitive, mating-type heterozygosity is acting to suppress *rad52-20*. Mating-type heterozygosity suppresses this allele even in haploids, since *sir* mutations, which result in expression of the normally silent mating-type cassettes, were identified among the extragenic revertants of *rad52-20*. Among the other extragenic revertants of *rad52-20* were a new allele of *srs2*, two alleles of the transcriptional regulatory gene *ccr4*, and two new or as yet unidentified genes (*srf1* and *srf2*, *srf* - suppressor of rad fifty two). Since other researchers have shown that the RAD51 and RAD52 proteins interact, *RAD51* on a high copy number plasmid was tested and found to suppress the *rad52-20* allele, but *RAD54*, *55* and *57* did not suppress. The *RAD51* plasmid did not suppress *rad52-1*. The *rad52-20* allele may encode a protein which has low affinity binding to the RAD51 protein, an interaction necessary for normal recombinational repair. Experiments are currently underway to clone and sequence *rad52-20* to determine if the mutation is in the region known to be involved in the binding of the RAD51 and RAD52 proteins. Mating-type heterozygosity and mutations in *srs2*, *ccr4*, *srf1* and *srf2* will be tested for whether they increases *RAD51* expression, in order to determine if one or more of the *rad52-20* suppressors suppress by causing an increased expression of *RAD51*.

C5-431 DOUBLE STRAND BREAK REPAIR IN YEAST: INTERACTIONS BETWEEN MEMBERS OF THE *RAD52* EPISTASIS GROUP, Sharon L. Hays, Antoine A. Firmenich and Paul Berg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

The repair of DNA double strand breaks (DSBs) in *S. cerevisiae* requires members of the *RAD52* epistasis group. Repair is presumed to occur via a recombinational mechanism, inferred in part from the fact that mutations in genes in the *RAD52* epistasis group confer recombination defects as well as sensitivity to X-rays or other DSB mimetic agents. However, the specific function of most of these gene products is not clear. *Rad51* is a notable exception in that it was recently demonstrated to possess strand exchange activity similar to that of the *E. coli RecA* protein (Sung, Science 265:1241). In addition, *Rad51* and *Rad52* have been shown to interact directly on the basis of results from column affinity chromatography (Shinohara et. al., Cell 69:457) and two-hybrid analysis (Milne and Weaver, Genes and Development 7:1755).

Our results suggest that other interactions between members of the *RAD52* epistasis group exist as well. Specifically, we have previously shown genetic interactions between *RAD52* and *RFA1*, the large subunit of the yeast SSB, which was recently identified as a member of the *RAD52* epistasis group (Firmenich et. al., submitted). Here we provide evidence for genetic interactions of *RAD55* and *RAD57* with *RAD51* and *RAD52*. Our data indicate that overexpression of *RAD51* and/or *RAD52* can, to varying degrees, overcome the recombination defect and X-ray sensitivity of *rad55* and *rad57* mutant strains. Some of these interactions are direct as judged by the two-hybrid system.

These results suggest that at least some members of the *RAD52* epistasis group may act and assemble as part of a complex, which we call a recombinosome, to effect the repair of DSBs.

C5-432 THREE *rad* TALES OF THE WORM *C. elegans*, Phil Hartman, V. Dwarakanath, Clark Jones and Dan DeWilde, Biology Dept., Texas Christian U., Fort Worth, TX 76129

C. elegans has been employed widely to address a plethora of biological issues, including those which relate to DNA repair and mutagenesis. We report three such ongoing projects from our laboratory:

1. Damage-resistance DNA synthesis. UV radiation fluences as high as 250 Jm^{-2} minimally affected DNA synthesis and cell division in wild-type (but not *rad* mutant) embryos. Yet DNA repair was saturated and few embryos hatched. Electron microscopy indicated that *C. elegans* embryos had very small replication bubbles, whose size and distribution were unaffected by irradiation. This is consistent with the correlation between mammalian replicon size and the ability to recover DNA synthesis after UV irradiation (**BBA** 739, 207). Conversely, the decreased DNA synthesis observed after UV irradiation in *rad-3* embryos was due largely to inhibition of elongation.

2. The use of fem-3 *to study UV-induced mutations.* The sex-determination gene *fem-3* has been developed for the isolation and molecular characterization of mutations. To test this system, eighty-six UV-induced *fem-3* mutations were isolated. Twenty of these were initially thought to be deficiencies, deleting *fem-3* and at least one essential gene. However, they were subsequently shown to be "coincident mutations" (*i.e.*, separate mutations in *fem-3* and a linked, essential gene), which occurred at a much higher frequency than predicted.

3. Cloning a DNA repair gene. On the basis of their abilities to render animals UV hypersensitive, two allelic mutations were isolated in a strain characterized by high levels of genetic transposition. From one of the strains, the putatively offending transposon (Tc1) and continuous DNA were isolated. The latter was employed to screen a genomic library, which in turn yielded a 2.9 kb clone from a cDNA library. We are currently sequencing this clone.

C5-433 3-D RECONSTRUCTIONS SUGGEST A GENERAL STRUCTURE AND MECHANISM FOR MANY HELICASES IN DNA REPAIR, RECOMBINATION, TRANSCRIPTION AND REPLICATION, Edward H. Egelman and Xiong Yu, Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455

The RuvB protein is induced in *E. coli* as part of the SOS response to massive DNA damage. In reactions with model Holliday junctions, RuvB promotes a branch migration associated with the hydrolysis of ATP (reviewed in West, Cell **76**, 9-15, 1994). RuvB has also been shown *in vitro* to have a helicase activity (Tsaneva *et al.*, P.N.A.S. **90**, 1315-1319, 1993), and it has been identified as having the sequence motifs of a large superfamily of helicases (Lloyd and Sharples, N.A.R. **21**, 1719-1725, 1993). We have determined the three-dimensional structure of RuvB on double stranded DNA (Stasiak *et al.*, P.N.A.S. **91**, 7618-7622, 1994), and shown that RuvB forms hexameric rings around the DNA, in which subunits are all oriented in the same direction. We have now determined (Egelman, Yu, Wild, Hingorani and Patel, submitted) that the bacteriophage T7 gp4 proteins form nearly identical hexameric rings around single stranded DNA. The gp4 proteins are the primary helicases involved in T7 DNA replication, and they have extremely weak sequence homology to RuvB. The nearly identical structures suggests that the structure and mechanism have been conserved far more strongly than sequence for these proteins that have very different functions. Data on other helicases involved in DNA replication, recombination, repair and transcription will be presented, suggesting that hexameric structures that walk along DNA while hydrolyzing ATP may be widespread.

C5-434 STALLED REPLICATION FORKS AT UV-INDUCED LESIONS RESULT IN THE GENERATION OF LONG-LIVED DOUBLE STRAND DNA BREAKS IN HUMAN CELLS AND CHROMOSOME RECOMBINATION IN RODENT CELLS, Shoshana Squires, Juan F. Gimenez-Abian, Anderson J. Ryan, Ann M. Mullinger and Robert T. Johnson, Cancer Research Campaign DNA Repair Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK.

DNA replication is stalled at UV-induced lesions in both human and rodent cells, but their responses are substantially different. We assess stalling at the major primary lesions, the cyclobutane pyrimidine dimers (CPDs), by measuring the double strand DNA breaks (DSBs) produced by the T4 DenV endonuclease in DNA newly replicated during the first hour after UV. The level of these breaks in nascent DNA is similar in wild type human and hamster cells, and also in cells with ERCC-2 gene mutations (XPD, UV5). In all the hamster cells, and in wild type human, the level of DSBs generated at stalled replication sites diminishes with time, indicating either that the DNA polymerases bypass the lesions without leaving a gap in the daughter strand, or that the CPDs have been repaired before replication is resumed. In human cells UV-irradiation also induces DSBs without DenV treatment, predominantly at the replication forks, and their level varies linearly with the UV sensitivity of different repair-defective mutants. Our results indicate that these DSBs are formed at or near stalled replication sites. They are long-lived, and in XPD, but not in UV5, a large number of DSBs accumulate. XPD cells arrest in G_2 where they probably die. In hamster cells, the replication fork damage is highly recombinogenic leading to S phase-dependent exchange aberration in the first mitosis. By contrast in human cells, stalled replication forks result predominantly in the generation of chromatid breaks.

C5-435 TESTIS- AND MALE GERM CELL EXPRESSION OF *Xrcc-1*, Christi A. Walter[1], Zi-Qiang Zhou[1], Larry H. Thompson[2] and John R. McCarrey[3], [1]Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7762, [2]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551-0808, [3]Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78228

The DNA repair gene *XRCC1* has been shown to be involved in DNA strand-break repair based on its ability to restore the wild type level of DNA repair to the Chinese hamster ovary cell mutant EM9. Human and murine genes have been cloned. The purpose of this study was to develop animal models to characterize the expression of *Xrcc-1* in tissues and cell lines to determine if there is tissue-specific expression and to provide a baseline of information for future studies that may involve altering *Xrcc-1* expression in mice. Mice were selected as one model system because they are the most likely vehicle for studies that involve altering DNA repair gene expression via transgenics and germline inactivation by homologous recombination and because reproduction and embryology are well characterized. Baboons were selected as an additional model system because they are evolutionarily closely related to human and serve as a primate model. RNA was prepared from select tissues of young adult male C57Bl/6 mice, young adult W/W mouse testes which are deficient in germ cells, enriched populations of adult pachytene spermatocytes and round spermatids, a variety of cultured mouse cell lines, and select tissues from 4- to 5-year old baboons. Normal young adult male testis and enriched populations of pachytene spermatocytes and round spermatids displayed significantly higher levels of *Xrcc-1* expression than other mouse tissues, although *Xrcc-1* transcripts were found in low abundance in all tested tissues. Cultured mouse cell lines displayed levels of expression similar to male germ cells which is a striking contrast to the levels of expression obtained in somatic tissues from the mouse. Baboon testis displayed significantly higher levels of *XRCC1* expression than other baboon tissues, similar to the pattern of expression found in mice. The relatively high levels of expression identified in mouse and baboon testis and mouse pachytene spermatocytes and round spermatids suggest Xrcc-1 may have an important role in male germ cell physiology.

This work was supported by March of Dimes Foundation, NIH grant HD-23216 and a portion of this work was done under the auspices of the U.S. DOE by LLNL under contract number W-7405-ENG-48.

C5-436 p53 BINDS SINGLE STRANDED DNA ENDS THROUGH C-TERMINAL DOMAIN AND INTERNAL DNA SEGMENTS VIA THE MIDDLE DOMAIN, Galina Selivanova*, Georgy Bakalkin#, Tatjana Yakovleva#, Elena Kiseleva^, Laszlo Szekely*, Lars Terenius#, and Klas G. Wiman*., *Microbiology and Tumor Biology Center, and Departments of #Drug Dependence Research and ^Cell and Molecular Biology, Karolinska Institute, S-17177, Stockholm, Sweden.

We have previously shown that wild type p53 can bind single stranded (ss) DNA ends and catalyze renaturation of ss complementary DNA (1). Using p53 deletion mutants we have mapped the DNA end binding site to the C-terminal domain of p53 protein (residues 320-393). Besides the end of the ss DNA molecule, p53 can also bind internal segments of long ss DNA molecules via the binding site (DNA int site) distinct from the binding site for DNA ends (DNA end site). The DNA int site of the p53 protein was mapped to the central region of the molecule (residues 99-307). The carboxy-terminal domain alone was sufficient to catalyze DNA renaturation, although the central domain was also involved in promotion of renaturation by the full length protein. Importantly, that in the presence of excess of ssDNA ends we observed stimulation of p53 binding to ssDNA via its int binding site. The observation that p53 binds to ssDNA ends have led us to suggest that p53 may serve as an intracellular sensor of DNA strand breaks in vivo (1). It is conceivable, that the interaction of the C-terminal domain with ss ends of damaged DNA can activate ss DNA binding by the central domain of p53 in vivo, thus enhancing the ability of p53 to promote ss DNA reassociaton. Our results raise the possibility that p53 can participate in DNA repair in a more direct way, namely by promoting appropriate joining of protruding ss ends of damaged DNA through its ability to bind ss DNA ends and promote reannealing of ss complementary DNA.

1. Bakalkin, G., Yakovleva, T., Selivanova G., Magnusson, K.P., Szekely, L., Kiseleva, E., Klein, G., Terenius, L., and Wiman, K.G. (1994). Proc. Natl. Acad. Sci. USA 91, 413-417.

C5-437 COMPLEMENTATION OF FANCONI ANEMIA GROUP C (FACC) CELLS WITH FACC cDNA CORRECTS SPECIFIC DRUG-INDUCED CYTOTOXICITY AND CELL CYCLE ARREST, Gary M. Kupfer and Alan D. D'Andrea, Department of Pediatric Oncology, Dana-Farber Cancer Institute and Children's Hospital, Harvard Medical School, Boston, MA 02115.

Fanconi anemia (FA) is an autosomal recessive disease marked by developmental defects, bone marrow failure, and cancer susceptibility. The diagnostic test for FA depends upon the extreme sensitivity of FA cells to DNA cross-linking agents such as mitomycin C (MMC) and diepoxybutane (DEB). Recently, the gene for one of the four known complementation groups, known as FACC, was cloned, but the function for the encoded protein, now known to be cytoplasmic, remains unknown.

To test the hypothesis that heterologous expression of FACC cDNA corrects MMC sensitivity, we compared two isogenic cell lines: HSC536N, a FACC cell line exquisitely sensitive to MMC, and the same cell line transfected (corrected) with wild type FACC cDNA. The assay was performed using the MTT test, a colorimetric test that relies upon bioreduction of dye as an indication of cytotoxicity. The corrected and uncorrected cell lines were exposed to various dilutions of MMC over 4 days and then assayed. The corrected cells showed a 10 fold diminution in cytotoxicity in the 1uM dose range, as compared to the uncorrected cells. In addition, to test the hypothesis that FACC cDNA also corrects a cell cycle defect, we analyzed cell cycle distribution of the uncorrected and corrected cell lines treated with MMC, using propidium iodide staining and FACScan in order to measure DNA content. Corrected cells showed a two fold lower G2 accumulation under equivalent conditions as the MTT test, correlating with those results. A known cross-linking agent, cisplatin, which does not require the same bioactivation that MMC does, exhibited the same magnitude of effect by MTT and cell cycle assay. Cytosine arabinoside, an anti-metabolite, produced no difference in cytotoxicity, and etoposide, a topoisomerase II inhibitor that causes DNA strand breaks, had only slightly less cytotoxicity (0.5 fold) in the corrected cells.

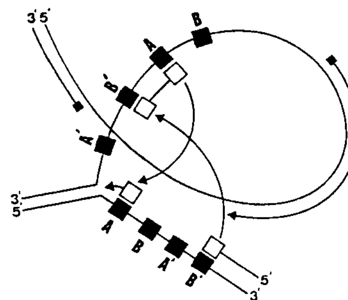
We conclude that DNA cross-linking agents demonstrate the largest differences in cytotoxicity between corrected and uncorrected FACC cells, that this difference correlates with the degree of G2 accumulation, a measure associated with genomic instability, and that other drugs' cytotoxic actions with different mechanisms are not protected to the same extent.

C5-438 Hairpin DNA metabolism and a possible role in genome maintenance in higher eukaryotes. S. M. Lewis and S. Suh, Division of Biology, California Institute of Technology, Pasadena, CA 91001 (ACS grant IM599C).

Murine cells were found to efficiently resolve a dimeric circular DNA molecule comprised of a 15.4kb perfect inverted-repeat. The test DNA, prepared *in vitro* from a polyoma-based shuttle vector plasmid, was reproducibly and site-specifically reduced to a 7.7kb monomer circle after transfection into various cell lines. The resolution of the inverted-dimer circle is readily understood on the basis of a scheme involving the nicking of hairpin structures, which might be imagined to form, for example, upon cruciform extrusion at each axis of symmetry. This is because, by DNA sequence analysis, the resolution junctions closely resembled those obtained when linear vector DNA with "hairpin" ends was likewise transfected into murine cells. In a previous study, the fine-structure of rejoined hairpin linear molecules indicated that the hairpin ends (bearing a covalent 5' to 3' interstrand connection) were nicked in a characteristic fashion near, but not exactly at, their termini prior to joining (S. M. Lewis, *PNAS* **91**:1332-36, 1994). Because neither inverted-dimer resolution, nor hairpin linear re-circularization has been described for *E. coli*, the possiblity exists that an ability to carry out these operations reflects a special feature of DNA metabolism in higher eukaryotes. The charactaristic and somewhat unusual pattern of cleavage of hairpin DNA structures in these studies suggests that hairpin DNA is specifically targeted, perhaps by a DNA repair process. These results have implications with regard to 1) the "hotspot" instability of inverted-repeat DNA , 2) the origin of "snap-back" DNA in vertebrate genomes, and 3) DNA amplification and replication mechanics.

C5-439 INVERSIONS WITH DELETIONS AND DUPLICATIONS A MODEL INVOLVING CONCURRENT REPLICATION. Alasdair Gordon[1] and Jennifer Halliday,[2] Institut Curie[1] and Institut Monod,[2] Paris France.

Inversion mutations have recently been seen in model eukaryotes and in humans suffering genetic disease and include inversion leaving flanking sequence intact, inversion in conjunction with deletion of sequence at one end or at both ends and inversion of sequence, some of which is duplicated, with deletion of sequence at one end. The junctions of the juxtaposed regions exhibit an immediate complementary sequence nature. Although a variety of models have been proposed none appears sufficiently robust to explain all such events. We suggest a strand misalignment-realignment model involving concurrent DNA replication (see Fig.). Leading strand misalignment onto the lagging strand template followed by chain growth and realignment onto the leading strand template would invert the sequence replicated. The inversions can be modelled as the rearrangement of blocks of sequence defined by inverted repeat series whose order of replication is determined by leading strand misalignments and realignments.



C5-440 MOLECULAR ANALYSIS OF DELETIONS FORMED AT THE ENDOGENOUS HPRT LOCUS IN FANCONI ANEMIA CELLS, Agnès Laquerbe, Ethel Moustacchi, James C. Fuscoe and Dora Papadopoulo, URA 1292 du CNRS, Institut Curie-Biologie, Paris, France

A high level of chromosomal breakage, either spontaneous or induced by DNA cross-linking agents, is an important feature of Fanconi anemia (FA), an inherited DNA repair disorder characterized by progressive bone-marrow failure, developmental abnormalities and predisposition to leukemia. We have previously reported that in contrast to normal cells in which the vast majority of spontaneous and psoralen photoinduced *HPRT*$^-$ mutations are base substitutions, in FA lymphoblasts a high proportion of *HPRT*$^-$ mutants are due to intragenic deletions (D. Papadopoulo, C. Guillouf, H. Mohrenweiser and E. Moustacchi, Proc. Natl. Acad. Sci. USA 87, 8383-8387, 1990). Taken together with the increased chromosomal instability, these observations indicated that the wild type FA gene(s) play an important role in the maintenance of the genomic integrity.

In order to obtain informations on the mechanism(s) underlying the genomic rearrangements in FA, the breakpoint sites of deletions in 11 FA-derived *HPRT*$^-$ mutants were analysed. The pattern of psoralen photoinduced FA deletions indicates that the action of a site-specific mechanism may take place since the rearrangements in independent mutants involving a given exon are identical and since deletions of different size have the same breakpoint. Interestingly, at some of the 3'breakpoint junctions we observed an heptamer motif identical to the essential element of the recognition signal sequences which direct cleavage and joining in the V(D)J recombinational process, a mechanism that normally controls immunoglobulin and TCR genes assembly during development. Our observations suggest that the wild type FA gene(s) may be involved in the control of an aberrant site-specific cleavage activity. The question arises whether an illegitimate V(D)J recombination may represent a secondary repair pathway activated in some critical situations for cell survival.

C5-441 THE EFFECT OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C (FACC) PROTEIN ON NITROGEN MUSTARD (HN2)-INDUCED CYTOTOXICITY, APOPTOSIS, G2/M ARREST AND DNA INTERSTRAND CROSS-LINKING. Marathi, U.K., Reagh, S.H., Brent, T.P. Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN., 38101. Hypersensitivity to DNA cross-linking agent-induced cytotoxicity and chromosomal aberrations has suggested that the underlying defect in Fanconi anemia is in DNA interstrand cross-link (ISC) repair. The FACC protein can correct the mitomycin C and diepoxybutane hypersensitivity in Fanconi anemia lymphoblast cell lines belonging to the complementation group C. However, the mechanism by which the FACC gene product protects cells is yet unknown. In the current study, we have attempted to discern the cytoprotective mechanism by comparing HN2-induced cytotoxicity, apoptosis, cell cycle arrest and DNA damage in the mutant HSC-536N and the corrected HSC-536N+pFACC3 cell lines. The IC50 values for the mutant and corrected cell lines were 10nM and 150nM, respectively. Treatment of asynchronous cultures with 25-250nM HN2 resulted in dose and time-dependent G2/M arrest. Whereas the corrected line escaped G2/M block within 24-48 hr after treatment with 250nM HN2, the mutant line was arrested in G/M for at least 96 hr. The protracted G2/M block in the mutant line was associated with DNA degradation characteristic of apoptosis. Preliminary measurements of HN2 induced ISC formation and repair indicate no differences between the mutant and corrected cell lines, suggesting that the FACC protein may not be involved in the initial steps of ICS repair. (This work was supported by NIH grant CA60178 and by the ALSAC.)

C5-442 REGULATED EXPRESSION OF RESTRICTION ENDONUCLEASE ACTIVITY IN MAMMALIAN CELLS

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DNA double-strand breaks (dsbs) are the primary lethal lesion in cells exposed to ionizing radiation (IR). Detecting cellular responses specific to the dsbs formed by IR is hampered by a broad spectrum of damage. Transfer of restriction enzymes (REs) into mammalian cells affords access to dsbs in the absence of other lesions; however, this approach is not quantitatively radiomimetic due to the variable introduction of REs into cells in quantities ranging from none to much greater than average (Bryant, *I.J. Rad Biol* 54:869, 1988). While this effect might be eliminated by regulated, homogeneous RE gene expression in mammalian cells, such a strategy has been limited by leaky transcriptional control (e.g. Morgan, *Mol. Cell. Biol.* 8: 4204, 1988). **Methods:** Braselmann *et. al.* reported stringent regulation by estrogen of a chimeric transcription factor::estrogen receptor (ER) fusion protein (*PNAS* 90: 1657, 1993). We have used this approach to regulate RE activity in mammalian cells in an effort to create a radiomimetic dsb model system tightly controlled by estrogen. PCR was used to modify a RE gene (Pvu II) to allow efficient translation in eucaryotic cells; expression of the modified gene in *E. coli* verified function by phage restriction. This gene was installed in a mammalian expression system expected to afford estrogen-dependent regulation; the resulting construct has been transferred into rodent cells. **Results:** Recovery of robust, G418-resistant stable transfectants at similar frequencies for RE gene-containing and control constructs (in the absence of estrogen) indicates that any basal Pvu II expression in the former case occurs at a level tolerated by the cells. Preliminary results suggest cytotoxicity associated with estrogenic steroid exposure in the former, but not the latter, cells. The dependence of killing on estrogen exposure duration is currently under investigation, as is the ability of putative RE-induced dsb to function as sublethal damage with respect to subsequent IR exposure. Direct measurement of dsb yields will be performed using pulse field gels. **Conclusion:** The Pvu II gene has been engineered for regulated mammalian cell expression. This system apparently affords tight control of intracellular RE activity by estrogen, providing a means to study quantitative DNA dsb effects in the absence of other changes generated in X-irradiated cells. Current results will be presented.

C5-443 CLONING OF THE HUMAN *XRCC3* DNA REPAIR GENE, WHICH AFFECTS CHROMOSOME STABILITY AND BROAD-SPECTRUM MUTAGEN SENSITIVITY,

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The CHO line irs1SF was isolated as an X-ray sensitive mutant and characterized as having elevated spontaneous chromosomal aberrations and hypersensitivity to UV radiation, ethyl methanesulfonate, and especially DNA crosslinking agents such as mitomycin C (MMC) (Fuller & Painter, Mutat. Res., 193:109-121, 1988). No other members of this complementation group are known. The *XRCC3* gene was identified by somatic cell hybrids as a gene that conferred MMC resistance to irs1SF. To clone the *XRCC3* cDNA, we transfected irs1SF with a human pEBS7 cDNA expression library provided by Dr. Randy Legerski. We isolated primary transformants having resistance to MMC and to hygromycin from the plasmid dominant marker. The DNA from one primary clone was used to obtain a secondary transformant, from which a cosmid library was made. Library screening using the hygromycin gene as a probe gave several cosmid clones that conferred MMC resistance. The correcting activity was localized to a 4.2 kb *Eag*I fragment that includes the CMV promoter of the pEBS7 vector. The cDNA is ≈ 2.5 kb in size. Both genomic and cosmid transformants showed only partial correction for resistance to MMC (≈ 20%). Interestingly, the slow growth rate of irs1SF (T_d = 22 hr) was significantly improved in the transformants, and both the low plating efficiency and chromosomal aberrations were efficiently corrected, i.e. ≈ 90%. These results suggest that the damage causing the chromosomal aberrations is different from that produced by mitomycin C and show that the response to both kinds of damage is improved by the cDNA. Among 28 MMC-resistant hybrids made between irs1SF and human lymphocytes, chromosome 14 was the only human chromosome present in all hybrids. Fluorescence in situ hybridization localized the 4.2 kb *Eag*I fragment to chromosome 14q32.3, suggesting that the gene conferring resistance to MMC in hybrids corresponds to the cloned cDNA. (This work was done under the auspices of the US. DOE by LLNL under contract No. W-7405-ENG-48 and supported by NIH grant no. CA34936.)

C5-444 recA-DEPENDENT DOUBLE-STRAND BREAK REPAIR OF PLASMID AND CHROMOSOMAL DNA IN THE RADIATION RESISTANT BACTERIUM DEINOCOCCUS RADIODURANS,

Michael Daly and Kenneth Minton, USUHS, Bethesda, MD 20814

D.radiodurans is a bacterium which is characterized by its extreme resistance to the lethal and mutagenic effects of both ultraviolet and ionizing radiation. To date, D.radiodurans is the most radiation resistant organism discovered, showing full survival at exposures as high as 3,000,000 rad.

We have shown that the extraordinary DNA repair capabilities of this organism require recA-dependent functions. Following an exposure to ionizing radiation (1.75 Mrad), wild-type repairs >100 double-strand breaks per chromosome, whereas a recA-defective mutant does not repair any.

A two-plasmid system was developed to provide physical evidence for recombination after 1.75 Mrad irradiation. Each plasmid was designed to contain different mutant alleles of a drug resistance gene as well as physical polymorphisms. It is evident that recA-dependent homologous recombination is extensive and plays an integral part in D.radiodurans' plasmid repair as demonstrated by the appearance of cross-over products visualized in gels.

A chromosomal study-system was developed to monitor repair of the chromosomes following 1.75 Mrad. After irradiation, recA dependent homologous recombination between chromosomal reporter sequences was extensive, irrespective of location; with clear evidence of recombination in regions as small as 100bp. We predict >700 recombinational repair events per chromosome.

We report a remarkable phenomenon of irradiation-resistant plasmid purified from D.radiodurans. When identical plasmids are CsCl-purified from D.radiodurans and E.coli, D.radiodurans' plasmids are more damage-resistant. Upon irradiation of D.radiodurans' plasmids, higher order structures become apparant. Structural models accounting for the radiation resistance of D.radiodurans plasmid will be presented.

C5-445 THE GENETIC REQUIREMENTS FOR SINGLE-STRAND ANNEALING PATHWAY OF DOUBLE-STRAND BREAK REPAIR IN YEAST,

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In *S. cerevisiae*, there are two major mechanisms for repair of a DNA double-strand break (DSB): (i) gap repair, a conservative mechanism leading to a gene conversion event (Szostak et al. 1983), and (ii) single-strand annealing (SSA) mechanism, in which repair of a DSB is accompanied by a deletion of one of the flanking direct repeats along with the loss of intervening sequences (Fishman-Lobell et al., 1992). We are interested in the genetic requirements for the SSA pathway of repair.

In our experiments, we induced DSBs by galactose-driven expression of a site-specific HO-endonuclease. As a substrate for HO-induced recombination, we used a plasmid which carries two directly repeated copies of the *E.coli* lacZ gene, one of the two being interrupted by a 117-bp fragment containing HO recognition site. By physical monitoring of recombination, we found that only *RAD52* gene is required to repair HO-induced DSBs by SSA mechanism, whereas *RAD51*, *RAD54*, *RAD55* and *RAD57* are not. *RAD50* and *XRS2* genes are also not required, however, *rad50Δ* and *xrs2Δ* mutations markedly retarded the kinetics of 5' to 3' degradation of the DSB end.

Earlier, we showed that the *RAD1* gene is involved in the SSA pathway to remove nonhomologous sequences from the 3' ends of recombining DNA (Fishman-Lobell and Haber, 1992). We extended this observation to show that *RAD10*, but not other excision-repair genes (*RAD2, 3, 4, 7, 14, 16, 25*) are required for the completion of recombination.

Currently, we are introducing the lacZ direct-repeat sequences into a chromosome to see whether the genetic requirements for SSA are different for plasmid *vs.* chromosome substrates.

C5-446 Evidence for Ku end binding activity in DNA double-strand break (DSB) repair and V(D)J recombination: Sexi Mutants. Sangeun Lee and Eric A. Hendrickson, Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912.

Four ionizing radiation sensitive (IR^S) and DSB repair mutants, sexi-1 to sexi-4, were isolated from the Chinese hamster lung V79-4 cell line. We have further demonstrated that all these mutants are severely impaired in V(D)J recombination. Cell fusion experiment showed that sexi-2 and sexi-3 belong to the XRCC5 complementation group, whereas sexi-1 defined a novel mutation. Recently, representatives of the XRCC5 complementation group were shown to be severely deficient in a DNA end-binding activity, similar to a previously described activity of the autoantigen Ku. Additional experiments have demonstrated that Ku, (a p70/p86 heterodimer) activity in xrs cells can be functionally complemented by a p86 Ku cDNA. To extend this finding to the sexi mutants, we performed DNA mobility shift assays. As expected, sexi-2 and sexi-3 lack Ku DNA end binding activity. Moreover, they express no or very low levels of p86 Ku mRNA, and on introduction of a p86 Ku cDNA, IR^S, DNA end-binding and V(D)J recombination defects were overcomed. Finally, we also studied DNA end-binding activity in sexi-1 cells, and showed that they too were deficient in Ku DNA end-binding. Introduction of a p70 Ku cDNA complemented the IR^S, DNA end binding, and V(D)J recombination defects of sexi-1. Interestingly, northern and western analyses indicated that endogeneous p70 mRNA and p70 protein are expressed in sexi-1 cells, suggesting that the sexi-1 defect may be a subtle missense mutation. Currently, sequencing analysis of Ku70 cDNAs in wild-type and sexi-1 mutant cells is underway.

C5-447 ENRICHMENT OF A DNA END-JOINING ACTIVITY FROM *XENOPUS LAEVIS* EGG EXTRACTS

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DNA double strand break (DSB) termini with single strand protrusions (< 10 nt) are efficiently rejoined by Xenopus laevis egg extracts. Using linear plasmid substrates carrying noncomplementary restriction ends, nonhomologous DNA end joining is detected by the formation of monomeric circles (head-to-tail H/T junctions), whereas simultaneously occurring multimers mostly result from simple ligation of complementary ends generating head-to-head (H/H) or tail-to-tail (T/T) dimers. Sequence analysis of H/T junctions indicates that joining in the egg extracts includes precise alignment of DNA termini, gap filling, exonucleolytic removal of mismatched 3'-ends and ligation.

We have constructed novel plasmid substrates carrying non-palindromic and therefore non self complementary 3'-protrusions (NSCP) which prohibit simple ligation by DNA-ligases alone and thus allow to detect nonhomologous end-joining by dimer product formation. Here we report the enrichment of an activity from Xenopus egg extracts which rejoins the NSCP plasmid substrates by formation of H/T, T/T and H/H dimers. In crude extracts, dimers are mainly joined H/T, whereas dimers in joining aktive protein fractions are predominantly joined T/T. Sequence analysis of some of the few H/T junctions formed in these protein fractions shows, that these junctions contain small deletions whose breakpoints coincide with a 5bp sequence homology between the tail terminus and the flanking sequence of the head terminus. Comparison of the terminal sequences in each possible dimer orientation reveals, that the highest degree of homology (6 to 12 bp) is found in the T/T orientation in 18 to 23 bp distance from the termini which could be responsible for preferential T/T formation. A possible mechanism for dimer formation is discussed.

C5-448 REPAIR OF DNA DOUBLE-STRAND BREAKS IN MAMMALIAN CELLS BY HOMOLOGOUS RECOMBINATION AND END-JOINING MECHANISMS, M. Jasin, P. Rouet, and F. Smih, Cell Biology and Genetics Program, Sloan-Kettering Institute, 1275 York Ave., New York, NY 10021. FAX - (212) 717-3317; email - m-jasin@mskcc.org

To study the repair of DSBs introduced into mammalian chromosomal DNA, we have developed expression vectors for rare-cutting, site-specific endonucleases from *S. cerevisiae*. We used the universal code equivalent of the mitochondrial intron-encoded endonuclease I-*Sce* I to build the mammalian expression vector, pCMV-I-*Sce* I. (The I-*Sce* I sequence was provided by B. Dujon, Pasteur Institute.) In addition to providing a consensus Kozak sequence for efficient translation, the I-*Sce* I ORF was modified by fusing sequences encoding a nuclear localization signal and a hemagglutinin epitope tag.

Our initial assay for *in vivo* cutting and enhanced recombination measures extrachromosomal recombination, since this form of recombination is very efficient in mammalian cells and sensitive to DSBs. The assay utilizes RSVCAT plasmid substrates consisting of overlapping chloramphenicol acetyltrasferase (CAT) gene fragments transiently transfected into cells. The RSVCAT plasmids were modified by the insertion of a synthetic I-*Sce* I site at the end of the homology repeats and cotransfections were carried out in COS1 cells. We observed a substantial increase of CAT activity in cotransfections of pCMV-I-*Sce* I with CAT substrates containing the I-*Sce* I site but not with plasmids lacking the site. Southern analysis verified *in vivo* cleavage, as well as recombination. Constitutive expression of the endonuclease is not toxic to mouse 3T3 cells. These results have been recently published (P. Rouet, F. Smih, & M. Jasin, 1994, *Proc. Natl. Acad. Sci. 91,* 6064).

We have recently observed efficient cutting of introduced chromosomal I-*Sce* I sites in 3T3 cells and have found that they are recombinogenic, stimulating gene targeting two to three orders of magnitude. However, they are also repaired efficiently by end-joining mechanisms (P. Rouet, F. Smih, & M. Jasin, *Mol. Cell. Biol.*, in press). Results of these studies will be presented. It is expected that expression of rare-cutting endonucleases in mammalian cells will provide a powerful tool for the analysis of the repair of chromosomal DSBs, as well as for molecular genetic manipulations, such as chromosome fragmentation and, potentially, gene-targeting.

C5-449 TRANSCRIPTION OF DONOR ALLELES INCREASES DOUBLE-STRAND BREAK (DSB)-INDUCED GENE CONVERSION TRACT LENGTHS, Jac A. Nickoloff and Laura Gunn, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115

The mechanism by which transcription enhances homologous recombination in yeast and mammalian cells is unknown. Transcription may increase the frequency of initiating events (i.e., strand separation might facilitate pairing), or it may increase conversion tract lengths (perhaps by driving Holliday junction branch migration). In the latter model, recombination would be enhanced because markers are more likely to be included in conversion tracts as tract lengths increase. We constructed an *ARS1/CEN4* plasmid-based recombination system to test the latter model in yeast. A recipient *ura3* allele contained six silent restriction fragment length polymorphisms (RFLPs) at approximately 100 bp intervals flanking an HO nuclease recognition sequence. Donor alleles either lacked a promoter (Δ*pro-ura3*) or were regulated by the *GAL1* promoter (*GAL-ura3*). Homology lengths were identical in the two crosses. DSBs in recipient alleles were created *in vivo* by HO nuclease (also controlled by the *GAL1* promoter; *GALHO*) stimulating conversion to Ura^+ about 100-fold. We presume that the *GAL-ura3* donor allele is activated before broken alleles are converted since HO nuclease is produced only after the *GALHO* is transcribed and the mRNA is translated. Events involving the chromosomal *ura3* locus were not detected because it was mutant at the same location as the HO site, and crossovers were not detected because these produce dicentrics. Ura^+ plasmids from ≥60 independent products from each cross were transferred to *E. coli* and conversion tracts were measured by RFLP analysis. Donor allele transcription levels had no effects on the proportions of unidirectional tracts (extending 5' or 3' from the HO site), the proportions of unidirectional vs bidirectional tracts, or frequencies of discontinuous tracts. However, the average tract length with a silent donor was shorter than with an active donor (103 bp vs 154 bp). Strikingly, 34% of products from the silent donor had tracts <54 bp, but only 7.4% from the active donor were of this class (significant at the $P = 0.0005$ level; Fisher exact test). These results support the hypothesis that transcription enhances recombination by increasing tract lengths, although they do not rule out the possibility that transcription also enhances initiation. This research was supported by grant CA 55302 from the National Institutes of Health.

C5-450 MODIFICATION OF THE CELLULAR RESPONSE TO IONIZING RADIATION IN CHINESE HAMSTER CELLS BY HUMAN CHROMOSOME 4,
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Hypersensitivity to ionizing radiation (IR) and radioresistant DNA synthesis (RDS), which is a hallmark of ataxia-telangiectasia (A-T) cells, have also been observed in two complementation groups of X-ray-sensitive Chinese hamster cell mutants. To assign the defective gene in these mutants to a human chromosome, microcell-mediated chromosome transfer was performed. We found that the presence of human chromosome 4 suppresses the RDS in both groups of mutants. Moreover, the inhibition of DNA synthesis following IR was also enhanced in the presence of human chromosome 4 in wild-type V79 cells. Surprisingly, chromosome 4 did not improve the cellular survival following IR in either the mutant or the wild-type cells. The level and type of chromosomal aberrations after X-irradiation in G_1 phase, were not notably changed by the presence of chromosome 4 in hamster cells. This indicates that the gene which is defective in the AT-like hamster cell mutants is not located on human chromosome 4, and that the sensitivity to IR, in terms of cell killing and chromosomal instability, is not associated with the RDS, suggesting that radiosensitivity and RDS are two pleiotropic effects of one mutation. In human AT-D cells, which are considered to have two normal copies of chromosome 4, no effect of trisomy 4 on the rate of DNA synthesis following IR, was observed. These results suggest that the gene involved is not responsible for the cellular sensitivity to IR, but regulates the replicative DNA synthesis following IR. PCR analysis of chromosome 4-specific loci in a panel of hamster-human hybrids indicates that this gene, most probably, is located on 4q.

C5-451 PROGRESS IN MAPPING/CLONING THE *IRS1* (*XRCC2*) DNA-DAMAGE REPAIR GENE.
John Thacker, Cathryn Tambini, Andrew George, [1]Paul Simpson, [2,3]Lap-Chee Tsui and [2,3]Stephen Scherer. DNA Repair & Mutagenesis Group and [1]Chromosome Damage Group, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, England; [2]Dept. Molecular & Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8 and [3]Dept. Genetics, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

The *irs1* hamster cell line was selected as sensitive to X-rays but was found also to have sensitivity to a number of other DNA-damaging agents especially those forming cross-links in DNA (Jones et al., 1987, *Mutat. Res.* **183**:279-286). Following on from unsuccessful attempts to clone the gene by DNA transfer, we have pursued a positional cloning strategy. A provisional assignment of the human gene complementing the *irs1* line (the *XRCC2* gene) was made previously to chromosome 7 (Jones et al. 1990, *Mutagenesis*, **5**:15-23).

A series of fusion hybrids was made between *irs1* and a normal human lymphoblastoid line, and hybrids with complementation of sensitivity were analysed in detail. Each of these hybrids has as its major human component part or all of chromosome 7, as revealed by chromosome painting and the presence of chr.7 DNA markers. One of the complementing hybrids has only a small fragment of chromosome 7, and the same fragment was found in an *irs1*/human cell hybrid from a different fusion series (kindly supplied by Dr. L.H. Thompson, Lawrence Livermore Labs.). This chr.7 region appears to be contiguous and has been extensively mapped using *Alu*-PCR-derived probes on hybrid panels, previously mapped probes for this region, and microsatellite markers.

Further, the analysis of radiation-reduced hybrids both retaining and losing the putative *XRCC2* gene has narrowed down the location of the gene to the sub-band level (about 3-5 Mb). Work has started on the use of a library of YACs for this region to complement the defect by fusion to *irs1* cells, and thus to localize and clone the gene.

C5-452 THE REJOINING OF DNA DOUBLE STRAND BREAKS WITH NON-HOMOLOGOUS ENDS *IN VITRO*,
Rebecca Mason, John Thacker and Micaela Fairman, DNA Repair and Mutagenesis Group, MRC Radiobiology Unit, Didcot, Oxon, UK.

The repair of double strand breaks in DNA is critical for cell survival. Here we describe a system to identify factors involved in the rejoining of DNA with non-homologous ends. Two defined pieces of DNA (pUC18 and λ) are cut with separate restriction enzymes so that different end combinations are produced. These are then incubated in cellular extracts, and the product of non-homologous joining identified by size after gel electrophoresis. It has been found that mammalian extracts will join non-homologous ends with differing degrees of efficiency, with 3'-3' mis-matches showing the highest levels of rejoining. This activity is heat labile, salt sensitive and dependant on either Mg^{2+} or Mn^{2+} ions. The rejoining activity has been considerably purified and is different from the enzymes involved in the rejoining of homologous breaks. Several of the non-homologous products have been analysed by DNA sequencing to reveal the rejoining mechanisms employed. To date, fill-in synthesis, base removal followed by blunt end ligation and the involvement of short (1-4 bp), direct repeats have all been detected using this system.

C5-453 THE FIDELITY OF DNA DOUBLE-STRAND BREAK REJOINING IS ENHANCED UNDER PLD REPAIR CONDITIONS,
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This study contributes to the elucidation of the molecular mechanism underlying potentially lethal damage (PLD) repair. Repair of DNA double-strand breaks (dsb) is involved in PLD repair, i.e. in the enhanced survival of cells due to postirradiation treatment under nongrowth conditions before plating cells on nutrient agar (growth conditions). However, dsb are rejoined when cells are kept either in nongrowth *or* growth medium. One possibility to explain the enhanced survival of cells after postirradiation treatment in nongrowth medium might be an enhanced fidelity of dsb rejoining under nongrowth relative to growth conditions. We have addressed this problem by using a plasmid-mediated assay. Into one of the two selectable plasmid markers a single dsb was introduced by a restriction enzyme. The cut plasmid was transfected into an appropriate yeast mutant. Transformants which had correctly rejoined the dsb were selected on the basis of restoration of the *function* of the cut gene. The yeast mutant was allowed to rejoin the cut plasmid under either nongrowth or growth conditions. The results show that the fidelity of dsb rejoining is higher in cells kept under nongrowth relative to growth conditions.

C5-454 AN ASSAY FOR DETERMINING DOUBLE-STRAND BREAK DISTRIBUTIONS AND REJOINING QUALITY IN SPECIFIC GENOME LOCATIONS, Markus Löbrich, Björn Rydberg and Priscilla K. Cooper, Life Sciences Division, Building 934, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

DNA double-strand breaks (dsb) are quantitated by hybridizing radioactively labelled single-copy DNA-sequences/probes to NotI restriction fragments of interest which have been separated according to size by the use of pulsed-field gel electrophoresis. While the production of dsb causes a decreased intensity of the hybridization band compared to an unirradiated control sample, dsb rejoining results in restitution of the band. Since a random rejoining of DNA ends changes the hybridization pattern but reconstitutes the original band with only a negligible probability, our method records almost exclusively correct rejoining events. With the use of DNA-probes to particular restriction fragments we are able to investigate specific regions of the genome with regard to induction as well as repair. A 3.2 Mbp NotI fragment on the long arm of chromosome 21 of a primary human fibroblast cell line was studied after 80 Gy and 160 Gy X-irradiation. The determined induction rate of 5.7 x 10^{-3} breaks/Mbp/Gy compared well with the values for the total genome which have been reported by other authors using different techniques. However, the repair studies after both initial doses yielded a high percentage of breaks that were not correctly rejoined (between 25 and 30% even after incubation times as long as 24 h). In contrast, the corresponding value for the total rejoining events of the whole genome as measured with a conventional pulsed-field electrophoresis technique ranged between 2 and 5%. This difference of at least 20% is likely to represent mis-rejoining events that increase the average molecular weight but do not reconstitute the original 3.2 Mbp NotI fragment. The possibility that there is heterogeneity in the genome with regard to extent and/or fidelity of rejoining dsb is particularly interesting in the light of potential differences between heterochromatic DNA and actively expressed regions. Furthermore, the application of our hybridization method to radiosensitive mutant cells having different defects in dsb rejoining can contribute to an understanding of the pathways for dsb repair.

C5-455 **CpG METHYLATION HAS NO EFFECT ON MAMMALIAN EXTRACHROMOSOMAL RECOMBINATION AND DNA END-JOINING,** Feng Liang and Maria Jasin, Program of Molecular Biology and the Cell Biology & Genetics Program, Sloan-Kettering Institute and Cornell University Graduate School of Medical Sciences, 1275 York Ave., New York, NY 10021. FAX (212) 717-3317, email m-jasin@mskcc.org.

CpG methylation places a protruding methyl group in the major groove of DNA, affecting various biological functions and the metabolism of DNA (for reviews see Doerfler,W, 1983, Ann. Rev. Biochem. 52, 93-124; Razin and Cedar, 1991, Microbiol. Rev. 55, 451-458). Even though abundant information exists regarding the relationship between CpG methylation and its effects on biochemical properties of DNA, gene transcription, mutagenesis, and chromatin structure, it is unknown whether CpG methylation in mammalian cells affects either homologous recombination or DNA end-joining, two major DNA repair pathways. To address this question, a sensitive and quantitative extrachromosomal recombination assay was developed based on bacterial transformation. Extrachromosomal recombination constructs are created from truncated Tn5neo genes, which express only in bacteria, so as to bypass transcriptional effects in mammalian cells. The two intermolecular recombination substrates containing either 5' or 3' truncated Tn5neo genes have an overlap of 300 bp of homologous sequences and can undergo recombination via the single-strand annealing mechanism. The plasmids were cleaved in vitro near the end of homology to stimulate recombination, then methylated by a CpG methylase. The modified substrates were electroporated into COS1 cells. After 4 hours, extrachromosomal DNA was recovered, and analyzed by Southern blotting and by bacterial transformation. Our results showed no difference in either homologous recombination or DNA end-joining between methylated and unmethylated substrates. Therefore, CpG methylation appears to have no or a very limited effect on either extrachromosomal recombination or DNA end-joining.

C5-456 THE REJOINING OF DNA DOUBLE STRAND BREAKS WITH HOMOLOGOUS TERMINI *IN VITRO*, Micaela P. Fairman and Andrew Johnson, DNA Repair and Mutagenesis Group, MRC Radiobiology Unit, Didcot, Oxon, UK.

Numerous studies have shown that double strand breaks in DNA lead to a wide variety of cellular endpoints such as chromosomal aberrations and cell death. However, the enzymes responsible for the rejoining of double strand breaks are poorly defined. Although several DNA ligases have been isolated from mammalian sources, there is increasing evidence that additional factors are required for full activity of these enzymes. By using defined DNA cut with a restriction enzyme to produce homologous ends, in conjunction with biochemical fractionation of mammalian extracts, we have identified multiple proteins involved in the rejoining of double strand breaks. Using the rejoining of the plasmid pUC18, cut with the restriction enzyme Sal I as an assay, we have been able to identify several fractions that will join such breaks. One of these fractions has been highly purified, and has been shown to be free of ligase I. Paradoxically, no adenylation product, which is diagnostic for ligases, can be demonstrated in this fraction. We have also identified two factors which contain no break joining activity themselves, but will greatly stimulate a purified DNA ligase. One of these factors (REP 1), has been extensively purified but is of very low abundance. The second factor (REP 2) also been has been purified over several chromatographic steps. This appears to have similar properties to REP 1, but elutes at a different molecular weight on sizing columns. The further purification of these factors is in progress.

C5-457 THE PROCESSING OF DNA DOUBLE-STRAND BREAKS (DSBS) DURING HOMEOLOGOUS RECOMBINATION IN MAMMALIAN CELLS , Abdellah Belmaaza, Josée-France Villemure and Pierre Chartrand, Research and Development, Canadian Red Cross Society, Montreal Center, 3131 Sherbrooke St. E., Montreal, Canada, H1W 1B2 and Departments of Microbiology-Immunology and Molecular Biology, Université de Montréal.

Studies done in prokaryotes and eukaryotes have indicated that DNA sequence divergence decreases the frequency of spontaneous homologous recombination. To determine if repair of DSBs by homologous recombination is also sensitive to DNA sequence divergence in mammalian cells we have used an assay that does not rely on the recovery of functional products. The assay is based on the acquisition by homologous recombination of endogenous mouse LINE-1 sequences (L1Md) by exogenous LINE-1 sequences. We introduced into mouse cells four gapped plasmid-borne LINE-1 sequences. In two plasmids, the gap is flanked on both sides by LINE-1 sequences either from the mouse, L1Md-A2 (95% average homology to L1Md) or from the rat, L1Rn-3 (less than 85% homology to L1Md). In the other two chimeric plasmids, the gap is flanked on one side by L1Md-A2 sequences and on the other side by L1Rn-3 sequences in both possible configurations. All four plasmids gave *similar frequency of homologous recombination*. However, recombinants with precise gap repair were obtained only when L1Md-A2 sequences were present at least on one side of the gap. In the imprecise recombination events, gap repair was initiated from either side of the gap and was either partial or extended to various *distances in the L1Md sequences homeologous* to those flanking the gap. The simplest interpretation of these results is that DNA sequence divergence between the LINE-1 elements does not interfere with the early step(s) of DSB repair but rather influences later step(s) of this process. The results will be discussed in terms of homology search, pairing, strand-invasion, the processing of recombination intermediates, the stability and evolution of the mammalian genome.

C5-458 LETHALITY, CHROMOSOME LOSS OR DELETION CAN RESULT FROM A SITE-SPECIFIC DOUBLE-STRAND BREAK WITHIN A DISPENSABLE HUMAN YAC IN YEAST. Craig B. Bennett, Tammy J. Westmoreland, Joyce R. Snipe and Michael Resnick. Lab. of Molecular Genetics, Nat. Inst. of Environmental Health Sciences, Research Triangle Park, NC 27709
Lethality resulting from unrepaired genomic double-strand breaks (DSBs) has generally been attributed to the loss of essential genetic material. Recently, we demonstrated that an unrepaired, site-specific DSB between nonhomologous DNA sequences within a centromere-containing plasmid (YZ-CEN) could lead to lethality in a haploid yeast (*Saccharomyces.cerevisiae*) strain even though the DNA was dispensable (1). We proposed that a persistent DSB *per se*, not just loss of essential information, can be an important source of lethality. To examine this idea further, we integrated a YZ site (from *MAT*) into a yeast artificial chromosome containing 360 kb of human DNA (YAC12) within a diploid yeast strain by Alu targeting. An inducible plasmid-borne endonuclease (HO) fused to a *GAL1-10* promoter was used to produce a site-specific DSB at the YZ target when cells were incubated on galactose. Cell survival, YAC loss and the kinetics of DSB induction were examined for independent isolates carrying YZ integrated at different sites. All YACs exhibited loss of the YZ-associated genetic marker *URA3*. For most YACs, there appeared to be a simple deletion, presumably due to recombinational repair between flanking Alus induced by the DSB (cells were plated to medium nonselective for the YAC). As expected the repaired DSB did not affect survival. However, for three of the YACs (A8, A17 and A26), the consequences of the YZ break were more profound. Unlike the others, a persistent site-specific break was observed in the three YACs based on Southern blotting of chromosomes separated by TAFE gel electrophoresis. Two of them (A8 and A17) exhibited reduced survival (~30%) compared to cells containing the original YAC12. Most (>95%) of the survivors had lost the entire YAC. Among the survivors a few had YACs with large deletions surrounding the original YZ site and some had terminal deletions. The low survival, and YAC loss, with A8 and A17 is similar to results with the YZ-CEN plasmid (1) supporting our original proposal that an unrepaired DSB in nonessential genetic material--in this case human DNA--can lead to lethality. The high survival of the A26 YAC-containing strain appears to be due to slow production of the DSB.

1. Bennett et al. Proc. Nat. Acad. Sci. 90 (1993) 5613

Mutagenesis/Genomic Instability/Cell Cycle

C5-500 DUAL REGULATION OF THE *RECA* GENE OF *BACILLUS SUBTILIS*. Ronald E. Yasbin, David Cheo, Sun Ji, and Ken Bayles. Department of Biological Sciences, UMBC, Baltimore, MD. 410-455-3668, yasbin@umbc.edu.

The SOS regulon of *Bacillus subtilis* responds to DNA damage as well as to developmental signals. An important effector molecule for this regulon is the RecA protein. Transcription of the *recA* gene is induced following DNA damage via a repressor-type of mechanism that requires a functional RecA protein. However, during the development of the competent state the *recA* gene is induced via an activator mechanism dependent upon ComK but independent of RecA. Using a mutagenesis strategy, we have identified sequences upstream of the structural *recA* gene that are involved in the DNA damage and/or competence induction of this key regulatory protein. Additional results have demonstrated that a nucleotide change within the -35 recognition sequence for the σ^A containing RNA polymerase enhances the transcription of the *recA* gene from non-competent cells while this same mutation decreases transcription in competent cells. On the other hand, mutations within the promoter region of the *recA* gene have been isolated and sequenced that depress competence transcription while not effecting the DNA damage induction. Collectively, the data indicate that the *recA* gene is under tightly controlled transcription mechanisms that are specifically related to the developmental stages.

C5-501 THE ROLE OF *DINR* IN THE SOS PHENOMENA OF *BACILLUS SUBTILIS*, Kevin W. Winterling and Ronald E. Yasbin, Department of Biological Sciences, UMBC, Baltimore, MD 21228
The induction of the SOS regulon of *B. subtilis* results in the derepression of a variety of damage inducible or *din* genes. All of the known *din* genes have at least one copy of the regulatory element, 5'-GAAC-N_4-GTTC-3' (Cheo Box), upstream of the transcription start site. Mutagenesis and deletion analysis of the Cheo Box have demonstrated its importance to the regulation of SOS induction. The DinR protein had been suggested as the cellular repressor of the SOS system, (J. Bacterol., 173; 7084.) and recently, Lovett *et al*., have shown that DinR binds to the Cheo Box (J. Bacterol., 175; 6842.). In order to continue to elucidate the mechanisms that regulate the four distinct phenomena of the SOS regulon of *B. subtilis* , we have begun to examine the role of the *dinR* gene. Specifically, we are examining the regulation of the *dinR* gene itself as well as the role of DinR on bacterial survival and mutagenesis. Our characterizations have revealed that inactivating one copy of the *dinR* gene in strains that are merodiploid for this gene has no adverse effects. However, inactivating *dinR* in a true haploid strain results in a mutant that grows very slowly. Furthermore, these mutants are sensitive to DNA damaging agents.

C5-502 CHARACTERIZATION OF THE *umu*-COMPLEMENTING OPERON FROM R391

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Genetic and molecular experiments have shown that in addition to conferring resistance to antibiotics and heavy metals, certain R-factors carry genes involved in mutagenic DNA repair. These plasmid encoded genes are structurally and functionally related to the chromosomally encoded *umuDC* genes of *Escherichia coli* and *Salmonella typhimurium*. To date, three such plasmid operons, *mucAB*, *impCAB*, and *samAB*, have been characterized at the molecular level. Recently, we have identified three additional *umu*-complementing operons from the IncJ plasmid R391, and two IncL/M plasmids R446b and R471a. We report here the molecular characterization of the R391 *umu*- complementing operon. The nucleotide sequence of the minimal R-plasmid *umu*-complementing (*rum*) region revealed a LexA-regulated operon consisting of two genes, $rumA_{(R391)}$ and $rumB_{(R391)}$, that were separated by seven nucleotides. Although plasmid encoded, the $RumAB_{(R391)}$ proteins showed greatest homology to the chromosomal *E. coli* and *S. typhimurium* UmuDC proteins. Genetic characterization of the $rumAB_{(R391)}$ operon revealed a split phenotype. In *recA*+ and *recA1730* backgrounds the $rumAB_{(R391)}$ operon was phenotypically indistinguishable from *mucAB*. However, in a *recA430* strain, the $rumAB_{(R391)}$ operon gave levels of mutagenesis that were intermediate to *mucAB* and *umuDC*. Characterization of the highly purified $RumA_{(R391)}$ protein suggests that the latter phenotype correlates with the reduced posttranslational processing of the $RumA_{(R391)}$ protein. Thus, the $rumAB_{(R391)}$ operon possesses characteristics that are reminiscent of both chromosomal and plasmid encoded *umu*-like operons and may provide an evolutionary link between them.

C5-503 ACTIVATION OF *DIN1* EXPRESSION IN YEAST

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The yeast *Saccharomyces cerevisiae* has many DNA damage inducible (*DIN*) genes that are specfically expressed in response to DNA damaging agents. *DIN1* is one of two genes encoding alternative forms of the regulatory subunit of ribonucleotide reductase. *DIN1* is not normally expressed in mitotically growing cells, but is strongly induced by DNA damaging agents. In order to study the regulation of expression of *DIN1*, I have screened a library of yeast sequences on a high copy vector for trans-acting genes that would activate or repress expression of a *din1-lacZ* fusion. One such trans-acting gene, *DCX1* (din constitutive expression), when on a high copy vector causes constitutive and hyperinduced expression of *DIN1* in normal and DNA-damaged-induced states, respectively. Multiple copies of *DCX1* however, have no effect on expression of a second damage inducible gene, *DIN3*. These data suggests that *DCX* acts as a positive regulator of *DIN1* and that its action may be specific to *DIN1*.

To further analyze *DCX1* function, I constructed a *dcx1* deletion mutation in the chromosomal gene. The *dcx1* mutant is viable and normal in its growth and its sensitivity to damaging agents at 23°C or above, but it is more sensitive than normal to hydroxyurea at 16°C. Damage-inducible expression of *DIN1* in the *dcx1* mutant as analyzed both by northern analyses and by monitoring reporter gene expression is only slightly decreased in the mutant when grown at 30°C. I am currently analyzing the effects of the *dcx* mutation on the expression of *DIN1* and the *RNR1* and *RNR2* genes at 16°C.

C5-504 EFFECTS OF *recA* MUTATIONS IN THE PHOSPHATE-BINDING LOOP MOTIF ON RECOMBINATIONAL DNA REPAIR AND COPROTEASE FUNCTIONS,

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The bacterial RecA protein plays 2 major roles related to its function as a DNA repair enzyme: 1) it catalyzes DNA strand exchange used in recombinational DNA repair, and 2) it mediates the autocleavage of the LexA repressor protein thereby regulating the derepression of SOS genes that respond to DNA damage. We have used mutagenesis methods to create many unique *recA* mutants with single amino acid substitutions in the P-loop motif of the ATP-binding site and have compared their recombination and coprotease (LexA cleavage) functions *in vivo*. The results show that position Pro67 is unique in that multiple substitutions here differentially affect these two functions. A Trp substitution results in high constitutive coprotease activity and a modest reduction in recombination activity. A Glu substitution results in moderate constitutive coprotease activity and causes a 90% loss in recombination activity. We have also identified a new recombination-proficient/coprotease-deficient *recA* mutant class. Arg or Lys substitutions abolish LexA cleavage but allow for a low level of recombination activity. *In vitro* LexA cleavage assays show that purified 67Trp RecA protein demonstrates a completely relaxed specificity for NTP cofactors and it is also capable of using shorter oligonucleotide cofactors for cleavage of lambda cI repressor. Our findings, interpreted in light of the RecA/ADP crystal structure, are consistent with the idea that Pro67 is located at a position such that mutations here affect the interaction of RecA with both LexA repressor and DNA.

C5-505 INVOLVEMENT OF α-KETO GLUTARATE ALDOLASE IN RESPIRATION DURING THE SOS RESPONSE IN *E. COLI*.

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The SOS regulatory network is induced in *E. coli* by treatments that damage DNA or interfere with its replication. A great number of diverse physiological responses are members of this complex system and most of them share the characteristics of being regulated by RecA protein and LexA repressor. Several reports indicate now that genes only regulated by activated RecA protein are part of the SOS response. Inducible proteins under RecA control were detected in *lexA*(Def) strains by two-dimensional electrophoresis. Microsequencing of one of these has shown that the α-keto-glutarate aldolase (KHG) was part of the SOS network. A disrupted mutant in KHG aldolase was constructed. This mutant is unable to resume respiration after DNA damaging treatment. The KHG aldolase is thus an inducible protein controlled by activated RecA protein and involved in respiration during the SOS response of *E. coli*.

C5-506 IDENTIFICATION OF NEW *umuC* HOMOLOGS BY DEGENERATE PRIMER PCR AMPLIFICATION

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The *umuC* gene product is an essential component for a mutagenic process in *E. coli* known as SOS or error-prone repair. Sequence analysis of at least eight functional *umuC* homologs, residing on bacterial chromosomes or on transmissible plasmids, has revealed six unique groups based on sequence conservation. We have designed degenerate primers directed towards conserved amino acid regions within the UmuC protein (TKTLAK and ERTVRE) to identify new *umuC* homologs in *Citrobacter freundii, Klebsiella pneumoniae, Proteus vulgaris*, and, on the IncT plasmids R394 and Rts-1. Comparison of the DNA sequence of a 280 bp amplicon revealed two new homolog groups, distinct from all previously sequenced members of the *umuC*-like family: The plasmid-encoded IncT Rts-1 and R394 genes (95% nucleotide similarity) and the *K. pneumoniae* and *P. vulgaris* genes (91% nucleotide similarity). *C. freundii umuC* sequences were found to resemble those of *E. coli* (79% nucleotide, 92% amino acid). This approach is being expanded to include other gram negative and gram positive bacteria and should provide insights into the evolutionary relationship of the *umuC* -like family.

C5-507 **REGULATION OF THE MULTIPLE PHENOMENA THAT CONSTITUTE THE SOS REGULON OF *Bacillus subtilis***, Sun Ji and Ronald E. Yasbin, Department of Biological Sciences, UMBC, 5401 Wilkens Avenue, Baltimore, MD 21228 (410-455-3668, Yasbin@umbc.edu).

Bacillus subtilis is a Gram positive soil organism that is capable of several differentiation and developmental events. Previously we had established that the SOS regulon of this bacterium was induced during the development of the competent state (the ability to bind and take-up exogenous DNA). Based on the DNA damage induction, on the competence induction, and the dependence of these processes on a functional RecA protein, the phenomena that constitute the *B. subtilis* SOS system have been divided into four groups or types. Type I phenomena are damage and competence inducible and are dependent upon the presence of RecA. On the other hand, Type III phenomena are dependent on RecA for DNA damage induction but the competence induction is independent of RecA. We have begun to characterize the DNA sequences involved in the two types of regulation through mutagenesis and deletion analysis. Essentially, the DNA damage inducible (*din*) genes of both Type I and Type III have at least one copy of the consensus sequence GAAC-N4-GTTC (Cheo Box) to which the DinR protein (the cellular SOS repressor) binds. However, the Cheo Boxes among the Type III *din* genes (*recA, dinA, dinR*) are found upstream of the -35 σ^A recognition site while the Type I *din* genes (*dinB, dinC*) have their Cheo Boxes located between the -10 and -35 recognition sites. Furthermore, the Type III genes have consensus sequences for the binding of the Competence Transcription Factor (CTF or ComK) and sequences of inverted repeats surrounding their Cheo Boxes. We have isolated mutations within the -10 and -35 recognitions sequences that adversely effect the regulation of either the DNA damage induction or the competence induction of selected *din* genes. Taken collectively, the data suggests that the conformations of the DNA sequences, as well as specific DNA recognition sequences, upstream of the Type III structural genes play an important role in the competence regulation of these genes.

C5-508 ANALYSIS OF THE MOLECULAR MECHANISM OF UV MUTAGENESIS IN *E. coli*, Sumati Murli and Graham C. Walker, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Studies of mutagenesis in *Escherichia coli* have indicated that mutagenesis by UV and many chemicals is not a passive process but rather requires the induction of the SOS system. Genetic analysis has revealed that the products of three genes regulated by the SOS system, *umuD*, *umuC* and *recA*, as well as DNA polymerase III are required for most UV and chemical mutagenesis. Translesion synthesis has been demonstrated *in vitro* by Echols and colleagues using purified UmuD', UmuC, RecA, DNA polymerase III and a substrate carrying a single abasic site.

Analysis of the mechanism of *umuDC*-dependent translesion synthesis has been seriously hampered by the difficulty of purifying UmuC from cells that overproduce UmuC. We are employing an alternative approach which involves a modest overproduction of a derivative of the protein carrying a histidine tag, accompanied by the simultaneous production of other SOS regulated proteins and activated RecA. In this manner, limited amounts of a UmuD'-UmuC complex have been purified without the use of denaturing agents. The biochemical activities and protein-protein interactions of this complex are under investigation.

Consistent with an interaction of UmuD' and UmuC with the replication apparatus are the observations that a strain overproducing the *umuDC* operon is cold-sensitive and that the conditional lethality is associated with an inhibition of DNA synthesis. The nature of this replication defect is being investigated by analyzing the interactions between UmuD'/C and the subunits of DNA polymerase III.

Mutations in the chaperonins *groES* and *groEL* suppress the cold sensitivity of a strain overexpressing UmuD and UmuC. *groES* and *groEL* mutants have been found to have a deficiency in UV mutagenesis and GroEL has been shown to co-immunoprecipitate with UmuC. GroEL and GroES could play a role in mediating the correct folding of UmuC or in stabilizing a complex containing UmuC required for UV mutagenesis. The plasmid pKM101 encodes analogs of UmuD and UmuC called MucA and MucB. Recently we have demonstrated that, in contrast, *mucAB* - dependent UV mutagenesis does not have a dependence on GroEL and GroES.

C5-509 STRUCTURAL ANALYSES AND PROTEIN-PROTEIN INTERACTIONS OF UmuD AND UmuC, SOS MUTAGENESIS PROTEINS OF *E. coli*. Timothy Opperman, Melissa Lee, Angelina Guzzo, and Graham Walker. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The expression of the *umuDC* operon, which is part of the DNA damage-inducible SOS regulon, is required for SOS mutagenesis in *E. coli*. The *umuD* gene product (UmuD) of this operon is post-translationally modified to UmuD', which is active in mutagenesis. UmuD' and UmuC, are thought to interact with RecA* (the activated form of RecA), and DNA polymerase III holoenzyme in such a way that leads to mutagenic bypass of DNA lesions that normally block DNA replication. In this model of SOS mutagenesis, several protein-protein interactions are predicted. To learn about these protein-protein interactions and the relationship between the structural and functional properties of the UmuD and UmuC proteins we have taken several approaches.

A series of UmuD derivatives that contain a single Cys residue has been constructed to study the structure and molecular interactions of the UmuD homodimer. The reactivity of the unique thiol group of each UmuD derivative was used to assess its solvent exposure. In addition, the ability of the thiol group of each unique Cys residue to form disulfide bonds within the UmuD homodimer after treatment with oxidizing agents was examined in order to determine the relative proximity of various positions in UmuD to the dimer interface. A strategy of attaching photo-reactivatable crosslinking agents to the unique thiol groups is being used to analyze the interaction between UmuD and RecA*.

To study the protein-protein interactions between UmuD or UmuD', and UmuC, RecA* and DNA polymerase III holoenzyme we are using affinity chromatography. Purified UmuD or UmuD' has been covalently linked to Affi-gel 15 resin and the retention of UmuC, RecA*, and DNA polymerase III holoenzyme by these columns is being analyzed. In another approach to study protein interactions in mutagenesis we have fused UmuC to Glutathione S-transferase (GST). The interaction between this fusion protein and other proteins involved is SOS mutagenesis is also being analyzed. In addition, we are performing deletion analyses on the fusion protein to determine the sites on UmuC required for interaction with UmuD.

C5-510 EPISOMAL MUTATIONS ACCUMULATING IN *E. coli* UNDER SELECTIVE CONDITIONS: A ROLE FOR CONJUGAL TRANSFER, J. Pablo Radicella, Peter U. Park and Maurice S. Fox, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

When bacteria are subjected to starvation as a consequence of certain non-lethal selections, mutations that allow colony formation accumulate with time. These mutations have been called "adaptive". This observation challenges the established view that mutations occur spontaneously, independent of their consequences. Recent work has shown that "adaptive" reversions of a particular *lacZ* frameshift in *Escherichia coli* have a different spectrum of DNA sequence changes than those arising during exponential growth and suggests that they are the product of DNA replication errors. The origin of this synthesis has been the subject of several models. We propose that, for an indicator strain carrying the *lacZ* frameshift mutation in an F' episome, the appearance of "adaptive" revertants under lactose selection can be associated with the transfer of the episome. We show here that under the selection conditions transfer of the episome occurs during several days of incubation. The revertant lac^{+} allele can be found in scavenger cells that did not originally contain the revertible episomal *lac* allele. After six days of incubation on minimal lactose plates up to 50% of the revertant episomes are found in the scavenger cells. Moreover, the number of late arising colonies from a fixed number of indicator bacteria plated on minimal lactose medium increases with the scavenger cell density. When the same *lacZ* frameshift allele is transferred to the chromosome, the appearance of late arising mutants is reduced more than 20-fold. Episome transfer could be providing a basis for localized DNA replication, perhaps error prone, as proposed by Stahl, that is responsible for the appearance of "adaptive" mutants.

C5-511 EFFECT OF OXIC VERSUS ANOXIC IRRADIATION CONDITIONS ON THE γ-RADIATION-INDUCED $lacI^{d}$ MUTATION SPECTRUM IN *Escherichia coli* WILD-TYPE AND *umuC* STRAINS, Neil J. Sargentini[1] and Kendric C. Smith[2], [1]Dept. of Microbiology and Immunology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501, and [2]Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305

The results obtained from sequencing 285 dominant *lac*-constitutive mutations from wild-type cells that had been ^{137}Cs-γ irradiated (300 Gy) in the presence of oxygen were as follows: 29% G·C→A·T, 21% G·C→T·A, 14% A·T→T·A, 11% G·C→C·G, 9% A·T→G·C, 5% A·T→C·G, 5% *lacO* (mostly long deletions), 2% multiple mutations, 2% multi-base deletions, 1% single-base deletions, and essentially no additions. While deletions of up to 830 bases were frequently detected in the spontaneous mutation sets, base substitutions comprised, by far, the most common radiation-induced mutations (oxic, 89%; anoxic 82%). Overall, the oxic (300 Gy) and anoxic (750 Gy) base substitution spectra were very similar, with A·T→G·C mutations showing the greatest difference between the two sets (oxic, 10%; anoxic, 21%). When the oxic and anoxic sets of base substitutions in the wild-type strain were examined for site-specific differences (greater than twofold), only A·T→T·A mutations (at 7 sites) were specifically enhanced by oxic irradiation, while with anoxic irradiation A·T→G·C (8 sites) and A·T→C·G (3 sites) showed this enhancement. The results obtained from sequencing 461 mutations (300 Gy, oxic irradiation) from *umuC* mutant cells were as follows: 19% G·C→A·T, 7% G·C→T·A, 3% A·T→T·A, 7% G·C→C·G, 16% A·T→G·C, 9% A·T→C·G, 7% *lacO* (mostly long deletions), 1% multiple mutations, 8% multi-base deletions, 21% single-base deletions, and 2% additions. After taking into account that the *umuC* strain produced only 22% as many *lac*-constitutive mutants as the wild-type strain (both irradiated with 300 Gy and yielding surviving fractions of about 8%), it was concluded that the *umuC* strain yielded only 5 to 40% of the wild-type level for most mutation classes, but the *umuC* strain was as proficient as the wild-type strain in producing multi-base deletions, and it was hypermutable (4.3-fold) for the production of single-base deletions.

C5-512 MECHANISMS INVOLVED IN THE ASYMMETRY OF AAF-INDUCED MUTAGENESIS

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In vivo experiments using pUC8 derived plasmids with single AAF adducts have revealed a strong strand specific bias in the induction of frameshift mutations. Indeed ≈20 fold higher mutation frequency is found when the AAF adduct is located in the lagging strand template as compared to the leading strand template. In an attempt to understand this strand bias in mutagenesis we have constructed single adducted plasmids containing strand markers that allows us to analyse the segregation of the DNA strand upon transformation *in vivo*. In all cases, a large majority (>90%) of transformed colonies result from the replication of only the non adducted strand. The remaining colonies contain plasmid progeny derived from both strands. These latter events will referred to as events where translesion synthesis (ie replication through the adduct: TLS) has occured. A careful analysis of the frequency of TLS events led to the following conclusions : i) TLS is about 10 fold more frequent when the adduct is located in the lagging strand as compared to the leading strand giving thus a partial account for the observed difference in mutation frequency, ii) the induction of the SOS system increases ≈ 10 fold TLS frequency in both strands, and iii) the SOS mediated increase in TLS is umuD/C dependent.

In the experiments described above, the single AAF adduct was located at ≈400 nt from the ColE1 origin of replication. Using another set of constructions in which the AAF adduct is located at ≈2000 nt from the origin, the strand bias in mutation frequency was no longer observed. Both leading and lagging strand construction gave rise to the same mutation frequency which in turn was equal to the frequency observed in the lagging strand when the adduct was near the origin. These results will be discussed in terms of the polymerases involved in leading and lagging strand replication in ColE1 plasmids.

C5-513 DEVELOPMENTAL CHOICES: THE LIFE OF A TEMPERATE PHAGE AND THE MAINTENANCE OF LYSOGENY DURING COMPETENCE AND DNA DAMAGE

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The observation that certain lysogenic strains of *Bacillus subtilis* have depressed levels of transformation, led to the characterization of the SOS response in this organism. Lysogens of bacteriophage Ø105 are induced during competence leading to lysis of the competent cells. This lysis leads to the reduced levels of transformation in these lysogens. In contrast, lysogens of Ø3T are induced fully by DNA damage but are not induced during competence and can be transformed at levels comparable to non-lysogenic strains. Both Ø105 and Ø3T prophage are induced in vegetatively growing cells exposed to mitomycin C, yet Ø3T prophage can not be induced in cells which have differentiated to competence prior to mitomycin C exposure. Thus, the "smart" prophage Ø3T can distinguish between SOS caused induction and competence directed induction. On the other hand, the "naive" phage, Ø105 can not distinguish between these two stress conditions.

C5-514 MOLECULAR HANDLES ON ADAPTIVE MUTATION, Susan M. Rosenberg[1], Reuben S. Harris[2], Simonne Longerich[2] and Anne M. Galloway[1], [1]Department of Biochemistry, and [2]Department of Biological Sciences, 4-74 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7 Canada.

An unexpected way of making mutations in bacteria has challenged concepts of the genetic mechanisms behind evolution for the past six years. Mechanistic studies of these so called "adaptive" mutations are rapidly revealing a novel molecular mechanism involving DNA double-strand breaks, genetic recombination, probable DNA polymerase errors, and the possible suspension of mismatch repair during the reversion of a *lac* frameshift mutation in *Escherichia coli.* The molecular details of this process are altering our understanding of how mutations form in non-dividing cells.

C5-515 ROBERTS SYNDROME: MUTAGEN SENSITIVITY ASSOCIATED WITH ALTERED CHROMATIN STRUCTURE. X.M. Shang[1], E.L. Schultz[1], V. Tonk[3], D. Tomkins[4], R.A. Schultz[1,2]. McDermott Center[1] and the Department of Pathology[2], University of Texas Southwestern Medical Center, Dallas, TX; Department of Pediatrics[3], Texas Tech University, Lubbock, TX, and Department of Pediatrics[4], McMaster University, Hamilton, Ontario, Canada.

Roberts syndrome is a rare autosomal recessive human disease which is clinically manifested in the newborn by mental and growth retardation, tetraphocomelia, and a variety of craniofacial abnormalities. Cell lines derived from RS patients exhibit cytogenetic abnormalities which include random chromosome loss and the splaying of heterochromatic chromosomal regions. Quantitative data will be presented illustrating the sensitivity of RS cells to a spectrum of mutagens including UV, MMC, DEB, EMS, and cisplatin. Additional data will be presented for experiments examining a number of parameters related to metaphase chromatin structure in RS, with the following observations: 1) Quantitative evaluations using fluorescence image analysis revealed that RS metaphase chromosomes bind DAPI less efficiently than chromosomes from normal cells; 2) Denaturation of chromosomal DNA with either a C-banding procedure or 70% formamide at 70°C each produced an aberrant hybridization pattern on RS chromosomes in FISH experiments employing biotinylated total human DNA as probe; and 3) RS cells exhibited a >3-fold increase in sensitivity to VM-26, a potent inhibitor of topoisomerase II. Collectively, the data illustrate an altered metaphase chromatin structure in RS. Mutagen sensitivity as a consequence of DNA repair on an altered chromatin context will be discussed.

C5-516 MOLECULAR ANALYSIS OF MITOTIC YEAST CELLS TREATED WITH 8-METHOXYPSORALEN PLUS UVA. Michèle Dardalhon, Bernard de Massy, Alain Nicolas and Dietrich Averbeck. Institut Curie, Section de Biologie, URA 1292 du CNRS, 26 rue d'Ulm, 75231 Paris cedex 05, France.

Mitotic recombination after treatment of yeast cells with the recombinogenic agent 8-methoxypsoralen (8-MOP) plus UVA was analysed. We examined recombination (gene conversion and crossing-over) within the *ARG4* gene using a diploid strain heteroallelic for the *RV* and *Bgl* mutations separated by 1015 nucleotides. Similarly, recombination induced by 8-MOP plus UVA was monitored in a haploid strain containing ectopic *arg4* duplication on chromosome V. Furthermore, we tested the appearance of double strand breaks (DSB) in the *ARG4* region during post-treatment incubation.

The results obtained after 8-MOP plus UVA treatment indicate that, in mitotic cells, *(1)* recombination in the *ARG4* locus is increased 30 to 500 fold per 10^5 survivors depending on the strains and the doses used, *(2)* the increase of recombination results essentially from gene conversion events which involve twice more often the *RV* allele located in the 5' region of *ARG4* gene than the *Bgl* allele, *(3)* gene conversion in the ectopic configuration strain is associated with a low frequency of reciprocal translocation (10%), *(4)* DSB occur during post-treatment incubation in the 5' region of *ARG4* gene and in other intergenic regions. Implications of these results with respect to the sites of action of 8-MOP plus UVA will be discussed.

C5-517 CANCER AS A CONSEQUENCE OF INFORMATION ERASURE THROUGH THE ACTION OF DAMAGE TOLERANCE MECHANISMS, Primavara Grigoriu de Buendia, Instituto de Genetica, Universidad de los Andes, Bogota, Colombia

Cancerous cell characteristics recall those of primitive cells. As a consequence, cancer can be seen as a process of information loss and undifferentiation. Studies with *Xeroderma pigmentosum* cells have shown that carcinogenesis may be a result of increased activity of damage tolerance mechanisms when there are deficiencies of excision repair mechanisms.

I am developing a model which identifies damage tolerance mechanisms as archaic repair mechanisms which maintain cellular viability through patching DNA with primeval monotonous sequences and, in this way, erasing evolved information which was present before damage. This process will return the cells to a primitive state known as cancer.

This research studies the following predictions of this model:

1. Damage tolerance mechanisms should be more efficient than excision repair mechanisms for cellular viability maintenance.
2. Mutations produced by damage tolerance mechanisms should show a more monotonous spectra than those produced by excision repair mechanisms or those produced when there is a decrease of DNA replication fidelity.
3. Nucleotide changes in non-informative parts of the genome must be more monotonous than those in the informative parts of the genome.
4. Mutations in cancerous cells should show a tendency to a monotonous spectra.

Interaction studies between DNA repair genes in *Drosophila* and mutation spectra studies in several species are in agreement with these predictions.

It is expected that this model could improve knowledge about the cancer process, which is still poorly understood.

C5-518 MUTATIONAL SPECTRA INDUCED BY ALKYLATING AGENTS IN CHINESE HAMSTER CELLS IN CULTURE AND IN T-LYMPHOCYTES AND SKIN FIBROBLASTS FROM RATS TREATED IN VIVO, Albert A. van Zeeland, Jacob G. Jansen, Christel W. op het Veld, Malgorzata. Z. Zdzienicka and Harry Vrieling, MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

Mutations induced in the *hprt* gene of cultured Chinese hamser cells and in T-lymphocytes and skin fibroblasts of rats treated with a series of monofunctional alkylating agents were analyzed at the DNA sequence level. These chemicals include EMS, ENU, ENNG, MMU, MMS, and hydroxy-ethyl-nitrosourea (HOENU). Chemicals with a high nucleophilic selectivity (EMS, MMS, MNU), *i.e.* those which do not or very inefficiently react with the oxygens of thymine generate primarily GC to AT transitions which are probably caused by mispairing of O^6-alkylguanine during DNA replication. Chemicals with a low nucleophilic selectivity (ENU, ENNG, HOENU) cause a mixture of GC to AT transitions and AT to TA transversions in cultured Chinese hamster cells which are deficient in alkylguanine transferase activity. In AGT positive rat cells, the class of GC to AT transitions is minor and almost 50% of the base pair changes are AT to TA transversions which are most likely caused by O^2-ethylthymine. In virtually all cases the T involved in this transversion is located in the non-transcribed strand of the *hprt* gene. We hypothesize that this strand bias is caused by difference in error rate of replication past O^2-ethylthymine between the two DNA strands. The mutational spectrum of ENU and HOENU in rat T-lymphocytes were very similar, suggesting that the mutagenic properties of ethyl- and hydroxy-ethyl-DNA adducts are the same. The mutational spectrum of EMS in Chinese hamster EM-C11 cells, which carry a mutation in the XRCC-1 gene, showed a class of deletions that might be caused by a reduced inability of EM-C11 cells to replicate DNA containing 3-ethyladenine.

C5-519 GENOME ORGANISATION OF THE HAMSTER *HPRT* GENE INFLUENCES MUTATION INDUCTION BY UV-IRRADIATION, Judith G. Tasseron-de Jong, Hans den Dulk, Marcel Tijsterman, Piet van de Putte, Laboratory of Molecular Genetics, Leiden Institute of Chemistry, Gorlaeus Laboratories, P.O.Box 9502, 2300 RA Leiden, The Netherlands.

Comparison of mutation induction by UV-irradiation in the endogenous *hprt* gene and in a single copy of *hprt* cDNA on retroviral integrants in hamster cells has shown that the mutation spectra of both forms of the gene were different both in repair deficient cells and in repair proficient cells. The different position of both forms of the gene on the chromosomal DNA does not seem to play a role in the origin of this difference as the position in the genomic DNA of retroviral cDNA integrants has no influence on the mutability(1). Also a difference in the target sequence for lesion induction can not be the cause for the different mutation induction as in both forms of the gene this sequence is exactly the same. This suggests that both lesion induction and repair are influenced by the genomic organisation of the gene.

In contrast to the endogenous gene where mutation induction in repair deficient cells is mainly the result of 6/4 photoproducts(2) this induction in cDNA integrants seems to be mainly the result of cyclobutane dimers. Repair of cyclobutane dimers is strand specific in the endogenous gene(3) but no effect of strand specific repair is observed in the mutation induction in the cDNA integrants. Transcription of both strands of the cDNA seems unlikely as no antisense RNA could be detected by rt-pcr.

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3. Vrieling et al., Mol.Cell.Biol., 9, 1277, 1989.

C5-520 PROXIMAL AND DISTAL EFFECTS OF SEQUENCE CONTEXT ON ULTRAVIOLET MUTATIONAL HOTSPOTS, Michael Seidman[1] and Dan Levy[2], [1] Otsuka Pharmaceuticals, 9900 Medical Center Dr., Rockville, Md. 20850, and [2] NCI, NIH, Bethesda, Md., 20892

Base substitution mutagenesis induced by DNA damage occurs non randomly across a gene. Although it is generally agreed that sequence context is a major determinant of mutational hotspots and coldspots, progress towards defining the critical sequence determinants has been slow. This is because of the difficulty of constructing reporter genes whose sequence can be freely manipulated without destroying gene activity, which is necessary for the mutation assay. We have used a shuttle vector system carrying a suppressor tRNA marker gene to study UV hotspots in repair deficient Xeroderma Pigmentosum (A) cells. We developed a manipulable reporter gene by taking advantage of the structure of tRNA, which permits some sequence alteration while preserving function if appropriate pairing in stems is maintained. An 8 base palindromic sequence, which was the site of two UV hotspots in the interior of the gene, was built into the acceptor stem and pretRNA gene. The new sites were as active mutagenically as the endogenous sites. A series of constructions were prepared which contained all possible bases at the three positions of the palindrome which were in the pretRNA, adjacent to the new hotspots. The UV mutational spectra for these constructions revealed both suppression and enhancement of activity at the new hotspots, but also striking effects on sites as much as 80 bases away. These data indicate that in addition to the traditional focus on bases in the immediate vicinity of a mutational site, attention will have to be paid to the distance over which a sequence element can influence mutagenesis.

C5-521 DNA DAMAGE AND MUTATION INDUCED BY ULTRAVIOLET AND SIMULATED SOLAR RADIATIONS, Evelyne Sage[1], Emmanuel Brulay[1], Ethel Moustacchi[1] and Elliot Drobetsky[2], [1] CNRS URA 1292, Institut Curie Section de Biologie, Paris, France and [2] Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Qué., Canada.

Epidemiological and molecular evidence show that DNA damage and subsequent mutations generated by ultraviolet (UV) radiation within the sunlight spectrum are critical components in the aetiology of skin cancer. In order to investigate the nature and the processing of sunlight-induced premutagenic lesions, two approaches have been taken.

Firstly, we have characterized the mutational specificity of simulated solar light (SLL) at the adenine phosphoribosyltransferase (*aprt*) locus of Chinese hamster ovary cells deficient in excision repair ($ERCC1^-$). This has been compared to the mutation spectrum obtained previously in the same gene, in a repair proficient background (1). The majority of mutations were "UV-like", but in comparison to the wild type (wt) strain, they were very differently distributed along the gene, i.e. the hot spots found in wt (in exon 2) are abolished, and mutations are dispersed in different exons. This emphasizes the role of repair rate for different photolesions in the mutagenicity of SLL.

Secondly, various classes of photolesions induced *in vitro* by UVC (254nm), UVB (300-320nm), UVA (320-400nm) and simulated solar radiation have been revealed using specific DNA repair endonucleases. Cyclobutane pyrimidines dimers (Py< >Py) are the major photolesions induced by these radiations, including UVA. Oxidative DNA damage at pyrimidines (NTH-sensitive sites) and at purines (FPG-sensitive sites) are also observed. The ratio of purine oxidation products to Py< >Py is 1/100 after UVB and UVC, and 1/3 after SSL. After irradiation with UVA, only Py< >Py and single strand breaks are detected at doses up to 1000kJm-2. In solar light, UVB radiation is the major component contributing to photolesions produced *in vitro*. Meanwhile, *in vivo* the UVA component is likely to contribute to the induction of solar photolesions, due to the presence of endogenous photosensitizers which absorb UVA and generate activated oxygen species.

1. Drobetsky E., Moustacchi E., Glickman B. & Sage E, (1994) Carcinogenesis 15, 1577-1583.

C5-522 REDUCED FRAMESHIFT FIDELITY OF A REPLICATIVE POLYMERASE FROM HIV-1 CONTAINING MUTATIONS IN THE THUMB SUBDOMAIN, Katarzyna Bebenek*, William A.Beard§, Thomas A. Darden*, Samuel H. Wilson§, and Thomas A. Kunkel*, *Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, §Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555-1068

HIV-1 reverse transcriptase is a heterodimer composed of a 66- and 51-kDa subunit. Structural studies have revealed that both subunits consist of common subdomains and that the subdomains in p66 are spatially organized to form a cleft which accommodates the nucleic acid. By analogy with a right hand the subdomains have been referred to as fingers, palm and thumb. Based on the crystallographic data, amino acids 250-300 comprise the thumb subdomain which interacts with the template-primer. These interactions may be critical for determining rates for strand slippage-initiated errors involving intermediates containing one of more unpaired nucleotides in the template-primer stem. To test this hypothesis we have changed each residue from position 253 to 271 one by one to alanine. This region includes the residues that comprise alpha-helix H in the thumb subdomain of the RT, suggested by X-ray crystallography to interact with the primer strand. We have analysed the kinetic properties and the fidelity of the mutant RTs. Two of the mutant enzymes, with changes at residues (G262 and W266) on the side of helix H that faces the minor groove of the DNA, exhibit an elevated dissociation rate constant (koff) for template-primer and an increased inhibition constant (Ki) for AZTTP. They also have an increased error rate for template-primer slippage initiated errors during both DNA- and RNA-dependent DNA synthesis. Thus, defined, single amino acid substitutions in a replicative polymerase can confer a mutator activity for errors involving strand slippage.

C5-523 GENOMIC SITE-SPECIFIC MUTAGENESIS IN *SACCHAROMYCES CEREVISIAE* USING AN EXCISABLE MARKER, Françoise Längle-Rouault and Eric Jacobs, Department of Molecular Genetics, TRANSGENE S.A., 67082 Strasbourg Cédex, France.

This method involves a two-step procedure . A DNA fragment generated by PCR which consists of a *URA3* selective marker surrounded by short directly repeated sequences homologous to the genomic target is used to transform a *ura3* yeast strain . These two sequences are included within the synthetic oligonucleotides used for PCR amplification , and carry the desired sequence modifications to be introduced in the genomic target. The *Kluyveromyces lactis URA3* is used because it has only 72% homology to the *Saccharomyces cerevisiae URA 3* and therefore gene conversion events of the genomic *ura3* mutation giving rise to Ura3$^+$ transformants occur at low frequency. After homologous recombination , *URA3* is found inserted in the genome at the target site, surrounded by the two short sequences with one , both or neither carrying the expected mutation Subsequently excision events resulting from homologous recombination between the direct repeats can be selected in the presence of fluoro orotic acid . The resulting Ura3$^-$ clones are harvested for subsequent analysis. A high frequency of genomic site-directed mutagenesis in yeast can be obtained using this strategy.

C5-524 A ROLE FOR ULTRAVIOLET-A IN SOLAR MUTAGENESIS, Elliot A. Drobetsky[1], Johanne Turcotte[1], Anne Châteauneuf[1], Bruno Lamolet[1], Ethel Moustacchi[2], and Evelyne Sage[2], [1]Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Que., Canada, and [2]Institut Curie, Section de Biologie, Paris, France.

It is well established that exposure to solar UVB (290-320nm) gives rise to mutations in oncogenes and tumour suppressor genes which initiate the molecular cascade towards skin cancer. Although UVA (320-400nm) has also been implicated in multistage photocarcinogenesis, its precise role remains much less clear. We have determined the DNA sequence specificity of mutations induced by rigorously purified UVB and UVA at the adenine phosphoribosyltransferase locus of Chinese hamster ovary cells. This has been compared to results previously obtained for broad spectrum simulated sunlight (295-4000nm) and 254nm UVC in the same gene. The overall data reveal novel clues about the nature and processing of premutagenic damage induced by UVA in rodent cells, and, most importantly, elucidate the relative contribution of this wavelength region to broad spectrum solar light mutagenesis. We demonstrate that T→G transversions, a generally rare class of mutation, are induced at high frequency (up to 50%) in UVA-exposed cells. Furthermore, this event comprises a significant proportion of the simulated sunlight-induced mutant collection (25%), but is not prevalent in cells irradiated with either UVB (10%) or UVC (5%). We conclude that the DNA sequence specificity of solar light-induced mutations in rodent cells cannot be adequately explained by the direct effects of the UVB component alone, and that UVA also plays an important role. This highlights the inadequacy of narrow spectrum UV sources as models for sunlight-induced skin cancer.

C5-525 **CORRELATION OF THE UV-INDUCED MUTATIONAL SPECTRA AND THE DNA DAMAGE DISTRIBUTION OF THE HUMAN *HPRT* GENE.** G. Kotturi, J.G. de Boer and B.W. Glickman. Centre for Environmental Health, Department of Biology, University of Victoria, Victoria, B.C., Canada V8L 3S1.

Automated DNA sequencers and capillary electrophoresis instruments can be readily adapted for various types of sequence-based nucleic acid analysis. We have used both of these instruments to determine the *in vitro* UV-induced DNA damage distribution of the human *hprt* gene. The distribution of the 6-4 pyrimidine-pyrimidone photoproducts and cyclobutane dimers were determined. The overall, and DNA-strand specific formation of the two photoproducts was compared. The UV-induced damage distribution in the *hprt* gene was compared to the mutational spectra in human cells. A comparison of DNA damage and mutations at specific DNA base positions revealed a better correlation of cyclobutane pyrimidine dimers and mutation than was seen for the 6-4 photoproduct. In addition, a capillary-based system was used as an alternate method of detection which reduced the separation times and offered the potential of recovering the fragments.

C5-526 DNA REPAIR AND SOMATIC MUTATION IN BREAST AND DISTAL TISSUES FROM BREAST CANCER PATIENTS,
Jean J. Latimer[1,3], Kelly Beaudry-Rodgers[3], Amal Kanbour-Shakir[2] & Stephen G. Grant[1,4], [1]Departments of Obstetrics, Gynecology and Reproductive Sciences and [2]Pathology, Magee-Womens Research Institute; Departments of [3]Human Genetics and [4]Environmental and Occupational Health and Toxicology, University of Pittsburgh, PA 15213
Breast cancer may arise as a multistep progressive process involving mutation of oncogenes, tumor suppressor genes and possibly mutator genes, consistent with the paradigm developed in colorectal cancers. One proven mechanism for the mutator phenotype is loss of DNA repair capacity, which has been shown to occur as the loss of mismatch repair in the etiology of 16% of sporadic colon cancers and in all colon tumors in cancer prone families with Lynch syndrome II (LSII). Other genetic conditions with defects in DNA repair are the repair deficiency syndromes including xeroderma pigmentosum, Bloom syndrome and ataxia telangiectasia, which are characterized by a high risk of developing cancer and increased somatic mutation. Therefore, early loss of DNA repair is associated with an abnormally high frequency of somatic variation or "genomic instability", as well as a direct involvement in the process of oncogenesis and hereditary cancer predisposition. LSII includes breast cancer, which has not yet been associated with the loss of DNA repair. We are analyzing the role of mismatch repair and nucleotide excision repair (NER) in the etiology of breast cancer using microsatellite instability PCR assays on DNA derived from breast tumor and non-tumor adjacent tissue from the same patient, and the unscheduled DNA synthesis assay (UDS) on primary explants of breast tumor and non-tumor adjacent epithelial tissue. Concurrently we are applying the glycophorin A (GPA)*in vivo* erythrocyte assay of somatic mutation and segregation on breast cancer patients. Preliminary studies have included tumor cell lines and have shown that breast (MDA and MCF-7) and colon tumor lines are 2-3x lower in NER compared with normal foreskin fibroblast cells. In terms of the primary epithelial cultures, we show that lineage specific differences exist in adult tissue cultures i.e., breast tissue in general is lower in NER than foreskin fibroblasts. These breast cancer patients prior to genotoxic therapy, show a significant increase in somatic mutation using the GPA assay, over age-matched controls. These studies will yield important information involving the role of genomic instability in the molecular etiology of sporadic and hereditary breast tumors.

C5-527 MUTAGENIC MECHANISMS IN *SACCHAROMYCES CEREVISIAE*: IN VIVO STUDIES WITH VECTORS CARRYING A SINGLE LESION AND ANALYSIS OF CLONED *REV* GENES,
Christopher W. Lawrence, Peter E.M. Gibbs, John R. Nelson and David C. Hinkle, Departments of Biophysics and Biology, University of Rochester, Rochester, NY 14642-8408
To get a better understanding of the mechanisms of induced mutagenesis in the model eukaryote organism, budding yeast, we need to determine the mutagenic properties of individual mutagenic lesions *in vivo*, identify and characterize the genes whose functions are required for mutagenesis, reconstruct translesion synthesis *in vitro* using the purified products of these genes, and reproduce the *in vivo* results. Recent work directed towards this long term goal has been concerned with examining the mutagenic properties of individual UV photoproducts *in vivo*, and also those of abasic sites. Both single-stranded vectors and duplex vectors carrying the lesion centrally placed within a 28 nucleotide single-stranded region have been used for this purpose. The frequency of translesion synthesis in yeast, and also the error frequency of this process, are often very different from those found in *E. coli*, indicating that these properties are dependent on the particular replication proteins and conditions used, as well as on lesion structure. In contrast, the types of mutations induced are usually very similar, indicating that they are determined largely by lesion structure. However, the types of insertions opposite an abasic site in the two species are dissimilar, perhaps because they are produced by different mechanisms, a possibility that is currently under investigation. In addition, we have been examining the properties of the *REV3* and *REV7* genes, whose functions are needed for induced mutagenesis. Assays with partially purified REV3 protein are consistent with the prediction that it encodes a DNA polymerase. Methods to purify the protein to homogeneity and definitively establish this conclusion are currently in progress. The *REV7* gene has been cloned and its properties, together with those of the *REV1* gene, are being studied.
Supported by NIH grant GM21858.

C5-528 IN-VITRO REPLICATION OF UV-IRRADIATED DNA BY HUMAN CELL EXTRACTS:EVIDENCE THAT XERODERMA PIGMENTOSUM VARIANT (XP-V) CELLS BYPASS LESIONS IN AN ABNORMAL, ERROR-PRONE MANNER,
W. Glenn McGregor, Krisztina Nadas, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI
Despite a normal rate of excision repair, XP-V cells are extremely sensitive to UV mutagenicity and abnormally slow in replicating DNA containing photoproducts. Furthermore, the kinds of mutations induced by UV_{254} in the endogenous *HPRT* gene differ significantly from those of normal cells. In XP-V cells, there is a higher than normal proportion of mutations that occur at thymine photoproducts, and a very high proportion of C to A transversions that arise solely from cytosine photoproducts in the transcribed strand. To test the hypothesis that the DNA replication complex of XP-V cells is abnormally error-prone when bypassing photoproducts, we are using the DNA replication fidelity assay developed by Kunkel and coworkers. This assay detects mutations in the lacZ*α* gene that occur when SV40 *ori*-containing Form I M13mp2 phage are replicated in vitro by cell-free extracts. Replication of undamaged templates by HeLa or XP-V cell extracts yielded low background mutant frequencies. An average of one dimer per Form I phage reduced synthesis by HeLa cell extract to 40% of the control and increased the mutant frequency 3-fold. With XP-V cell extract, these values were 85% and 11-fold. The presence of dimers in the replication product obtained with HeLa cell extract indicated that bypass had occurred. Sequence analysis of the mutants derived using HeLa cell extract showed that 14/17 (82%) of the base substitutions were C –> T transitions, and the cytosine was located in either strand. In contrast, analysis of the mutants derived using XP-V cell extract showed that only 3/17 (18%) were C –> T transitions; 8/17 (47%) were C to A, and 7 of these 8 resulted from cytosines located in the leading strand. The rest of the base substitutions, 6/17 (35%), were T –>A transversions. These results suggest that the hypermutability of XP-V cells results from error-prone replicative bypass of DNA containing photoproducts. (Research supported by DHHS Grant CA01747 to WGM and by Grant CA21253 from NCI.)

C5-529 GENOTOXIC EFFECTS OF α-DEOXYADENOSINE ASSESSED *IN VITRO* AND *IN VIVO*,
Hiroshi Ide, Hironori Shimizu, Yoshiharu Kimura, and Keisuke Makino, Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan

Hydroxyl radicals produced in cells generate a number of DNA lesions including base damages and strand breaks etc. These lesions are generally restored by cellular repair enzymes. However, if unrepaired, they exhibit genotoxic effects by either constituting a replication block or directing misincorporation of incorrect nucleotides. Hydroxyl radicals react with deoxyadenosine to abstract the anomeric hydrogen atom at C1'. The sugar radical thus formed undergoes anomerization to produce an α-anomer of deoxyadenosine (αdA) as well as a parental β-anomer. Radiolysis of deoxyadenosine in aqueous solution has shown that absence or low concentration of oxygen appear to be crucial for the formation of this lesion.

In the present study, αdA was site specifically incorporated into oligodeoxynucleotides and its influence on DNA synthesis was accessed both *in vitro* and *in vivo*. Templates containing αdA were replicated by several DNA polymerases *in vitro* and newly synthesized products were analyzed by denaturing PAGE. The analysis revealed that DNA synthesis was temporally arrested at the lesion. The nucleotide incorporated opposite αdA in translesional synthesis was determined by the primer extension assay and Maxam-Gilbert method. Either T, C, or A was incorporated αdA, but G was barely incorporated under these conditions. Oligonucleotides containing a single αdA, a model abasic site (tetrahydrofuran, F) as a control lesion, or normal β-deoxyadenosine, were synthesized and ligated into single-stranded M13mp18 DNA. These vectors were replicated in *E. coli*. DNA was extracted from progeny phage and sequenced to determine the lethal and mutagenic effects of these lesions. These results will also be presented at the meeting.

C5-530 ULTRAVIOLET HYPERMUTABILITY IN MELANOMA-PRONE FAMILIES

Kenneth H. Kraemer[1], Shin-Ichi Moriwaki[1], Robert E. Tarone[2], Margaret A. Tucker[3] and Alisa M. Goldstein[3], [1]Laboratory of Molecular Carcinogenesis, [2]Biostatistics Branch, and [3]Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD 20892

Members of cutaneous melanoma (CM) families with dysplastic nevi (DN) are at high risk of developing CM. We utilized a shuttle vector plasmid, pSP189, to assess the ability of cells from 6 familial CM kindreds to repair damaged DNA. Ultraviolet treated pSP189, containing the bacterial suppressor tRNA gene *supF* as a marker for mutations, was transfected into the human cells and 2 days later replicated plasmids were harvested and the plasmid mutation frequency (MF) was determined by transformation of indicator strains of *E.coli*. The post-UV plasmid MF was found to increase with donor age in lymphoblastoid cell lines from 16 normal donors ranging in age from 4 years to 98 years. The calculated post-UV plasmid MF was 2.9% at birth and rose about 0.03% per year to 5.5% at age 98 ($p=0.001$). Cell lines from 13 of 13 patients with CM ($p=1.5 \times 10^{-8}$) and from 5 of 8 patients with DN without CM ($p=0.001$) showed elevated post-UV plasmid MF . However, cell lines from 0 of 2 patients with CM without DN had elevated post-UV plasmid MF. Cells lines from 6 of the CM patients ($p=0.004$) and 3 of the DN patients ($p=0.02$) also showed elevated spontaneous plasmid MF. Thirteen additional cell lines were studied from unaffected (6) or indeterminate (2) blood line family members, from normal spouses (4) and from a spouse with DN (1). Of 27 cell lines with elevated post-UV plasmid MF only 8 were from donors who did not have CM or DN (19/24 vs 8/28, $p=0.0003$). This study indicates that post-UV plasmid hypermutability is a laboratory marker for members of melanoma-prone families, and suggests that patients with familial CM have defective DNA repair.

C5-531 REPLICATION OF O6-METHYLGUANINE CONTAINING DNA,

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O^6-methylguanine (O^6mG) in DNA is linked with increased mutagenesis and chromosome instability. The mutagenesis is due to increased G→A transitions produced by misincorporation of TMP across from O^6mG. Chromosome instability appears to be caused, at least in part, to futile cycles of mismatch repair which leave persistent single-stranded regions in the DNA. We are studying the replication of defined O^6mG DNA substrates by both repair and replicative DNA polymerases in order to determine how O^6mG containing DNA is replicated. We find that repair and replicative DNA polymerases perform with different efficiencies depending on the O^6mG DNA substrate.

O^6mG in DNA is a premutagenic and precarcinogenic lesion. Since 20 to 30 % of human cancer cell lines lack O^6mG-methyl transferase and thus the ability to repair this DNA damage, aberrant replication of O^6mG containing DNA my contribute to the development of a cancer cell.

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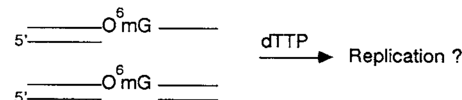

C5-532 PROTRACTION OF RADIATION DOSE AND MUTATION INDUCTION IN GROWING POPULATIONS OF HUMAN CELLS,

Sally A. Amundson and David J. Chen, LS-1, Mail Stop M888, Los Alamos National Laboratory, Los Alamos, NM 87545.

In order to investigate further the effects of recombinational proficiency on cell survival and mutation by ionizing radiation, we have exposed the syngenic cell lines TK6 and WTK1 to continuous low dose-rate γ-irradiation. We previously demonstrated that acute X-ray exposure results in lower survival and lower mutation induction at both the thymidine kinase (*tk*) and hypoxanthine-guanine phosphoribosyltransferase (*hprt*) loci in TK6 cells compared to WTK1 cells. These differences were attributed in part to reduced levels of recombination in the TK6 cell line relative to WTK1. We have exposed asynchronous growing populations of these cells to ^{137}Cs γ-rays at dose rates of 99.8 cGy/min., 14.3 cGy/hr, 6.7 cGy/hr, and 2.7 cGy/hr. Both cell lines exhibited a dose-rate effect for survival, with a more pronounced effect in WTK1. Compared to acute doses, the low dose-rates also tended to protect against mutation induction at the *hprt* locus, slightly in TK6, and more dramatically in WTK1. In both cell lines we observed an "inversion" of the dose-rate effect for mutation, with the smallest induction of mutants resulting from the highest of the chronic dose-rates used. This phenomenon previously has been linked to cell cycle effects and has not been observed in non-cycling cells. Interestingly, at the lowest dose-rate used, although there was not a significant protective effect on mutation in TK6, mutation induction in WTK1 was reduced to the levels of TK6.

(This research is supported by the US DOE under contract KP0400/005181 to LANL and by NIH grant CA50519.)

C5-533 MICROSATELLITE INSTABILITY AND INCREASED MUTATION RATES IN HUMAN CELL LINES TOLERANT TO METHYLATION DAMAGE,

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Mammalian cell clones tolerant to the cytotoxic effects of methylating agents have been isolated and characterized. Methylation tolerance is associated with the ability to endure the presence of O^6-methylguanine (O^6-megua) in DNA. One model for methylation tolerance suggests that the lethality of O^6-megua arises through the cell's attempts to process the methylated base by DNA mismatch correction. Cells acquire tolerance through the loss of this pathway. We have recently found that tolerance is sometimes associated with the loss of a DNA mismatch binding protein and oss of the binding activity confers a spontaneous mutator phenotype. We have analysed spontaneous mutations in the *aprt* gene of a tolerant mismatch binding defective hamster cell line. The rates of minus frameshifts and AT:TA transversions were strikingly elevated. These mutations were in repeated elements and a large number were dinucleotide deletions in DNA sequences resembling microsatellites. In agreement with a role in preventing minus frameshifts, defective binding was associated with microsatellite instability. We are determining how many genes are involved in methylation tolerance and/or mismatch correction. The results indicate that tolerance is a recessive trait and N-methylnitrosourea (MNU) sensitivity in tolerant/normal cell hybrids is similar to the normal level. Determination of MNU sensitivity of hybrids between independent clones defines two complementation groups. The spontaneous mutation rates of clones representative of the two complementation groups have been measured at the *hprt* gene and at microsatellite sequences. Preliminary data indicate that one of the two complementation groups has the microsatellite instability and elevated rates of spontaneous mutation consistent with a defect in mismatch correction.

C5-534 MULTICOPY SUPPRESSORS OF THE COLD-SENSITIVE PHENOTYPE OF THE *pcsA68* MUTATION IN *E. coli*

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The *pcsA68* mutant strain cs2-68 of *E. coli* was isolated as a cold-sensitive mutant which formed a long filament with a large nucleoid mass in the central region. We have recently demonstrated that the *pcsA68* mutation occurred in the *dinD* gene, which was identical to orfY upstream of the *pyrE* gene at 82 min. The SOS response was induced when the *pcsA68* mutant cells were exposed to low temperatures. The cs phenotype was suppressed in *recA* null and *lexA* induction-defective mutants. The *pcsA* gene could be disrupted without any defect for normal cell growth, indicating that its gene product is not essential for cell viability. The *pcsA68* mutant protein appears to generate a toxic effect on cell growth at low temperatures. To understand how the *pcsA68* gene product confers such an effect, we screened for multicopy plasmids carrying chromosomal fragments that could suppress the cs phenotype. At least three different *Bam*HI fragments were identified to exhibit such an activity. One was found to carry the *lexA* gene, and another to carry the *dinG* gene, whose product shows similarity to yeast RAD3 and human XpD DNA helicases. The other was found to carry the *dinI* gene. The complete sequencing of it revealed that DinI is homologous to ImpC coded by TP110 plasmid and also to YRF7 coded by EC-retron67. Expressions of the genes coding for these proteins are also under the control by LexA. We present a model to explain why the *pcsA68* mutant is cold-sensitive. We describe newly found SOS genes in *E. coli*, one of which codes for a protein similar to YLW6 of *C. elegans* and also to UmuC of *E. coli*.

C5-535 **Study of the action of 7-methoxy-2-nitro-naphtho[2,1-b] furan (R7000), an extremely potent mutagen.**

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Nitrofurans constitute a family of widely used, synthetic, broad spectrum antimicrobial agents as well as food preservatives and feed additives. Most of these compounds are mutagenic in bacteria and mammalian cells and tumorigenic in experimental animals. A strong relation between structure and genotoxic activity was demonstrated for compounds of the Nitronaphthofuran series. 7-methoxy-2-nitronaphtho [2,1-b] furan (R7000) ranked among the most potent mutagens of this series. We have previously reported that it is activated in bacteria to DNA-binding products. This DNA-adduct formation require a prealable metabolic activation (nitroreduction) of the product. The characterization of target nucleotides involved in this binding was investigated. DNA lesions were essentially detected in GC-rich rather than AT-rich sequences. In addition, we have demonstrated that R7000 is able to induce base pair substitutions, mainly GC-->TA, GC-->CG and AT-->TA transversions. This compound may be also a very efficient inducer of base pair deletions/insertions resulting in frameshift. The influence of the Uvr excision repair system on the mutagenic spectrum of R7000 is in progress.

In addition, in order to characterize one or several factors involve in the mechanism of R7000 toxicity, we have undertaken a two-dimensional gel analysis of the proteins synthesized in bacterial cells after exposure to this agent.

C5-536 **EVIDENCE FOR INVOLVEMENT OF THE p53 DNA-DAMAGE RESPONSE PATHWAY IN DNA REPAIR.** **Albert J. Fornace, Jr., Qimin Zhan, I-Tsuen Chen, and Martin L. Smith, Lab. of Molecular Pharmacology, DTP, DCT, NCI, Bethesda, MD 20892-4255.**

The p53 tumor suppressor and its downstream effector genes, like *CIP1*, *GADD45*, and *MDM2*, play a central role in modulating cell growth after genotoxic stress and in activation of a G_1 checkpoint[1]. This presumably allows time for the repair of DNA damage prior to entry into S phase. We now report evidence for a direct role for one or more p53 response genes in DNA repair. The human Gadd45 protein, which is DNA-damage inducible, was found to associate with PCNA, an abundant nuclear protein involved in DNA replication, DNA repair, and cell-cycle control. Gadd45-PCNA complexes were detected in extracts from γ-irradiated human cells, and recombinant Gadd45 (rGadd45) was found to associate with PCNA in vitro. Immunodepletion of rGadd45 from extracts of human cells reduced nucleotide excision repair (NER) using an in vitro assay. In contrast, the addition of rGadd45 was found to stimulate nucleotide excision repair (NER) in the same assay. Since PCNA is a necessary cofactor for NER, a reasonable interpretation of our results is that Gadd45 facilitates the assembly of PCNA in the functional repair complex. When Gadd45 expression was reduced in p53 *wt* cells by introduction of a *GADD45* antisense expression vector, there was a significant reduction in clonagenic survival after UV irradiation at doses producing high levels of photoproducts that are subsequently repaired by NER. Additional data will be discussed demonstrating that inhibition of activation of p53 effector genes, like *GADD45*, reduces both NER and cell lethality after exposure to UV radiation. These results establish *GADD45* as a link between the p53-dependent cell cycle checkpoint and DNA repair.

[1] Kastan, M.B., Zhan, Q, El-Deiry, W.S., Carrier, F, Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, A.J. Jr. Cell *71*: 587-597, 1992.

C5-537 CONFLUENT HOLDING RECOVERY IN IRRADIATED HUMAN DIPLOID FIBROBLASTS: POSSIBLE ROLE OF THE P53/WAF1 SIGNAL TRANSDUCTION PATHWAY.

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It has been shown previously with human diploid fibroblasts that a fraction of cells remains irreversibly blocked in the G(0)/G(1) phase of the cell cycle when they are irradiated in confluent, density-inhibited cultures, and released by subculture to low density (Little and Nagasawa, *Rad. Res.* 101, 81-93, 1985). This G(0)/G(1) block is significantly reduced when the cells are held in confluence for 2-24 hrs, suggesting the activity of a repair process that may be related to the repair of potential lethal damage (PLDR). For example, confluent holding for 12 hrs prior to subculture of GM6419 cells irradiated with 400 cGy reduced the fraction of cells blocked in G1 from 60% to 40%. Northern analysis of the WAF1/p21 gene product indicated that cells irradiated and held in G(0)/G(1)l have high levels of WAF1 mRNA expression up to 36 hrs post-irradiation, whereas expression in cells released by subculture to low density dropped rapidly to near background levels. WAF1 is a cyclin-dependent kinase inhibitor that suppresses DNA repair factor PCNA. Experiments designed to examine cyclin-CDK kinase activity and the phosphorylation status of the Retinoblastoma susceptibility gene (Rb) are underway.

C5-538 GENOMIC INSTABILITY DUE TO GERMLINE p53 MUTATIONS DRIVES PRENEOPLASTIC PROGRESSION TOWARD CANCER IN HUMAN CELLS

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Normal fibroblasts from 7 out of 7 affected individuals from five Li-Fraumeni families developed changes in morphology, chromosomal abnormalities, escape from senescence and development into cell lines that can be transformed by a *ras* oncogene. Based on these in vitro studies we found germline p53 mutations in five of five families. The immortal fibroblasts lose the wild type p53 upon escape from senescence and restoration of a wild type gene results in senescence and cell death. Cell cycle analyses indicated that p53 regulates a check point in G1 which can be restored in transfectants expressing wild type p53. Inappropriate entry into S due to expression of a mutant p53 protein may give rise to genomic instability.

Our data suggest that unlike transformation of rodent fibroblasts by cooperation of a *ras* oncogene and mutant p53, additional events other than loss or mutation of wild type p53 alleles are necessary for tumor formation in human cells. Here too, genomic instability drives further mutations in genes capable of suppressing *ras* leading to full tumorigenic transformation.

To test if mutant p53 directly induces genomic instability, we transfected mutant p53 genes into normal diploid fibroblasts from a normal donor and found chromosomal alterations and extended lifespan as was observed in Li-Fraumeni syndrome (LFS) fibroblasts. The extent of aneuploidy varied with the particular p53 mutant gene transfected. Stable transfection of a p53 expression plasmid with an amino acid 248 mutation gave rise to mostly hypodiploidy, a chromosomal change seen early in the development of aneuploidy in LFS cells. p53 expression plasmids with mutations at amino acids 175 and 273 induced genetic instability including aneuploidy, double minute and dicentric chromosomes.

C5-539 Selective Reduction of Cyclin D1 Expression and Subunit Association with PCNA and Waf1/ Cip1 in SV40 Large T Antigen Immortalized Human Fibroblasts

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Protein complexes containing cyclins and cyclin dependent protein kinases (cdks) have been shown to be rearranged in both spontaneous and viral tumor antigen transformed cells. In this report we have examined G1 and S phase cyclin/ cdk complexes as a function of the neoplastic progression of human diploid fibroblasts transfected with the SV40 large T antigen. We find that the expression of cyclin D1and its association with PCNA and Waf1/ Cip1 remain unchanged in pre-crisis human fibroblasts transfected with SV40 large T antigen. However, in these same cells the association of cdk4 with cyclin D1, PCNA and Waf1/ Cip1 is disrupted. Upon immortalization, cyclin D1 protein expression is decreased and binding of both PCNA and Waf1/ Cip1 with the remaining cyclin D1 is reduced. In contrast, large T antigen increased the expression of cyclin A and cyclin E proteins in both pre-crisis and immortal cells and did not reduce the binding of PCNA or Waf1/ Cip1 to either cdk2 or cyclin A proteins. These results show that large T antigen expression in human fibroblasts selectively uncouples cyclin D1 from cdk4 and subsequent immortalization of these cells results in further changes to the cyclin D1 dependent cell cycle regulatory pathways.

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C5-540 OVER-EXPRESSION OF WILD-TYPE AND A MUTANT HUMAN P53 IN THE LENS OF TRANSGENIC MICE.

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Transgenic αAhp53mt mice that over-express a mutant human p53 gene in the lens are characterized by a defect in primary lens differentiation that results in a mild nuclear cataract developing at late stages of gestation. By contrast, secondary fiber differentiation is unaffected, except for a defect in the process of fiber denucleation. No abnormal cell proliferation was ever observed in the lens of these mice. On the other hand, αAhp53wt mice that over-expressed wild type human p53 develop microphthalmia as a result of a defect in secondary lens fiber differentiation that sets in shortly after birth. We see abnormal fiber cells in the lens cortex suggestive of a cell state that precedes apoptosis. In an effort to directly link the observed phenotype to the activity of the wild-type human p53 transgene, we generated mice that over-express both wild-type and mutant human p53 alleles. Lens development in these double transgenic mice is almost normal, suggesting a mechanism of dominant negative interference of the mutant product with the function of wild-type product. This result suggests that targeting wild-type alleles to the affected tissues is problematic because p53-mutated tumor cells are more resistance to apoptosis than adjacent normal cells .

C5-541 14-3-3 AND CASEIN KINASE I MEDIATE AN ESSENTIAL LINK BETWEEN A NOVEL CELL CYCLE CHECKPOINT AND GROWTH CONTROL,

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Molecular analysis has demonstrated that many proteins involved in cell cycle control and DNA metabolism are highly conserved. Two functionally redundant homologs of the casein kinase I (CKI) protein kinase have been identified in fission yeast (*hhp1* and *hhp2*) which have distinct effects on cell cycle progression and the coordination of DNA repair. Also, two functionally redundant 14-3-3 protein homologues have been identified (*rad24* and *rad25*) which together encode an essential function and which are involved in cell cycle checkpoints and progression. CKI isoforms show cell cycle dependent distribution and one of the 14-3-3 proteins has a role in DNA double-strand break repair.

The phenotypes of the *rad24* and *hhp1* null mutants present an interesting contrast. While both are involved in DNA metabolism, *rad24* advances mitosis and presents a semi-*wee* phenotype, while *hhp1* delays mitosis and presents an elongated phenotype. The *hhp* mutant cell cycle progression defect is not dependent on either the DNA damage checkpoint or the S phase-Mitosis dependency checkpoint. The mitotic delay is therefore due either to activation of a hitherto undefined checkpoint or hhp function is required to activate $p34^{cdc2}$ directly.

hhp1 - rad24 double mutants are inviable, suggesting that *hhp1* is not involved in activating $p34^{cdc2}$. Furthermore, genetic analysis using *rhp51* (the *S. pombe* homolog of *recA* and *RAD51*) indicates that the *rad24 - hhp1* essential function is related to cell growth effects and not to its role in DNA metabolism. The delay to mitosis caused by *hhp1* mutation appears to be dependent on a novel checkpoint mediated by *rad24* which is distinct from the DNA damage checkpoint. This novel checkpoint may monitor chromosome segregation as *hhp1* mutants demonstrate a high level of minichromosome instability and are sensitive to inhibitors of microtubule polymerization. The genetic interaction between *rad24* and *hhp1* has demonstrated distinct functions for a single 14-3-3 protein isoform and a single CKI protein isoform in a genetically tractable system and suggests that these conserved elements might participate in similar functions in higher cells.

C5-542 CELL CYCLE-RELATED DNA REPAIR MECHANISMS IN HUMAN AND RODENT CELLS, David L. Mitchell and Roger R. Hewitt, Department of Carcinogenesis, The University of Texas M.D. Anderson Cancer Center, Science Park/Research Division, Smithvlle, TX 78957

The importance of different repair mechanisms for cell cycle recovery and their relationship to the expression of G1 checkpoint genes is considered by comparing the response of Chinese hamster ovary (CHO) cells with human cells. CHO cells are most sensitive to UV-irradiation in early S-phase; human cells display a negligible, or much less distinct, cell cycle response for cell killing. Attempts to induce or activate p53 protein in CHO AA8 and K1 cells by treatment with adriamycin or UV have been unsuccessful, consistent with published reports that CHO cells do not contain measurable p53 protein. This observation is consistent with the absence of radiation induced growth arrest in these cells. The rate and extent of (6–4) photoproduct repair is constant through the cell cycle in human fibroblasts and in xeroderma pigmentosum variant cells. In contrast, the CHO cells showed a marked reduction in excision repair in G1. As the cells progressed through early S phase and into S-phase the ability to repair (6–4) photoproducts increased significantly to near normal levels. As the cells reach G2 phase the rate of (6–4) photoproduct repair decreased. [This work was supported by NIH grant CA 04484-34].

C5-543 EFFECTS OF MIMOSINE BLOCKAGE IN CHO CELLS: IMPLICATIONS FOR REGULATION OF S-PHASE RADIATION DELAY, Joseph A. D'Anna, Joseph G. Valdez, Scott F. Peterson and Harry A. Crissman. Los Alamos National Laboratory, Los Alamos, NM 87545

Treatment of G1 CHO cells with mimosine arrests them near the G1/S boundary (P.J. Mosca, P.A. Dijkwel, & J.L. Hamlin Mol. Cell. Biol. 12, 4375-4383 (1992)). The cells do not initiate DNA synthesis, but they appear to lose their ability to inhibit the initiation of early replicating regions in response to ionizing radiation (J.M. Larner, H. Lee and J.L. Hamlin, Mol. Cell. Biol. 14, 1901-1908 (1994)). Larner et al. have suggested that the early replicating origins are already prepared for initiation in the mimosine-blocked cells so that they can no longer respond to DNA damage. Measurements of cultures synchronized with mimosine or aphidicolin are consistent with that hypothesis. Whereas CHO cells synchronized with mimosine do not initiate DNA synthesis, they undergo similar biochemical and structural changes as do cells synchronized with aphidicolin which allows initiation. These include time-dependent depletion of histone H1 from chromatin, histone H1 phosphorylation, phosphorylation of the cyclin dependent kinase cdk2, and unbalanced growth. The results indicate that the restriction point for unbalanced growth precedes the mimosine arrest point, and they are consistent with the notion that loss of H1 from replicons is a preparatory event for the initiation of DNA replication. They raise the possibility that inhibition of the initiation of DNA replication in response to ionizing radiation (Larner et al., 1994) may be regulated prior to or prevent cdk2 activation and the preparatory chromatin structural changes required for initiation at sets of replication foci during S phase. If this is so, then cdk2 activity and H1 phosphorylation may be down-regulated in response to irradiation of exponentially growing cells which exhibit minimal G1 delay. The latter agrees with the results of L.R. Gurley and R.A. Walters who found that ionizing radiation reduces histone H1 phosphorylation in CHO cells (Biochemistry 10, 1588-1593 (1971)).

C5-544 DNA FRAGMENTATON IN POSTMITOTIC DEVELOPMENT IN WI-38 AND IMR 90/1 FIBROBLASTS IN VITRO, P. Brenneisen[1] and K. Bayreuther[2], Department of Dermatology, University of Düsseldorf, 40001 Düsseldorf, F.R.G.[1], Institute of Genetics, University Hohenheim, 70599 Stuttgart, F.R.G.[2]

Normal human embryonic lung fibroblasts of the cell lines WI-38 and IMR 90/1 proliferate and differentiate in vitro along the cell lineage mitotic fibroblasts (MF) MF I- MF II- MF III, and the postmitotic fibroblasts (PMF) PMF IV- PMF V- PMF VI and PMF VII. PMF VI has been characterized to represent the terminally differentiated end cell of the fibroblast stem cell system. The development of mitotic and postmitotic WI-38 fibroblasts can be speeded up by two treatments with mitomycin C (MMC). Genomic DNA of whole cells of WI-38 and IMR 90/1 fibroblasts has been demonstrated by pulsed-field gel electrophoresis (CHEF) to become progressively cleaved to double-stranded fragments in the ranges of < 1.9 mb - 50 kb, and 100 - 20 kb in the advancing spontaneous and MMC induced accelerated development of PMF in vitro. These PMF populations reveal similar to identical [^{35}S]methionine labeled polypeptide pattern in the IEF range as the MF, when analyzed by two-dimensional polyacrylamide electrophoresis (2D-PAGE). PMF which spontaneously enter apoptosis or which are induced to enter apoptosis by chemical agents have been shown to undergo low molecular weight DNA fragmentation in the range of 150 - 4.3 kb and to exhibit an altered polypeptide pattern. The results demonstrate that increased genomic instability and DNA damage by spontaneous or MMC induced high molecular weight DNA fragmentation is a normal process in the postmitotic development and do not impair the normal protein synthesis of the postmitotic fibroblasts.

C5-545 REPLICATION TIMING OF THE GENOMIC REGION CONTAINING THE AMPLIFIED CTG REPEAT SEQUENCE RESPONSIBLE FOR MYOTONIC DYSTROPHY, J.M. Barceló, E. Rajcan-Separovic, R.G. Korneluk, Children's Hospital of Eastern Ontario and University of Ottawa, Ontario, Canada.

Myotonic dystrophy (DM) is an autosomal dominant genetic disorder affecting approximately 1/8000 individuals. Mutant alleles responsible for this disease contain an unstable CTG trinucleotide repeat sequence in the 3' untranslated region of the DMK gene at position 19q13.3.

The time within the S phase in the cell-cycle in which a gene is replicated has been shown to relate to its transcriptional activity. The presence of a mutation consisting of amplified CGG repeat at Xq27.3 has been demonstrated to result in delay of replication of the genes spanning at least 184 kb around the repeat. In those alleles the expression of at least the closest gene to the repeat is suppressed. In the case of DM, the unstable trinucleotide repeat is of the same nature and moreover DMK gene expression results are controversial. It has been suggested that the disease may be caused by the anomalous expression of genes in the vicinity of the mutation other than just DMK. We are conducting the study of replication timing of DMK in normal and mutant alleles by following two methodologically different but converging approaches. The first consists of the examination of the hybridization patterns of genomic probes containing DMK in interphase nuclei by fluorescence *in situ* hybridization. In the second method the estimate of the replication timing is based on the detection of BrdU substituted DMK sequences on a variety of synchronized cells, growing in culture, pulsed at defined intervals with this uridine derivate.

Determining the replication timing of mutant DM alleles may help answer some questions regarding the expression of DMK and surrounding genes as well as the pathophysiology of the disease.

C5-546 p53-MEDIATED APOPTOSIS IS THE PRIMARY CAUSE OF RADIATION SENSITIVITY IN ATAXIA-TELANGIECTASIA, M. Stephen Meyn, Lynne Strasfeld and Carl Allen, Departments of Genetics and Pediatrics, Yale University School of Medicine, New Haven, CT 06510

The autosomal recessive disease ataxia-telangiectasia (A-T) is characterized by ataxia, immune defects, genetic instability and cancer. A cardinal feature of A-T is a marked sensitivity to the killing effects of ionizing radiation. However, repair of DNA damage in A-T cells is grossly normal and the cause of the radiation sensitivity has remained puzzling despite numerous investigations. We now report that p53-mediated apoptosis is primarily responsible for the radiation sensitivity of A-T cells.

We exposed fibroblasts representing three different A-T complementation groups as well as two control fibroblast lines to 250 kv X-radiation and then examined irradiated and unirradiated cells by microscopy and DNA electrophoresis. Apoptosis was detectable in the A-T cells 24-48 hours after irradiation. By 72-96 hours 8-32% of the A-T cells were morphologically apoptotic and nucleosome "ladders" were detectable in their DNA. In contrast, control cells underwent minimal apoptosis (<1.5%). Similar results were obtained with streptonigrin, a radiomimetic mutagen. Exposure to ionizing radiation or streptonigrin induced widespread apoptosis in lymphoblasts representing the six known complementation groups of A-T, but not in control lymphoblast lines. Our DSN model predicts that the sensitivity of A-T cells to the killing effects of mutagens should be p53 dependent. Disruption of p53 function in an A-T fibroblast line by transfection of either the dominant-negative $p53^{143ala}$ mutant or an HPV16 E6 gene was associated with acquisition of near-normal streptonigrin and radiation resistance, while transfection and expression of the $p53^{143ala}$ mutant did not affect the streptonigrin or X-ray sensitivity of a control cell line.

Our results support our hypothesis that an unusually low threshold for activation of p53-mediated apoptosis by DNA damage may be the primary etiology for both *in vivo* and *in vitro* mutagen-sensitivity in A-T (Meyn, submitted). These data also suggest an etiology for the neurological deterioration and immune defects seen in A-T patients: Inappropriate activation of apoptosis by spontaneous DNA damage (Supported by grants from the A-T Children's Project and the N.C.I.).

C5-547 INFLUENCE OF DNA METHYLATION ON TOPOISOMERASE II ACTION AND GENOMIC STABILITY

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DNA hypomethylation is one of the possible consequences of mammalian neoplasia and also occurs in cultured cells after treatment with certain carcinogenic drugs. Genomic instability is also frequently observed in these cells. Impaired chromatid separation or other mitotic disturbances cause the formation of micronuclei, which have been discussed to play a role in tumorigenesis. Analysis of micronucleus frequencies in Syrian hamster primary cells and tumor cell lines revealed that the micronucleus frequencies are correlated with an altered methylation status in these cells. Changes in DNA-methylation may result in altered DNA conformation. Therefore, certain proteins may be disturbed in their interaction with DNA. The protein investigated in this study, topoisomerase II, is essential for chromatid separation. To elucidate whether altered DNA methylation acts as a topoisomerase II inhibiting factor, we compared the methylation status and topoisomerase II cleavage activity in the centromeric region of different Syrian hamster tumor and primary cell lines. After Southern blot analysis with a PCR amplified Syrian hamster specific probe following MspI/HpaII cleavage, hypomethylation in these DNA-regions could clearly be demonstrated. The topoisomerase II cleavage experiments showed unaltered location of etoposide-induced cleavage sites in these hypomethylated DNA regions, but decreased topoisomerase II action. These findings further confirm the relevance of DNA-methylation and topoisomerase II activity for the maintenance of genomic stability.

C5-548 INDUCTION OF G1 DELAYS AND p53 AND WAF-1 (p21) IN HUMAN FIBROBLASTS FOLLOWING EXPOSURE TO ACTINOMYCIN D, LOW-LET γ-RAYS, OR HIGH-LET α-PARTICLES, Donna M. Gadbois, David J. Chen, Harry A. Crissman, Sha-ke Wang, and Bruce E. Lehnert, Life Sciences Division I, Los Alamos National Laboratory, Los Alamos, NM 87545

Exposure of wild type p53 cells to low-LET γ-rays can cause delays in cell cycle transition across the G1/S boundary. Such delays appear to be due to an induction of p53, p53 mediated increases in p21, and a subsequent inhibition of the cdk2/cyclin E kinase by p21. The treatment of cells with Actinomycin D, a DNA intercalating agent that produces DNA strand breaks, also causes G1 arrest, presumably via a p53-mediated pathway. Although S and G2-phase delays caused by high LET α-particles have received some experimental attention, it remains to be demonstrated that α-particles like those associated with radon exposure can elicit G1 delays similar to those resulting from low LET radiation or drugs that cause DNA strand breaks. Moreover, information is currently lacking about the ability of α-particles to induce p53 and p21. Actinomycin D, γ-rays, and α-particles all damage DNA though different mechanisms so it is possible that they could affect cell cycle progression and p53 and Waf-1 induction differently as well. Using human foreskin fibroblasts (HSF) as a primary cell model, we have set out to compare the abilities of Actinomycin D, γ-rays (50-800 rads), and α-particles (19-171 rads) to cause G1 delays in the cell cycle, and to additionally assess how these different DNA-damaging stimuli compare in terms of inducing the p53 and Waf-1 proteins. Evidence for G1 delays was observed following all γ-irradiation. Actinomycin D (1.6 μM) was much more efficacious than γ-irradiation (400 or 800 rads) at inducing p53. However, γ-irradiation induced Waf-1 to a higher level than did Actinomycin D, which suggests that the signal for DNA damage induced by these treatments may be mediated, at least in part, through separate pathways. We have also observed that high-LET α-particles generated from ^{238}Pu cause G1 delays in G0-synchronized HSF cells, even after exposure to the lowest dose studied. Analyses of α-particle induction of p53 and Waf-1 are presently ongoing. These studies will help elucidate mechanistic differences in cell cycle responses to agents that cause different types of DNA damage. This work was funded by the U.S. Department of Energy under contract #KP0204000 and U.S. Army contract #KP400403202.

C5-549 THE IMPORTANCE OF THE p53 GENE FOR DNA DAMAGE RESPONSE, CELL CYCLE IN ATAXIA TELANGIECTASIA CELLS, Janet Hall and Marina Artuso, International Agency for Research on Cancer, 69372 Lyon Cedex 08, France.

The Ataxia Telangiectasia (AT) gene product has been implicated, together with those of the p53 and gadd 45 genes, in a signal transduction pathway that controls cell cycle arrest following exposure to ionising radiation (Kastan et al., Cell, 71, 587, 1992). In order to examine this relationship, changes in the expression of the p53 protein, following exposure to ionising radiation, are being measured in normal and AT lymphoblastoid cell lines. In contrast to the 6 normal cell lines studied, where both cell cycle changes and induction of p53 expression were seen after exposure to ionising radiation, the mean increase in p53 expression in the 8 AT cell lines studied was significantly reduced ($p<0.005$), the relative abundance of p53 protein being 1.58 ± 0.37 and 1.83 ± 0.42 in the AT cell lines compared to 2.96 ± 0.85 and 5.00 ± 1.87 in the normal cell lines 3 hrs after exposure to 2 Gy or 4 hrs after exposure to 5 Gy ionizing radiation. In contrast, the increase in p53 expression following exposure to the alkylating agent methylmethane sulphonate (MMS) (100 μg/ml) was 4.51 ± 3.46 and 4.52 ± 1.89 for the 3 normal cell lines and the 6 AT cell lines studied. The changes in the levels of gadd 45 and p21 mRNA and p21 protein were also reduced in the AT cell lines compared to the normal cell lines so far studied following exposure to ionizing radiation but were similar following exposure to MMS (100 μg/ml). The variability in responses may in part be due to differences in the expression of EBNA-5, an EBV-encoded nuclear antigen expressed in immortalised lymphoblastoid cell lines which has been shown to bind the p53 protein (Szekeley et al., PNAS USA, 90, 5455, 1993). The EBNA-5 profile of the cell lines following exposure to ionizing radiation has been investigated and it would appear that there is no correlation between the EBNA-5 profile and level of p53 induction observed.

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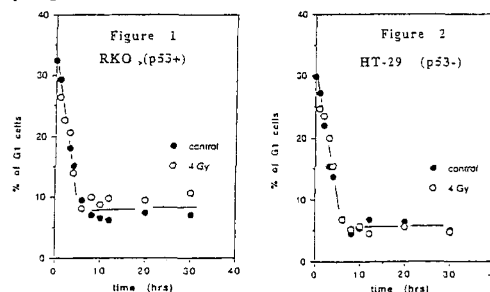
C5-550 ROLE OF THE p53 TUMOR SUPPRESSOR GENE IN THE CELLULAR RESPONSE TO DNA DAMAGE, Li-chun Huang and Geoffrey M. Wahl, The Salk Institute P.O.Box 85800 La Jolla, CA 92037
The p53 tumor suppressor is a transcriptional regulator that halts progression from G1 to S phase by inducing the synthesis of inhibitors of cyclin dependent kinases. p53 mediated induction of these inhibitors occurs in response to DNA damage. The number and specific types of breaks required to induce a p53 mediated arrest response cannot be assessed by current methods. We used mechanical microinjection of DNA into the nuclei of WS1 normal human diploid fibroblasts to gain insight into these issues. While supercoiled DNA had little or no effect on cell cycle progression, restriction endonuclease linearized DNA effectively prevented serum arrested cells from entering S-phase. By contrast, inactivation of p53 in WS1 cells stably expressing the E6 gene derived from human papilloma virus 16 proceeded into S-phase when injected with linearized DNA. This demonstrates that the arrest specifically triggered by double strand breaks is p53 dependent. p53 dependency was confirmed in a parallel set of experiments employing a glioblastoma cell line GM47.23, in which wild type p53 can be induced by dexamethasone treatment. This system has also enabled us to quantify the number of double strand breaks required to induce the arrest. As few as 15 injected double strand breaks prevented WS1 cells from entering S-phase. The arrest appeared to be of long duration since cells had still not entered S-phase by 48 hrs post injection. This result also indicates that in fibroblasts, the injected DNA was not repaired very effectively in G0 or early G1. This makes the hypothesis that p53 induces a G1 arrest to allow for DNA repair less likely in normal human fibroblasts. We also tested whether specific types of ends were more effective at inducing the arrest, and each appeared approximately equivalent even though blunt ends or ends with a 3' overhang are reportedly repaired less well than those of 5' overhangs. Consistent with previous reports that drugs that cause single strand DNA breaks do not induce p53 protein levels in the absence of DNA replication(Nelson and Kastan, MCB vol.14 p.1815-1823), we found that injecting nicked circular DNA had no effect on cell cycle progression. This system should enable us to dissect with far greater precision the repair potential of normal and mutant human cells, and to investigate the underlying signal transduction mechanisms involved in p53 mediated G1 arrest.

C5-551 RELATIONSHIP OF RADIATION-INDUCED G_1 PHASE ARREST AND P53 FUNCTION IN HUMAN TUMOR CELLS. Nagasawa, H., Li, C., Maki, C. and Little, J.B. Harvard School of Public Health, Harvard Medical School, Boston, MA 02115
Kastan et al (1991) reported that G_1 phase arrest after irradiation was dependent upon the status of p53 gene expression. However, it was not clear whether the arrest they measured occurred in the first or second G_1 phase. We examined progression from G_1 into S in 3 exponentially growing human tumor cell lines (HT29, p53-; MCF7 and RKO, p53+) by flow microfluorimetric (FMF) and autoradiographic techniques. Cells were incubated with 1µCi/ml of ^{3}H-TdR and 0.2 µg/ml of colcemid for 3 hours prior to irradiation to prevent contamination from second cell cycle G_1 phase cells. At 1-2 hour intervals, the cells were trypsinized from the culture flasks and prepared for analysis by FMF and continuous labeling indices. There was a very small delay in G_1 to S phase cell progression in all three cell lines. The fraction of G_1 phase cells decreased rapidly up to 8 hours after irradiation, based on analysis of FMF profiles, reaching a plateau at later times (Figs. 1 and 2). Based on these results, we conclude that there is no evidence for a radiation-induced arrest in the first post-irradiation G_1 phase related to expression of the p53 gene.



C5-552 CHARACTERIZATION OF G1 CHECKPOINT ARREST IN *S. cerevisiae* FOLLOWING TREATMENT WITH DNA-DAMAGING AGENTS, Wolfram Siede[1], Irina Dianova[1], Andrew S. Friedberg[1], Anna Friedl[2], Friederike Eckardt-Schupp[2] and Errol C. Friedberg[1], [1]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75235, [2]Institut für Strahlenbiologie, GSF Forschungszentrum für Umwelt und Gesundheit, 85758 Ober-Schleissheim, Germany
In *S. cerevisiae* G1 or G1/S cell cycle arrest in the presence of DNA-damage is an actively regulated response which depends on the checkpoint-activating genes *RAD9* and *RAD24* (1,2). We have shown that regulated arrest following exposure to UV or γ radiation can occur in at least two stages during G1/S transition, one of which is identical with START. Delayed entry into S-phase at low doses of UV radiation in mutants defective in nucleotide excision repair (NER) is independent of *RAD9*. However, the *RAD9*-dependency of G1 arrest after γ ray treatment is unchanged in these mutants. DNA strand breaks may therefore constitute the checkpoint-activating signal for G1/S arrest in the presence of DNA-damage. Such breaks may be induced directly by γ rays, or may be generated during the course of NER in UV-irradiated cells. We have investigated the yeast homolog of the human autoantigen Ku as a candidate protein that might sense DNA strand breaks and initiate a regulatory protein kinase cascade. Human Ku consists of two subunits and comprises a DNA-end-binding activity. The 80 kDa subunit has been implicated in the repair of double-strand breaks (3). A Ku-like DNA end-binding activity has been characterized in yeast cells and a gene homologous to the human 70 kDa subunit has been isolated (*HDF*) (4). Deletion of the yeast gene eliminates DNA end-binding activity in gel shift assays. However, it does not affect the sensitivity of haploid or diploid cells to UV radiation or γ rays (^{137}Cs or ^{60}Co, O_2 or N_2 conditions), nor does it impair checkpoint arrest in G1, S or G2. Hence, the Ku complex does not appear to be involved in repair or checkpoint control in yeast.

(1) Siede et al., Proc. Natl. Acad. Sci. USA 90 (1994) 7985
(2) Siede et al., Genetics 138 (1994) 271
(3) Taccioli et al., Science 265 (1994) 1442
(4) Feldmann and Winnacker , J. Biol. Chem. 268 (1994) 12895

C5-553 DEAMINATION OF CYTOSINE - CONTAINING CYCLOBUTYL PYRIMIDINE PHOTODIMERS: SIGNIFICANCE FOR UV MUTAGENESIS, Zvi Livneh* and Yoav Barak, Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100

The major type of DNA lesion caused by UV irradiation is the cyclobutyl pyrimidine dimer. The mechanism by which the lesion is processed into mutation is not fully understood. Recently it was suggested that cytosine in cytosine-containing dimers is rapidly and spontaneously deaminated to yield uracil-containing dimers. This implies that some of the ultimate mutagenic lesions may be different from the primary DNA lesions.

We have developed a bio-assay designed to measure the deamination of cytosine-containing dimers, in order to evaluate its significance in UV mutagenesis. In our assay, a reporter plasmid was UV-irradiated and incubated to allow deamination. Then the plasmid was treated with DNA photolyase, which specifically monomerized cyclobutyl pyrimidine dimers. If deamination of cytosine-containing dimers took place, uracils were formed in the DNA after the photolyase treatment. These uracils were assayed by their ability to cause mutations in the *cro* gene carried on the plasmid, after its introduction into an indicator strain. In order to prevent repair of the uracils, the assay was performed in an *ung* strain, that lacks uracil N-glycosylase. In addition, in order to eliminate contributions by the SOS system, the indicator strains carried *ΔrecA* and *ΔumuDC* mutations making them non-mutable by UV light and other SOS mutagens.

In the Ung$^-$ strain a high mutation frequency of Cro$^-$ mutations was obtained (up to 5×10^{-3}), and it varied with the dose of UV irradiation and the time and temperature of the deamination treatment. In contrast, with the isogenic Ung$^+$ strain, mutagenesis was constant and 100-fold lower, indicating that the mutations in the Ung$^-$ strain were indeed caused by uracils originating from deaminated cyclobutyl dimers. This assay was used to determine the rate constants of deamination under a variety of conditions. Our results suggest that deamination of cytosine-containing dimers occurs on a time scale of hours, and is thus likely to be more significant for mammalian systems than for *E. coli.*

C5-554 COMPUTER SYSTEM FOR ANALYSIS OF NUCLEOTIDE CONTEXT ROLE IN MUTAGENESIS (MutAn), Igor B. Rogozin and Nikolay A. Kolchanov, Theoretical Department, Institute of Cytology and Genetics of the Russia Academy of Sciences, Novosibirsk, 630090, Russia

The frequency of lesions and mutations in a site is influenced by the DNA sequence structure (polynucleotide context). Spontaneous and induced mutations and lesions occurs preferentially at a small number of sites, i.e., hotspots. Mutation and lesion hotspots studying can give a valuable information on the role of repair enzymes in mutagenesis.

The MutAn computer system is developed to study the nucleotide context influence on point mutations and lesions in DNA molecules. The system comprises programs for analysis of different statistic characteristics of point mutations and lesions. It makes possible to detect statistically significant correlations between mutations location and frequency and different features of nucleotide context. All the results of analysis are accumulated in Mutation Knowledge base. The context features detected for the set of mutations in the sequence may be compared with the previously obtained results from the Knowledge Base.

The important component of the MutAn system is the Data Base of Mutational Spectra. The format of this Data Base gives possibility for detailed description of experimentally obtained information on mutations and lesions in nucleotide sequences. Now Data base includes about 200 entries. Each entry describes a single experiment in which induced or spontaneous mutations were revealed.

The Data base of Mutational Spectra, the Mutation Knowledge base and the set of programs for mutations and nucleotide context analysis are integrated into MutAn computer system by the management block. Now the system includes different knowledges on analysis of nucleotide substitutions, deletions, insertions and lesions.

The system was applied to analysis of spontaneous and induced mutations in pro- and eukaryotic genomes. Different types of significant correlation between mutations and polynucleotide context have been revealed. The difference in mutation hotspot context features induced by Sn2 alkylating agents in different strains of *E.coli* (wild type and *uvrB*$^-$) was shown. Similar results were obtained also for ultraviolet light-induced mutations.

C5-555 ROLE OF *recBC* AND *xth* FUNCTIONS IN THE DNA DOUBLE-STRAND BREAKS REPAIR ACCOMPANIED BY FORMATION OF DELETIONS IN PLASMID DNA, Olga I.Sinitsina, Elena E.Vasyunina, Tatiana T.Timchenko, Grigory L.Dianov and Rudolf I.Salganik, Department of Molecular Genetics, Institute of Cytology and Genetics of the Russian Academy of Science, Novosibirsk, 630090, Russia

Uracil containing derivatives of plasmid pBR327 with the *tet* gene interrupted and inactivated by 165 bp direct repeats were constructed. In cells harboring these plasmids, deletion which restores the integrity of *tet* gene give rise to tetracycline- resistant colonies, providing thereby a simple phenotypic test for deletion formation. The frequencies of deletions in these plasmids were estimated in *Escherichia coli* strains proficient or deficient in excision repair or general recombination enzymes. It was demonstrated that location of uracil residues in opposite sites of plasmid DNA results in DNA double-strand breaks (DSB) effected uracil-DNA glycosylase and other repair enzymes ensuring simultaneous excision of modified nucleotides. The DSB, in turn, induces formation of deletions. The deletion occur as a result of base-pairing of direct repeats flanking the DNA broken ends. The frequencies of deletion in uracil containing plasmids increase tenfold in an *ung*$^+$ *E.coli* strain in comparison to *ung*$^-$strain. Mutations in *xth* and *recBrecC* genes reduce the frequencies of deletion formed. In *xth,recBrecC* mutant strain the number of deletion fall to the lowest spontaneous level, as in *ung*$^-$ *E.coli* strain. The data presented demonstrate that the enzymes responsible for the DNA repair interacting with heavily altered DNA carrying oppositely located modified nucleotides instead of DNA repair provide formation of deletion and probably, other DNA rearrangements.

C5-556 INTERPLAY OF STRESS SYSTEMS OF ESCHERICHIA COLI GAM^{r}444 MUTANT IN REPAIR OF γ-INDUCED DAMAGE, Valery N. Verbenko and Vitaly L.Kalinin, Molecular and Radiation Biophysics Division, Petersburg Nuclear Physics Institute, Gatchina, St.Petersburg, 188350, Russia

E. coli Gam^{r}444 mutant is super-radioresistant (survival S = 10^{-3} at dose 3D = 5.0 kGy) and contains likely three mutations relevant to enhanced repair. Kinetics of induction and elimination of RecA protein in mutant is more rapid than in wild-type cells and heat-shock proteins are hyperproduced at normal temperature (32°). Corresponding mutations were cloned in the wild-type strain AB1157 (3D = 1.1 kGy) using vector MudII4042 (Cm^r). Plasmid pgam12 which cannot confer radioresistance immediately after transformation is efficient after a single γ-irradiation of Cm^r transformants (3D = 2,3 kGy). We can assume that this plasmid carries a recessive Gam^r mutation which is active only after radiation-induced homozygotization between plasmid and host chromosome. The gam12 mutation influences expression of heat-shock regulon that we tested with lacZ gene under control of heat-shock promoter. In independent experiments four plasmids were isolated which cary dominant Gam^r mutations and can transfer enhanced radioresistance (3D = 2.1 - 2.3 kGy) directly after transformation of the wild-type recipient but not of the rpoH or recA mutants. A mutation-insertion into a chromosomal gene impaired by one of dominant mutations, gam^{r}18, causes drastic cell radiosensitization on the recBCsbcB background. gam^{r}18 mutation may be concerned with a helicase. Dominant plasmid of another type pgam43 lead to RecA-independent inhibition of DNA postirradiation degradation. Radioprotective effects of recessive and dominant mutations are additive. Our data show that enhanced radioresistance of Gam^{r}444 mutant is due to different types of mutations enhancing expression of heat-shock proteins genes, SOS-system and recombinational repair, moreover regulatory genes rpoH and recA or their products proved to be a subject for additive regulation.

Late Abstracts

NEW RecA MUTANTS RESISTANT TO UmuD'C PROTEINS, Adriana BAILONE, Raymond DEVORET, and Suzanne SOMMER, GEMC, Institut Curie, Bât. 110, F-91405 ORSAY, France

After DNA has been damaged by UV-light, three *successive* SOS processes take place in *E. coli*. (a) *Excision repair* removes around 80-85% of the lesions during the first 30 min; (b) in the next 40 min, *homologous recombination* puts aside around 50% of the remaining lesions; among the substrates acted upon by recombination are the 2 kb gaps produced downstream a lesion by a first round of replication; (iii) then *SOS mutagenesis* resolves half of the gaps left unrepaired by recombination by a second round of *error-prone* replication. The mutagenesis proteins UmuD and UmuC are induced late, slowly and parsimoniously. Furthermore, UmuD is matured by cleavage on a RecA filament to produce UmuD', which when bound to UmuC constitutes the active complex in mutagenesis.
The UmuD'C complex inhibits homologous recombination. This has been evidenced in Hfr x F⁻ crosses. We have isolated new suppressor mutations in the *recA* gene that allow bacteria to overcome the inhibitory action of UmuD'C proteins expressed at a high level. The new *recA* mutant bacteria are proficient in homologous recombination and in cleavage of LexA and UmuD proteins but they are unable to promote UV-mutagenesis. This is thought to be due to a decreased interaction between the mutant RecA filaments and the UmuD'C complex.

GENE CLONING OF NOVEL TYPE OF 3-ALKYLPURINE-SPECIFIC DNA GLYCOSYLASE FROM *Schizosaccharomyces pombe*, Magnar Bjørås, Davide Mainieri and Erling Seeberg, Biotechnology Centre, Univ. of Oslo, Norway and ESBS, Carcinogenese et Mutagenese Moleculaire et Structurale du CNRS, Universite Louis Pasteur, Illkirch, France.

Characterization of base excision repair mechanisms of alkylation damage in the fission yeast *Schizosaccharomyces pombe* has revealed the presence in this organism of an alkylation repair DNA glycosylase, TPG (three-alkyl purine DNA glycosylase), which is different from the other enzymes of this type described so far. Biochemically, the enzyme resembles the *E. coli* Tag enzyme because it efficiently catalyzes the removal of 3-methyladenines from DNA, whereas 7-methylguanines are not being removed. However, TPG also catalyzes very efficient release of 3-methylguanines, by kinetics similar to that of 3-methyladenines, and in this respect resembles the *E. coli* AlkA enzyme. Because of this property, expression of TPG completely suppresses the alkylation sensitive phenotype of *alkA* mutant cells. Sequence analysis of the *TPG* gene reveals an enzyme of 256 aa without sequence homology to any other 3-methyladenine DNA glycosylase previously described. Therefore, TPG represents a novel type of alkylaytion repair DNA glycosylase distinct from the three other classes of such enzymes so far characterized; the *E. coli* Tag type, the *E. coli* AlkA homologues, and the family of mammalian (e.g ADPG) and plant alkylation repair DNA glycosylases. Whether such an enzyme is specific for a narrow range of organisms closely related to *S. pombe* or is more universally distributed in distantly related organisms, remains to be investigated.

ENZYMATIC REPAIR OF FORMAMIDOPYRIMIDINES AND 8-OXOPURINES IN BACTERIA AND YEAST

Serge Boiteux, URA 147 CNRS, PR2-Institut Gustave Roussy, 94800 Villejuif, France

Formamidopyrimidines (Fapy) and 8-oxopurines are major lesions in DNA exposed to ionizing radiation and oxidizing agents. In E. coli, Fapy G, Fapy-A and 8-oxoguanine are excised by the Fpg protein (Fpg-E). The Fpg-E protein is a 30-kDa enzyme endowed with DNA glycosylase-, AP lyase- and dRpase activity. The fpg gene from the Gram-positive bacterium Lactococcus lactis was cloned and sequenced. The fpg-L gene is predicted to encode a 273 amino acids protein with 38 % identity with the E. coli Fpg protein. Comparison of physical, catalytic and biological properties of Fpg-E and Fpg-L shows that these two proteins have highly conserved structure and function.
The identification of analogues of bacterial Fpg protein is a matter of importance in eukaryotes. We have purified a DNA glycosylase that excises Fapy residues from the yeast Saccharomyces cerevisiae. Yeast Fapy DNA glycosylase is a 40-kDa protein that releases Fapy as a free base. Yeast Fapy DNA glycosylase does not excise N7-MeG, N3-MeA nor uracil. Yeast Fapy DNA glycosylase provokes the incision of 8-OxoG containing strand in DNA duplex. DNA duplex containing the 8-OxoG/G mismatch is incised at higher rate compared with 8-OxoG/C, 8-OxoG/T and 8-OxoG/A duplex. Another enzyme activity that preferentially incises the 8-OxoG containing strand in the 8-OxoG/C duplex has been identified and partially purified from S. cerevisiae. These repair activities do not incise duplex DNA that contains G/G, G/T or G/A mismatch.

STARVATION-ASSOCIATED MUTATION IN ESCHERICHIA COLI, Bryn A Bridges, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK.

When E. coli WU3610 tyrA (ochre) bacteria were incubated on plates lacking tyrosine, slow growing Tyr⁺ mutants distinct from those reported in logarithmic phase cells appear from day 7 in a time-dependent recA-independent manner. They could also be shown to arise when the cells were starved for requirements other than tyrosine; the process is thus not "directed". It is suggested that a DNA lesion capable of miscoding during transcription and replication accumulates with time. A "mutant" RNA transcript that allowed protein synthesis would trigger DNA replication and thus fix the mutation. Derivatives of WU3610 lacking mismatch correction showed elevated rates of starvation-associated mutation indicating that an abnormal base pair recognized by MutS protein may be an intermediate.

THE COMET ASSAY MODIFIED TO DETECT DNA BASE OXIDIDATION, AND REPAIR OF DNA DAMAGE IN CELLULAR AND SUBCELLULAR SYSTEMS,

Andrew R. Collins, Mária Dušinská, Ma Ai-guo and Iona Fleming, Rowett Research Institute, Aberdeen AB2 9SB, Scotland, UK.

Single cell gel electrophoresis (SCGE; the comet assay) is a very sensitive method for detecting DNA strand breaks; relaxed supercoiled loops of DNA in nucleoids embedded in agarose are attracted to the anode and form a "comet tail", the intensity of which indicates the number of breaks. We have modified the assay so that it detects oxidised bases. The nucleoids are digested (in the gel) with endonuclease III or formamidopyrimidine glycosylase to reveal oxidised pyrimidines and oxidised purines, respectively, as DNA breaks which then induce comet tails on electrophoresis.
We have examined the rate of repair of both these classes of lesion, as well as strand breaks, after treatment of human cells with H_2O_2. In HeLa cells, strand breaks are repaired within 1 hour; oxidised bases are more persistent, but significant repair is seen within 2 hours. Lymphocytes show incomplete repair of strand breaks, and no detectable removal of oxidised bases over 2 or more hours. It is possible that the persistent strand breaks are in fact intermediates in the repair of oxidised bases, held open because the synthetic stage of repair in lymphocytes is particularly slow.
We have also developed an *in vitro* repair assay based on SCGE. Agarose-embedded nucleoids from cells damaged with either H_2O_2 or UV provide the template for repair enzymes in a simple whole cell extract. Incision (in the presence of ATP) is indicated by the appearance of breaks and comet tails; resynthesis (stimulated by the provision of deoxyribonucleoside triphosphates) results in restoration of intact, supercoiled DNA and comets without tails.

REGIONS OF RAG-1 AND RAG-2 NECESSARY FOR INITIAL CLEAVAGE AND COMPLETION OFV(D)J RECOMBINATION.

Christina A. Cuomo, Susan A. Kirch, and Marjorie A. Oettinger. Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02114.

The recombinase activating genes, RAG-1 and RAG-2, are necessary to activate V(D)J recombination, and thus play an essential role in immune system development. We have carried out a mutational analysis of the RAG-2 protein and examined the effects of these mutations on the recombination of different substrates. Although the RAG-2 protein is highly conserved throughout evolution, the C-terminal one-quarter of the protein was dispensible for all types of recombination tested. This analysis defined a core active region of RAG-2 confined to the first three-quarters of the RAG-2 protein.

Recently, putative intermediates in the V(D)J recombination reaction have been observed in thymocytes (Roth, D. B., et al. Cell 69:41-53, 1992) and in pre-B cells (Schlissel, M., et al. Genes and Development 7: 2520-2532, 1993). At loci undergoing V(D)J recombination, double-strand DNA breaks have been identified at recombination signal sequences. We are developing a ligation-mediated PCR assay to detect double-strand DNA breaks at recombination signal sequences within substrates integrated into fibroblasts. Using this system, we will examine the effects of mutations in RAG-1 and RAG-2 on the occurence of these possible intermediates.

ACTIVATOR 1 MOVES ALONG TEMPLATE DNA IN A 3' TO 5' DIRECTION SCANNING FOR PRIMERS.

Frank Dean and Jerard Hurwitz, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021

Activator 1 (A1) is a 5-subunit accessory factor for DNA polymerase δ (pol δ). It functions as a DNA primer recognition factor required for assembly of the PCNA DNA clamp at 3'-OH DNA primer ends. Pol δ then binds to the complex and catalyzes processive elongation of the primer. A1 has an intrinsic DNA-dependent ATPase activity that is stimulated by the presence of DNA primers, PCNA, and the human single-stranded DNA binding protein (SSB).

It is possible that A1 locates DNA primer ends by either i) random diffusion through the solution, ii) binding to single-stranded DNA resulting in a relative increase in its concentration in the vicinity of primers, or iii) tracking along the DNA template until a primer is located. In order to distinguish between these possibilities we used a primer extension assay in order to measure the rate at which pol δ can find primers that are annealed to different DNA templates. The results demonstrated that pol δ utilizes primers that are annealed to single-stranded circular M13 DNA (7kb) more rapidly than primers that are annealed to 60 nt oligonucleotide templates. Pol δ also uses primers that are annealed near the 5' terminus of single-stranded linear M13 molecules 20 times more rapidly than primers that are annealed to the 3' ends of single-stranded linear M13. These reactions are dependent on the presence of A1, PCNA, pol d and SSB.

We suggest that A1 tracks along SSB-coated single-stranded DNA in a 3' to 5' direction until it finds DNA primer ends. PCNA then forms a complex with A1 and pol δ is recruited for primer elongation. The implications of these findings for models of coordinated leading and lagging strand DNA replication will be discussed.

EFFECT OF CODING END NUCLEOTIDES ON V(D)J RECOMBINATION,

Uthayashanker R. Ezekiel, Peter Engler, and Ursula Storb, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

The normal outcome of V(D)J recombination is the joining of two coding sequences, e.g. a variable (V) and a joining (J) region, into a coding joint, and the joining of two complementing rearrangement signal sequences (RSSs) into a signal joint. We show that the nucleotide composition of the coding ends strongly influences the efficiency of V(D)J recombination. The presence of two or more T's at the 5' end of either the 12mer or the 23mer RSS greatly decreases both coding joint and signal joint formation, and having T's at the 5' ends of both RSSs eliminates V(D)J recombination. This suggests that a step during the initiation phase of the recombination is affected, i.e. binding of the recombinase, synapsis, and/or cleavage at the heptamer/coding end border. A 5' T coding end can be rescued if the other coding end contains 5' G, C, or A, perhaps implying that synapsis may be a prerequisite for both signal joint and coding joint formation. Furthermore, the presence of As at the 5' end of the 12mer RSS, but not the 23mer RSS, affects coding joint, but not signal joint, formation thus presumably interfering with the processing of the coding end that was associated with the 12mer RSS. This is the first time that asymmetric processing of coding ends has been demonstrated and suggests that different protein complexes are bound to the 12mer and 23mer RSSs, and moreover, that these become transferred to the aligned coding ends for processing.
Supported by NIH grant AI24780. U.R.E. is the recipient of a Cancer Research Institute Fellowship.

Repair and Processing of DNA Damage

Expression of a *Drosophila MSH2* homolog, *spellchecker1* and phenotype of mutants.

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We have characterized a *MSH2* homolog from *Drosophila melanogaster* called *spellchecker1*. In contrast to yeast where *MSH2* mutants are viable, *spell* is an essential gene in the fly.

The DNA mismatch repair protein, MutS of *E. coli* as well as some of the MutS homologs (MSHs) of *Saccharomyces cerevisiae,* have been shown to play important roles in DNA replication, recombination and repair. The MutS protein binds to regions of mispaired or unpaired bases and recruits other proteins needed to resolve the contradiction. Such mismatches can arise in heteroduplex DNA formed during homologous recombination or through DNA damage. Mismatches can also arise during replication by misincorporation or by polymerase slippage in regions of sequence repeats. Simple DNA repeats are unstable in yeast mutant for *MSH2*. Humans with one defective *hMSH2* gene are prone to hereditary nonpolyposis colon cancer (HNPCC), and tumors from HNPCC patients show DNA repeat instability. No humans with mutations in both alleles of *hMSH2* have been found.

Drosophila homozygous for *spell* mutations survive embryonic and larval development to die as pupae. This is reminiscent of the phenotype of DNA damage caused either by massive somatic P element transposition or by exposure to ionizing radiation. We will present data on the expression of *spell* as well as details of the mutant phenotype.

RATES AND MECHANISMS OF LOSS OF HETEROZYGOSITY IN HUMAN LYMPHOBLASTOID CELLS, Micheline Giphart-Gassler, Vera v. Buuren- v. Seggelen and Arnolda v. Dalen, Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, 2333 AL Leiden, The Netherlands.

Genetic rearrangements resulting in loss of heterozygosity (LOH) are a major cause of the expression of recessive mutations and contribute to cancer progression. The HLA class I and class II genes of the human Major Histocompatibility Complex located on the p-arm of chromosome 6 are extremely polymorphic and provide for a natural heterozygous gene system covering 3 Mb of DNA. Mutants that have lost one of the HLA surface antigens can be immuno-selected with monoclonal antibodies and complement. Using this selection protocol we have found by fluctuation analysis that the rate of loss of HLA-A2 in lymphoblastoid cell lines is $1.8 \cdot 10^{-5}$ /cell/generation, a rate 50 fold that of *hprt* mutation. HLA typing of 30 independent spontaneous HLA-A2$^-$ mutants showed that 26 had also lost the HLA-B allele of the same haplotype, indicating that the far majority of the mutants have arisen by events causing LOH over a minimum length of 1 Mb of DNA. Next the extent of LOH was measured by allelotyping the 30 mutants using informative CA repeat loci spread over the entire chromosome 6. Non-disjunction leading to the loss of heterozygosity of all markers appeared the most frequent LOH event (40%), followed by LOH of all markers of the p-arm and mitotic recombination which resulted in LOH of all markers from the recombination breackpoint up to the telomer (23%). Only 2 mutants had retained heterozygosity at the markers tested which indicates that the contribution of point mutations to spontaneous loss of HLA-A2 is less than 10%.

ROLE OF DNA REPAIR IN PROTECTING MATURE NERVOUS TISSUE FROM DNA DAMAGE. G.E. Kisby, C. Sweatt, P.S. Spencer. Center for Research on Occupational and Environmental Toxicology; Oregon Health Sciences Univ., Portland, OR.

We are investigating the etiology of a disappearing Guamanian (Chamorro) neurodegenerative disease which features a combination of amyotrophic lateral sclerosis (ALS), Parkinsonism, and progressive dementia with Alzheimer-like neurofibrillary degeneration. While the environmental trigger for this disorder has yet to be identified, we have studied a genotoxic agent (cycasin, the β-D-glucoside of methylazoxymethanol, MAM) found in Chamorro food and medicine (both derived from the toxic cycad plant, which induces neuromuscular disease in animals). Cycasin and its aglycone MAM (both mutagenic carcinogens in mitotic cells) are rapidly taken up and widely distributed in the nervous system of animals, and both compounds selectively damage post-mitotic neurons in murine brain tissue slices. MAM induces specific DNA adducts (N^7-methylguanine, O^6-methylguanine) in human and murine neural tissue. This study examines MAM-induced DNA damage and specific DNA repair in neural cultures. MAM-induced neurotoxicity was potentiated by pre-treatment of mouse cerebral cortical explants with either O^6-benzylguanine (BG, courtesy of Dr. R. Moschel), an inhibitor of the DNA-repair protein methylguanine methyltransferase (MGMT), or the radiosensitizer bromodeoxyuridine (BrUrd). BG reduced MGMT levels (using a monoclonal antibody, MT3.1, courtesy of Dr. T. Brent). BG and BrUrd both potentiated DNA damage (*i.e.* O^6-methylguanine) in murine cortical explants and cerebellar neuronal cell cultures subsequently treated with MAM. In post-mitotic human SY5Y neuroblastoma cells differentiated with nerve growth factor, MAM significantly reduced MGMT and apurinic/apyrimidinic endonuclease levels. These findings point to the possibility that modulation of DNA repair in nervous tissue is associated with an increase in DNA damage and neurotoxicity induced by genotoxins. Findings from these studies (1) suggest post-mitotic neurons may be particularly sensitive to environmental genotoxins and (2) may provide important clues on the selective vulnerability and progressive degeneration of neurons found in human neuropathological conditions (*e.g.* ALS, Parkinson's and Alzheimer's diseases) related to the Guam disease. Studies are underway to characterize the role of other DNA-repair pathways in protecting the nervous system from insult by environmental genotoxic agents, including mustards. [Support: NIH grant NS 19611 and VA-CROET OR Environmental Hazards Research Center]

ROLE OF INTRAMITOCHONDRIAL GLUTATHIONE PEROXIDASE IN PRESERVATION OF mtDNA INTEGRITY

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There is growing evidence for an accumulation of mitochondrial (mt) DNA deletions in human brain with normal aging, which may be exacerbated in some pathologies including Alzheimer's disease. The relatively high rates of mutation which have been observed in mtDNA could result from an imbalance between intrinsic susceptibility of mtDNA to oxidative damage and mtDNA repair capacity. The potential role of the mitochondrial antioxidant defense in the preservation of mtDNA structural integrity has been investigated in human cell transfectants and transgenic mice which overexpress Se-glutathione peroxidase (GSHPx). GSHPx accounts for the major activity which can reduce H_2O_2 in mitochondria and therefore prevent the formation of hydroxyl radicals by Fenton-type reactions. Human T47D-GPx-2 cell transfectants, with enhanced nuclear DNA resistance to DNA strand breakage induced by an intracellular source of O_2^- and H_2O_2, menadione (Mirault et al., *J. Biol Chem.* **266**, 20752, 1991), were found to contain particularly high levels of GSHPx activity inside their mitochondria as compared to untransfected T47D cells. Immuno-electron microscopy revealed a ~20-fold higher concentration of GSHPx inside mitochondria than in cytoplasm of GPx-2 cells. Following 2-day exposure to antimycin, an electron transport chain inhibitor which stimulates production of O_2^- and H_2O_2 in mitochondria, these cells were significantly more resistant to the drug than untransfected or control transfectant cells. The extent of mtDNA strand breakage assessed by agarose gel electrophoresis, as measured by the ratio of linear to supercoiled and concatenated mtDNA forms, was remarkably reduced in both untreated and antimycin-treated GPx-2 cells, as compared to control transfectant cells (low GSHPx). These results suggest that the level of intramitochondrial GSHPx activity is an important determinant of mtDNA susceptibility to endogenous oxidative damage. We are currently further testing this hypothesis *in vivo* by comparing the age-dependent accumulation of mtDNA deletions in different brain regions of GSHPx transgenic and non-transgenic mice, by PCR analysis.

This work was supported by the National Cancer Institute of Canada and the Alzheimer's Association (Chicago).

THE REPLICATION ERROR (RER) PHENOTYPE AT MICROSATELLITE LOCI IN GERMINAL CENTRE CELL LYMPHOMA. Morgan GJ, Randerson J, HALL N, Jack A. Centre for Haematologic Oncology, The General Infirmary at Leeds, West Yorkshire, UK.

We have examined 24 cases of histopathologically well defined cases of Germinal Centre Cell lymphomas for allele imbalance at 28 different microsatellite loci. We have done this analysis using a fluorescent PCR technique combined with detection on a gene sequencer. The microsatellites are located on chromosome 6 and at the sites of 6 tumour suppressor genes. At these loci it has been possible to identify the phenomenon of replication error or microsatellite instability. This is where a novel allele is visible in the tumour tissue which is absent from the normal germline DNA. Six of the cases studied had evidence of microsatellite instability and 2 distinct patterns were seen. In 2 cases there was instability in a high percentage of the microsatellites. In the remaining 4 cases only 1 or 2 loci showed the phenomenon. The changes seen in the cases with frequent microsatellite instability are suggestive of an specific abnormality of DNA repair. Candidate genes which could be responsible for this are PMS1, hMLH and hMSH2 In the cases with replication error we have looked for allele imbalance of hMSH2 and hMLH using microsatellites. We did not detect any evidence for this at these loci and as with other studies it could be that mutation is the major cause of abnormality at these genes.

We have identified allele imbalance at a number of loci both on chromosome 6 and at the tumour suppressor gene loci P53, APC, DCC, NM23, WT1 and RB1. The suprising result is the frequency with which allele imbalance and microsatellite instability are see in this low grade malignancy which is brought about by the prevention of apoptosis by a deregulated BCL2. This frequent genetic instability is likely to contribute to transformation of these tumours which is seen in up to 60% of cases.

DNA MISMATCH BINDING ACTIVITIES IN METHYLATION TOLERANT AND COLORECTAL ADENOCARCINOMA CELLS. Edel O'Regan, Pauline Branch, and Peter Karran, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, EN6 3LD, UK.

Mismatch binding activities have been investigated in human cells that have been selected for resistance, or tolerance, to DNA methylation damage and in established human colorectal adenocarcinoma cell lines. Extracts of wild-type human cells contain two distinct mismatch binding activities: one binds preferentially to slipped/mispaired structures and to single G·T mismatches in an *in vitro* bandshift assay, a second activity preferentially recognizes A·C, T·T and T·C mispairs. Five cell lines, two selected for DNA methylation tolerance, the remainder identified among colorectal adenocarcinoma lines, are defective in G·T mismatch binding. All five lines bind normally to A·C mismatches. One G·T binding defective adenocarcinoma cell line, LoVo, has a deletion in the hMSH2 gene encoding the G·T binding protein. A·C binding therefore appears to be mediated by a distinct mismatch recognition activity. The two binding activities copurify through AcA34, heparin sepharose and DNA cellulose chromatography but can be separated by ammonium sulfate fractionation and FPLC on monoQ. Polycations affect the two binding activities of partially purified protein fractions in different ways: inclusion of spermine in the binding assay stimulates G·T binding and inhibits A·C binding.

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Mismatch binding activities have been investigated in human cells that have been selected for resistance, or tolerance, to DNA methylation damage and in established human colorectal adenocarcinoma cell lines. Extracts of wild-type human cells contain two distinct mismatch binding activities: one binds preferentially to slipped/mispaired structures and to single G·T mismatches in an *in vitro* bandshift assay, a second activity preferentially recognizes A·C, T·T and T·C mispairs. Five cell lines, two selected for DNA methylation tolerance, the remainder identified among colorectal adenocarcinoma lines, are defective in G·T mismatch binding. All five lines bind normally to A·C mismatches. One G·T binding defective adenocarcinoma cell line, LoVo, has a deletion in the hMSH2 gene encoding the G·T binding protein. A·C binding therefore appears to be mediated by a distinct mismatch recognition activity. The two binding activities copurify through AcA34, heparin sepharose and DNA cellulose chromatography but can be separated by ammonium sulfate fractionation and FPLC on monoQ. Polycations affect the two binding activities of partially purified protein fractions in different ways: inclusion of spermine in the binding assay stimulates G·T binding and inhibits A·C binding.

STUDY OF HETEROSPECIFIC INTERACTIONS BETWEEN MISMATCH REPAIR PROTEINS. HEXA OF *Streptococcus pneumoniae* AND MUTS-MUTL OF *Escherichia coli*. Marc Prudhomme, Stephane Duhamel, Paul Modrich* and Jean Pierre Claverys. MGM-CNRS 118 route de Narbonne 31062 Toulouse France. *Department of biochemistry Duke University Medical Center Durham NC 27710.

The Mut system from *E. coli* and the Hex system from *S. pneumoniae* are the two best characterized prokaryotic mismatch repair systems, sharing a similar specificity of mismatch recognition, and a similar mechanism of repair (long patch repair). In addition, the mismatch repair proteins MutS/HexA and MutL/HexB are homologous. Despite these similarities, attempts to complement Mut deficiencies by their Hex homologues *in vivo* have failed. Surprisingly it was shown that HexA has a dominant negative effect on the Mut system (1). Results of *in vivo* and *in vitro* investigations on this effect lead us to propose that HexA blocks Mut mismatch repair in two distinct ways. First, HexA competes with MutS for mismatch binding, and second, HexA interacts directly with MutL to inhibit MutL activity (2). These results prompted us to investigate which protein domains are required for interactions between mismatch repair proteins. With this aim, we devised a strategy for construction of *hexA/mutS* gene fusions, by homeologous recombination *in vivo* in *S. pneumoniae*. We will describe the preliminary characterization of a set of HexA/MutS chimeras and the study of their negative dominance and complementation abilities. The main features of *in vivo* homeologous recombination will also be discussed.

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REDUCED REPAIR OF DNA DOUBLE STRAND BREAKS IS ASSOCIATED WITH CHROMOSOMAL INSTABILITY IN MALIGNANT MELANOMA CELL LINES. T. M. Rünger, M. Kotas, M. Poot*, D. Dehner, K. Möller, A. Schwaaf, M. Leverkus, E.-B. Bröcker, Departments of Dermatology and Human Genetics*, University of Würzburg, Germany.
Genetic and chromosomal instability is a common feature of most malignant neoplasias, including malignant melanoma. It plays a pivotal role in multi-step carcinogenesis by facilitating the acquisition of the multiple genetic alterations necessary for complete malignant transformation. In order to study the role of abnormal DNA repair in the progression of malignant melanoma, we defined the chromosomal stability of three malignant melanoma cell lines (karyotype, DNA index, and micronucleus formation) and measured their ability to repair different kinds of DNA damage (pyrimidine dimers, oxidative DNA lesions, and DNA double strand breaks) using plasmid vectors. The chromosomal stability of the three melanoma cell lines LIBR, M1 and MeWo was characterized as being high, intermediate and low. Using UVB-irradiated plasmids pRSVcat, transfected into these three cell lines by electroporation, we found no significant differences in host cell reactivation of the plasmid's cat gene, indicating identical processing of pyrimidine dimers. Using singlet oxygen-treated plasmids pRSVcat (containing oxidative DNA damage), we found a slightly reduced host cell reactivation in M1 and MeWo: e.g. with 10 minutes illumination of the photosensitizer methylene blue (which generates singlet oxygen) the host cell reactivation was 100 % in LIBR, 56.5 ± 18.3 % in M1 (n.s.), and 27.8 ± 4.4 % in MeWo ($p < 0.01$). Using linearized shuttle vector plasmids pZ189, we found a very good ability of LIBR cells to ligate DNA ends (87.3 ± 19.9 %), reflecting proficient repair of DNA double strand breaks. Ligation ability was slightly reduced in M1 (54.1 ± 11.8 %, n.s.) and significantly reduced in MeWo (20.5 ± 3.4 %, $p < 0.01$). Mutation analysis of religated plasmids showed a normal mutational pattern with a preponderance of simple deletions with LIBR and M1, and of insertions and more complex rearrangements with MeWo. Our results indicate that in the three melanoma cell lines, an increase of chromosomal instability is accompanied by a slight decrease in the ability to repair oxidative DNA damage and a pronounced impairment in the ability to repair DNA double strand breaks. We conclude that an impaired ligation of DNA double strand breaks might constitute a molecular mechanism leading to chromosomal instability during melanoma progression.

OVEREXPRESSION OF THE E. coli aidB GENE RESULTS IN INCREASED MNNG RESISTANCE.
Michael R. Volkert, Laurel I. Hajec and Paolo Landini, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655-0122.

The E. coli aidB gene is a component of the adaptive response to alkylation damage and is induced by an ada-dependent mechanism when cells are treated with methylating agents. We have recently demonstrated that this gene encodes a homolog of the mammalian isovaleryl CoA dehydrogenase (IVD), an enzyme involved in the breakdown of leucine. We have cloned the aidB open reading frame onto the plasmid pTrc99A under the control of the IPTG inducible Trc promoter. This construction allows IPTG inducible expression of aidB and permits the examination of the phenotypic effects of aidB overexpression. These experiments suggest that aidB overexpression results in increased resistance to the mutagenic effects of N-methyl-N'-nitro-N-Nitrosoguanidine (MNNG). aidB-mediated MNNG resistance can also be seen in an ada-10::Tn10 mutant strain, indicating that aidB-dependent MNNG resistance does not require the induction of other adaptive response genes. This increased resistance appears to be specific for MNNG, since no detectable increase in resistance is seen when cells are treated with methyl nitrosourea, or methyl methanesulfonate. This suggests the aidB encoded IVD enzyme may either be involved in the metabolic inactivation of MNNG, or in repair of MNNG-specific lesions.

ANALYSIS OF MECHANISMS INVOLVED IN THE INHIBITION OF DNA REPLICATION AFTER ULTRAVIOLET LIGHT IRRADIATION BY USING A SV40 REPLICATION SYSTEM, Yi-Ching Wang and Ming-Ta Hsu, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R.O.C.
To study the mechanism by which UV light inhibits DNA replication, we examined the effects of UV_{254nm} irradiation on the replication of simian virus 40 (SV40) and SV40-based plasmid in monkey cells. The study was designed to examine what extent of replication initiation or elongation contributes to the inhibition of DNA replication after UV irradiation and to analyze the mechanism which mediates the rapid inhibition of DNA replication after UV. Kinetics analysis using two-dimensional neutral-alkaline electrophoresis technique showed that replication started to decline at 15 minutes post-irradiation at 90 J/m^2 with the concomitance onset of repair synthesis and that at one hour post-irradiation only repair synthesis was observed. When the tritiated thymidine label in SV40 replication intermediates before irradiation was chased for one hour, the majority of label was chased into mature Form I and II molecules. Furthermore, transfection technique using SV40-based plasmid showed that heavily irradiated plasmid can replicate efficiently if transfected cell was not irradiated. At 900 J/m^2, replication of irradiated plasmid at 48 hours post-irradiation was as efficient as non-irradiated plasmid. The replication product of irradiated plasmid exhibited a higher mutation frequency than that of non-irradiated control. We conclude that certain fraction of elongation proceeds on damaged templates albeit in an error-prone manner. We also observed that UV irradiation of host cell caused a dose-dependent inhibition of transfected non-irradiated plasmid. At 10 J/m^2, replication of plasmid was reduced to 38% of non-irradiated control at 24 hours post-transfection. Examination of chromatin conformation in irradiated cells by fluorescent microscopic analysis at different times after UV indicated that irradiation with 10-30 J/m^2 induced a rapid change in nuclear morphology. These data suggest that the chromatin conformation changes immediately after irradiation may mask the recognition of replication machinery to both genomic and extrachromosomal DNA and produce a rapid mechanism for inhibition of DNA replication.

T4 ENDONUCLEASE V-ENHANCED DNA REPAIR REDUCES ULTRAVIOLET RADIATION-INDUCED SUNBURN CELL FORMATION, ALTERATIONS OF LANGERHANS CELLS AND THY-1+ DENDRITIC EPIDERMAL T CELLS, AND LOCAL SUPPRESSION OF CONTACT HYPERSENSITIVITY IN MICE, Peter Wolf, Patricia Cox, Daniel B. Yarosh*, and Margaret L. Kripke, Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, and *Applied Genetics Inc., Freeport, NY, 11520
Exposure of skin to ultraviolet radiation (UVR) can lead to various biologic alterations, including inflammation (sunburn), sunburn cell formation (apoptosis), damage to cutaneous immune cells, and suppression of the induction of immune responses. Although the molecular mechanisms of these UVR-induced effects are not completely understood, DNA damage is thought to be involved. The topical application of liposomes containing T4 endonuclease V, a cylobutane pyrimidine dimer (CPD)-specific DNA repair enzyme, offers the opportunity to study the role of DNA damage in the effects of UVR on the skin. In the present study, exposure of C3H mice to 500 mJ/cm^2 of UVB radiation from FS40 sunlamps resulted in significant skin edema (inflammation), SBC formation and loss of the dendritic cell shape and a 48-67% decrease in the number of cutaneous immune cells, including ATPase+ and Ia+ Langerhans cells (LC) and Thy-1+ dendritic epidermal T cells (DETC), at 24 h after UVR exposure. The induction of local contact hypersensitivity, as assessed by sensitization of the mice three days after UVR exposure on UV-irradiated dorsal skin with 0.3% dinitrofluorobenzene (DNFB) and challenge with 0.2% DNFB five days later on the UVR-protected ears, was diminished by 80%. Whereas the topical application of T4 endonuclease V after UV irradiation negligibly affected UVR-induced skin edema, it partially protected against SBC formation and local immune suppression, and nearly completely inhibited the morphologic alterations and decrease of LC and DETC. The application of heat-inactivated control liposomes had no significant effect on any of these UVR-induced alterations. These results show that UVR-induced inflammation does not necessarily correlate with other effects of UVR. On the molecular level CPD seem to play a significant role in several effects of UVR, including SBC formation, alterations of cutaneous immune cells, and local immune suppression.

IN VITRO STUDIES OF THE ABILITY OF DNA POLYMERASE δ TO CATALYZE DNA REPAIR SYNTHESIS. Xiao Rong Zeng, Yunquan Jiang and Marietta Y.W.T. Lee, Department of Medicine, University of Miami, School of Medicine, Miami, Florida 33136.

Excision repair DNA synthesis is a complex biological process involving incision/excision of the damaged region followed by DNA synthesis and ligation. We have used an *in vitro* assay to assess the contribution of pol δ and PCNA in the repair of UV damage in model plasmids. Monoclonal antibodies were used to deplete HeLa cell nuclear extracts of pol δ and its auxiliary factor PCNA. These experiments show unequivocally that the ability of nuclear extracts to perform DNA repair synthesis is strongly dependent on pol δ and PCNA.

Using purified pol δ we are attempting to reconstitute the repair of UV damaged plasmids *in vitro*. As a first step we have examined the ability of pol δ preparations to effect DNA repair synthesis using T4 endonuclease V to provide the incision step. Surprisingly we have observed that human pol δ preparations can effect DNA repair synthesis on UV-irradiated DNA in the presence of T4 endonuclease V. The incorporation of radioactivity into UV-irradiated DNA by polymerase δ was dose dependent. The presence of PCNA, HSSB, and RFC all affect the DNA repair synthesis. In contrast, under the same experimental conditions, purified human polymerase α did not carry out such DNA repair synthesis. These findings suggest that a relatively simple system of components can be used to reconstitute an *in vitro* repair system, and also that this system is responsive to the known accessory proteins of pol δ that are involved in replicative DNA synthesis.