DNA REPAIR MECHANISMS

P. C. Hanawalt and E. C. Friedberg, Organizers

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Key Notes on DNA Repair

001 KEY NOTES ON THE FIELD OF DNA REPAIR, Paul Howard-Flanders, Yale University, New Haven, Connecticut, 06520

Some of the problems and key directions in research on DNA repair will be considered. Good progress is being made in the study of the endonucleases and other enzymes needed for the repair of DNA damaged in one strand only. In contrast, great difficulty has been experienced in approaching the problem of recombinational or postreplication repair in vitro. We have not so far succeeded in preparing useful amounts of substrate, such as DNA containing dimers and postreplication gaps, that could be used in vitro. An alternative system requiring DNA replication to make the substrate would be too complex to use. One approach to the problem stems from the observation that psoralen photocross-links in phage DNA (1) increase the frequency of genetic recombination in λ phage-prophage crosses in E. coli (λ) even in the absence of DNA replication (2). The increased recombination depends upon the damaged phage DNA being incised by the uvrA endonuclease and is dependent upon $recA^+$, but none of the other recombination genes so far tested. Psoralen cross-linked λ phage DNA injected into a wild type host cell is incised, but shows little sign of being subsequently repaired. As genetic recombination and recombinational repair are thought to depend upon cutting and joining of DNA molecules, experiments were performed to test whether psoralen-damaged λ DNA would cause other λ DNA in the same cell to be cut. In E. coli (λ) infected with labeled λ phages, the covalent circular λ DNA molecules so produced were not affected by superinfection with control λ, but were cut in response to superinfection with psoralen-damaged λ phages. This phenomenon of cutting undamaged molecules in response to damaged molecules - cutting in trans - required the incision of the psoralen-damaged DNA and the $recA^+$ host gene function. It occurred between homologous DNA, while nonhomologous DNA was untouched. Cutting in trans has also been observed in vitro using øX DNA and crude protein extracts of E. coli and is specific to homologous DNA. The most active extracts were from E. coli carrying λ $recA^+$ in which the level of the $recA^+$ protein was increased above the constitutive level. There appears to be an endonuclease activity in $recA^+$ cells that cuts intact superhelical DNA in response to interaction with incised homologous molecules (4).

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Enzymatic Recognition of DNA Damage

002 REPAIRABLE DAMAGE IN DNA, P. A. Cerutti, Dept. of Biochemistry and Molecular Biology J. H. Miller Health Center, University of Florida, Gainesville, Florida 32610.

Detrimental environmental agents play a major role in the health of cells and whole organisms. For many such agents chromosomal DNA represents the most sensitive and crucial target. The list of DNA damaging agents is long and the list of DNA lesions even longer since most agents induce more than one type of lesion. However, cells possess a limited number of modes to process and express DNA damage. This poses the following central questions. How does the structure of a specific class of lesions determine their repairability, i.e. the mode(s) and efficiency of lesion processing? How does the repairability determine the biological effects, i.e. lethality, mutagenicity, transformation and degeneration?

A classification of DNA damaging agents, e.g. into physical and chemical agents, is straightforward but its usefulness is limited since most damaging agents induce a wide spectrum of lesions. Physical and many chemical agents may in part induce structurally identical lesions, e.g. by indirect action via the intermediacy of active oxygen radical species. Classifications on the basis of the effect of a particular agent on DNA conformation, the patch-size induced by prereplication excision repair, the extent of unscheduled DNA synthesis or repair replication in repair-proficient and -deficient cells, etc. are of limited value. The application of these or similar criteria to individual lesions is expected to be more informative.

A useful classification of DNA lesions may best be developed from experimental data rather than theoretical considerations. The following properties of prototype lesions should be measured in standard cell systems (1) Repairability by error-free prereplication excision repair: Excision capacity under saturating and non-saturating conditions. (2) Effect on de novo DNA (and RNA) synthesis: Instructive lesions (normally coding or miscoding); Non-instructive lesions (causing a temporary or permanent block to DNA-replication and/or-transcription) (3) Regulatory activity: Inducing lesions (primary or secondary lesions inducing or modulating repair pathways); Non-inducing lesions. The structural properties of DNA lesions which determine these aspects of lesion processing are essentially unknown. The final goal remains, of course, the elucidation of the relationship of these structural and functional properties of prototype lesions to the major biological endpoints, i.e. lethality, mutagenicity, transformation and degeneration.

003 BASE EXCISION REPAIR, Errol C. Friedberg, Corrie Anderson, Thomas Bonura, Richard Cone and Rhona Simmons, Laboratory of Experimental Oncology, Department of Pathology, Stanford, California 94305.

The phrase "base excision repair" is used to define a repair mode in which damaged bases in DNA are excised as the free base. This event is apparently the initial step in the base excision repair pathway and is catalyzed by a specific DNA N-glycosylase (DNA-nucleosidase) that catalyzes hydrolysis of the bond linking the base to the deoxyribose moeity. At least two (and possibly three) different DNA glycosylases have been identified in extracts of prokaryote and eukaryote cells. One enzyme (endonuclease II of E. coli) is purported by Goldthwait and his colleagues[1] to catalyze the excision of 3-methyladenine and O^6-methylguanine from alkylated DNA and of arylpurines from arylated DNA. This enzyme is believed to have an associated apurinic endonuclease activity, hence its designation as endonuclease II. Lindahl and his colleagues[2] have described a 3-methyladenine DNA glycosylase that does not catalyze the excision of O^6-methylguanine from alkylated DNA and which has no detectable associated endonuclease activity. The relationship between these two enzymes remains to be determined. Uracil DNA glycosylase catalyzes the selective excision of uracil from DNA containing deoxyuridine. This enzyme is present in extracts of E. coli, B. subtilis and human cells. The activity is present both in nuclear and mitochondrial fractions of human KB cells, but we have been unable to detect this enzyme activity in extracts of drosophila cells grown in culture. Mutants of E. coli defective in uracil DNA glycosylase (designated ung^-) are abnormally sensitive to treatment with nitrous acid or sodium bisulfite, both of which promote the deamination of cytosine to uracil. This observation supports the postulated role of this enzyme in the excision repair of uracil from DNA in living cells.

Following base excision, the sites of base loss (purines or pyrimidines) are attacked by a specific type of enzyme termed apurinic endonuclease. Currently, five such activities have been described in extracts of E. coli (endonuclease II → VI), but it is not certain that all of these are distinct enzymes. Endonucleolytic incision at sites of base loss is presumably followed by exonucleolytic degradation resulting in excision of the deoxyribose-phosphate residue. Repair synthesis and DNA rejoining may then complete base excision repair as in the conventional model of nucleotide excision repair.

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004 NUCLEOTIDE EXCISION REPAIR - Lawrence Grossman, Department of Biochemistry, Johns Hopkins University, Baltimore, Maryland 21205.

The pyrimidine dimer specific endonucleases (Py<>Py correndonucleases) isolated from Micrococcus luteus and Escherichia coli act 5'-to T<>T, C<>T, or C<>C cyclobutane dimers on the damaged strand generating 3'-OH and 5'-P groups. Two such endonucleases are resolvable by phosphocellulose and DNA cellulose chromatography as well as by isoelectric focusing. These uniquely different proteins are required to confer UV resistance to bacterial cells. Py<>Py correndonuclease I and II, identifiable as the uvrA and uvrB gene products respectively act in uniquely different regions of lightly UV irradiated (10-15 J/m^2) DNA. The sequence specificity of the resolved correndonucleases is being precisely determined with an isolated region of the lac operon containing the RNA polymerase (RNAP) protected site (UV 5) and the repressor binding site. The potential of this approach for determining correndonuclease and restriction endonuclease specificity, "micro" clustering of T<>T and for C<>T + C<>C formation, effects of dimerization on RNAP and repressor binding and the protection by these proteins against UV damage at these sites will be presented.

The role of bacterial and mammalian correxonucleases in the excision process as well as the facilitative effects of DNA binding protein on DNA polymerase I excision will be discussed.

005 ENZYMATIC PHOTOREACTIVATION OF DNA, Betsy M. Sutherland, Department of Biology, Brookhaven National Laboratory, Upton, NY 11973

The distinguishing hallmarks of photoreactivation of DNA are the enzyme-mediated, light-dependent monomerization of pyrimidine dimers resulting in repair of the DNA and restoration of its biological integrity. Photoreactivation is differentiated from sensitized dimer monomerization by small molecules by the participation of the protein macromolecule and from dimer monomerization by tryptophan-containing peptides and proteins by their wavelength dependence. The photoreactivating enzyme shows three levels of specificty: first, for the length and kind of nucleic acid; second, for cis-syn cyclobutyl pyrimidine dimers; and third, for photoreactivating photons of wavelength 300-600 nm. Although all photoreactivating enzymes characterized to date show this specificity in function, current reports indicate possible heterogeniety in structure. Most PR enzymes [E. coli, T. domestica (silverfish), H. sapiens, S. griseus] are composed of a single polypeptide chain with a molecular weight of 35,000-40,000 d. However, the yeast enzyme is reported to be composed of two dissimilar subunits with molecular weights 60,000 and 85,000. In addition to the apoprotein portion photoreactivating enzyme holoenzymes contain a smaller molecular weight, non-protein cofactor which is essential for enzyme activity. In the case of the S. griseus enzyme, Eker reports that the cofactor absorbs light in the spectral region of action of the enzyme, and may thus be the chromophore responsible for absorption of photoreactivating light. For the E. coli, yeast and silverfish enzymes, however, the cofactor absorption is mainly at wavelengths less than 300 nm, and thus cannot mediation photoreactivation absorption in the range 300-600 nm. In the case of the E. coli enzyme, the photoreactivating absorption may well result from a new absorption band in the region 300-500 nm which appears when active enzyme binds to dimer-containing DNA.

The specificity of the photoreactivating enzyme for pyrimidine dimers offers great potential for use as an analytical tool: if potential biological damage is reversible by a true photoenzymatic reaction, pyrimidine dimers were important in production of the biological damage. Correlation of a light-mediated recovery event with enzymatic photoreactivation is essential before such a test can be applied. Three major approaches to such a correlation in a biological system are: 1. isolation and characterization of a photoreactivating enzyme from the cells; 2. characterization of the light-dependence and action spectrum of the recovery event in the cells; and 3. correlation of biological recovery and enzyme action (e.g. enzyme levels and extent of photoreactivation; action spectra). Such correlation of biology and biochemistry will provide powerful approaches for resolving the nature of chemical alteration leading to biological damage in complex biological systems, especially at damage levels too low to be analyzed by usual means. [Research supported in part by grants from Nat'l Cancer Inst. (CA14005), American Cancer Soc. (NP154B) and Research Career Develop. Award (CA-00009)].

Distribution and Quantitation of Damage in DNA

006 MEASURING THE PYRIMIDINE DIMER CONTENT OF UV IRRADIATED DNA WITH T4 ENDONUCLEASE V, A.K. Ganesan, E.C. Friedberg and P.C. Seawell, Department of Biological Sciences and Department of Pathology, Stanford University, Stanford, CA 94305.

The most sensitive methods currently available for detecting and measuring DNA damage employ specific endonucleases. The accuracy of this type of assay depends upon knowing precisely what lesions are recognized by the endonuclease used and how efficiently it incises DNA at the site of a lesion. Endonuclease V of bacteriophage T4 recognizes pyrimidine dimers, and is regularly used to estimate the dimer content of UV irradiated DNA in vitro and in permeabilized bacterial and cultured mammalian cells. We have used two methods to determine the efficiency with which this enzyme nicks DNA at pyrimidine dimers.1) Linear DNA from bacteriophage lambda was irradiated with UV (254 nm), treated with T4 endonuclease V and analysed by sedimentation velocity in alkaline sucrose gradients. The frequency of nicking by the endonuclease was calculated from the number average molecular weight (M_n). Results obtained with this method indicated an average of 0.7 nicks for each thymine converted into a pyrimidine dimer (determined by two dimensional thin-layer chromatography), or approximately 1 nick per pyrimidine dimer. 2) Superhelical DNA from colE1 and SV40 was irradiated with UV, treated with T4 endonuclease V and analysed by electrophoresis in agarose gels. The frequency of nicking was calculated from the proportion of unnicked molecules assuming a Poisson distribution of pyrimidine dimers. Results obtained with this method indicated an average yield of 1.3 nicks for each thymine converted into a dimer, or approximately 2 nicks per pyrimidine dimer. The apparent discrepancy between the two methods does not appear to be due to an artifact of agarose gel electrophoresis because similar results were obtained when superhelical colE1 DNA was analysed by sedimentation velocity in alkaline sucrose gradients or by sedimentation equilibrium in propidium iodide-CsCl gradients. Neither can the discrepancy be attributed to the conformation of the DNA at the time of irradiation, since linear colE1 DNA analysed by sedimentation velocity showed the same profile whether it had been irradiated in the linear form or in the superhelical form. Experiments are currently in progress to determine whether the endonuclease shows different efficiencies of nicking dimers in linear and superhelical DNA.

007 THE GENERATION OF CHAIN BREAKS AND ALKALI-LABILE BONDS UPON PHOTOLYSIS OF DNA CONTAINING IODINATED CYTOSINE RESIDUES, R. O. Rahn and R. S. Stafford, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

DNA containing ^{125}I-labeled cytosine was prepared by the Commerford method (1). This method preferentially iodinates cytosine, at the 5 position, in single-stranded DNA. The loss of iodine following either 254 nm or 313 nm irradiation was measured by precipitating the DNA on filter paper discs and then washing off the unbound iodine. A fluence of 3×10^3 J/m^2 at 254 nm and 3×10^4 J/m^2 at 313 nm removes ~20% of the iodine. The rate of loss is not appreciably affected by the presence of either hydrogen donors or oxygen. It is assumed that the loss of iodine results in the formation of cytosine. This assumption is based on absorbance studies done with iodinated poly-C whose absorbance spectrum changes to that of poly-C upon photolysis at 313 nm. Apparently, hydrogen is abstracted from a neighboring sugar since chain breaks and alkali-labile bonds also occur in irradiated iodine-labeled DNA. Sedimentation studies in alkaline sucrose gradients show that the number of breaks is one per six iodines lost. In neutral gradients half as many breaks are found, indicating that 50% of the breaks found in alkali are alkali-labile bonds. The number of breaks is approximately 4-fold less in the presence of either ethanol or cysteamine, which are good hydrogen donors. Breaks were also reduced 2-fold in renatured DNA with no concomitant change in the loss of iodine. A mechanism for these results will be presented and discussed in connection with the current model for the photolysis of DNA containing bromodeoxyuridine. (Research sponsored by the Department of Energy under contract with the Union Carbide Corporation.)

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008 ANALYSIS OF DNA LESIONS DETERMINED BY SUSCEPTIBILITY TO ALKALI, APURINIC ENDONUCLEASE OR OTHER DNA DAMAGE SPECIFIC ENZYMES FROM HUMAN CELLS, Thomas P. Brent, Department of Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, George W. Teebor, Department of Pathology, New York University Medical Center, New York, N.Y. 10016, and Nahum J. Duker, Department of Pathology and Fels Research Institute, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140.

A variety of lesions in damaged DNA may be analyzed by determining the susceptibility of such DNA to the strand-breaking action of either alkali, or DNA damage specific enzymes. DNA strand scission has been measured by monitoring the conversion of Form I PM2 DNA to Form II using alkaline or neutral pH velocity sedimentation gradients, or by a filter binding assay.[1,2] In earlier studies DNA damaged by ultraviolet light, ionizing radiation or heat at pH 5, was studied, while more recently DNA treated with alkylating agents has been examined. Each mode of damage introduces into DNA a certain number of apurinic or apyrimidinic (AP) sites that are detectable immediately following the treatment. The numbers of these AP sites determined by strand scission induced by purified human lymphoblast AP endonuclease corresponds closely, in all cases, with the number of strand breaks induced by a brief alkaline treatment (20 min at pH 12.2 at 37°C).[2] A more extensive alkaline treatment (4 hrs at pH 12.8 with 1.0 M glycine at 25°C)[3] although giving values that correspond well with enzymatically determined AP sites in acid heat depurinated DNA, results in inflated values for DNA alkylated with methylmethanesulfonate (MMS). It is well known that depurination of alkylated bases occurs at significant rates at physiological pH and temperature, however it has been reported that under alkaline conditions such bases are stabilized.[4] Our recent studies, however, show that significant depurination occurs during protracted alkaline treatment[3] of methylated DNA, leading not only to the over-estimation of AP sites, but also to the loss of a population of alkylated bases that is initially present. Estimation of initial AP site numbers is therefore best made utilizing brief alkaline treatment or purified AP-endonuclease.[2] This approach has led to the enzymatic detection of lesions in alkylated DNA other than AP sites. These lesions are converted to strand breaks by the combined action of a putative N-glycosidase activity, partially purified from human lymphoblasts, together with either AP endonuclease or alkaline treatment.[5] They represent a minority of the total alkylated bases in MMS-treated DNA, suggesting they are not 7-methylguanine. DNA treated with dimethyl sulfate or methylnitrosourea also contains such enzyme sensitive sites, while nitrogen mustard treated DNA does not. Identification of these enzyme susceptible alkylated bases is being actively pursued.

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009 QUANTITATION OF DNA DAMAGES INDUCED BY X-RAYS, Susan S. Wallace, Paul R. Armel, and Harold L. Katcher, Department of Microbiology, New York Medical College, Valhalla, NY 10595.

The ability to accurately determine by sedimentation velocity, the conversion of PM2 Type I closed circular supercoiled DNA to the open circular Type II form, has provided us with a very sensitive assay for strand breaks in x-irradiated DNA. Measurable strand breaks include those directly produced by x-rays, damages converted to strand breaks upon exposure to alkali, and damages converted to strand breaks upon incubation with repair endonucleases. The production of both strand breaks and alkali-labile lesions is greatly reduced when PM2 DNA is irradiated in the presence of the hydroxyl radical scavanger, potassium iodide. Thus it may be inferred, that when PM2 DNA is irradiated in air, in dilute aqueous solution, that both strand breaks and alkali-labile lesions are produced by hydroxyl radicals. Also, when alkali-labile lesions are examined in DNA x-irradiated in the presence of potassium iodide, two classes can be distinguished. One class, which converts immediately to strand breaks upon exposure to alkali, is also demonstrable when DNA is irradiated in the absence of potassium iodide. The second class, which is not measurable when DNA is irradiated in the absence of potassium iodide, requires 4 hours of incubation in alkali before completely converting to strand breaks. When x-irradiated PM2 DNA is incubated with partially purified Escherichia coli x-ray endonuclease, additional breaks are introduced into the DNA, presumably adjacent to damaged base residues. The number of damages per single strand break recognized by the E. coli x-ray endonuclease in PM2 DNA x-irradiated in the presence of potassium iodide, include 0.6 fast-converting alkali-labile lesions, 1.1 slow-converting alkali-labile lesions, and 2.7 non-alkali-labile base-damaged residues. The latter two classes of damages cannot be measured in PM2 DNA x-irradiated in the absence of potassium iodide. When PM2 DNA is x-irradiated in potassium iodide plus potassium nitrate, a scavanger of the aqueous electron, the non-alkali-labile base-damaged residues recognized by the E. coli x-ray endonuclease are almost completely eliminated. E. coli x-ray endonuclease, purified several hundred fold still exhibits the broad specificity defined above. The substrate specificity of enzyme preparations purified several thousand fold is currently being examined. Some of the base damaged residues recognized by the E. coli x-ray endonuclease in x-irradiated PM2 DNA could be of the 5,6 dihydroxy-dihydrothymine type, since osmium tetroxide-treated PM2 DNA containing these damages, is a substrate for the enzyme. An apurinic endonuclease, partially purified from Saccharomyces cerevisiae, has also been used to probe for apurinic/apyrimidinic damages in x-irradiated PM2 DNA. The majority of the alkali-labile lesions present in this DNA, both of the slow and fast-converting types, are recognized by the yeast apurinic enzyme.

Mechanism and Diversity of Photoreactivation

010 PHOTOSENSITIZED SPLITTING OF THYMINE DIMERS IN DNA BY PEPTIDES AND PROTEINS CONTAINING TRYPTOPHAN. Helene, C. Centre de Biophysique Moleculaire 45045 Orleans - FRANCE
Thymine dimers formed in DNA by UV irradiation can be split by irradiation at 300 nm in the presence of photosensitizers containing an indole ring: Serotonin and 5-methoxytryptamine, an oligopeptide containing a tryptophyl residue (Lys-Trp-Lys), a protein which exhibits a specificity for single stranded nucleic acids (protein coded for by gene 32 of phage T4). The mechanism of this photosensitized reaction has been investigated using different methods: fluorescence at room and at low temperature, flash photolysis, effect of electron scavengers, ionic strength dependence. In order to act as a photosensitizer, the indole-containing compound must be bound to UV irradiated DNA and stacked with nucleic acid bases and thymine dimers. Dissociation of the complex inhibits the reaction. The splitting arises as a consequence of an electron transfer from the excited indole ring to the dimer. In all cases, the splitting of thymine dimers regenerates intact thymine bases.

011 PHOTOREACTIVATING ENZYME FROM E. COLI: MECHANISM OF ACTION*, John Clark Sutherland, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

Purified photoreactivating enzyme (PRE) from E. coli has an absorption maximum at 257 nm, but no well defined absorption band for $\lambda > 300$ nm, the wavelength region which is required for photoreactivation. When PRE is mixed with ultraviolet irradiated DNA (uvDNA), there is an increase in absorption for $\lambda > 295$ nm and a decrease for $\lambda < 295$ nm compared to the sum of the absorptions of the separate components as monitored by absorption difference spectroscopy (ADS) (Wun, et al., 1977). These absorbance changes are not observed if PRE is mixed with unirradiated DNA nor is the increase for $\lambda > 295$ nm due to an increase in light scattering (ibid.). Irradiated of the PRE:uvDNA mixture with 365 nm radiation reverses the hyperchromicity at 350 nm and the hypochromicity at 257 nm with identical first order kinetics. The slope of the ADS reversal curve yields $\phi\varepsilon$, the product of the molar extinction coefficient of the complex at the wavelength of irradiation times the quantum yield for absorption reversal. Measurement of $\phi\varepsilon$ for a series of irradiation wavelengths leads to an action spectrum which resembles both the ADS (for $\lambda > 300$ nm) and the action spectrum for photoreactivation. With poly dA:dT qualitatively similar results were obtained but the absolute value of $\phi\varepsilon$ was lower. These results suggest that the long sought "chromophore" is, in fact, a molecular complex which exists only when E. coli PRE interacts with dimer-dontaining DNA and lead us to ask what are the moieties of the dimer and the PRE which interact to produce the new absorption band. A preliminary step in identifying the interacting moieties of the dimer is determining whether the PRE approaches the dimer from the major or the minor groove of the DNA. If the PRE approaches from the major groove it can interact with the cyclobutyl ring while if it approaches from the minor groove, it might interact with the two carbonyl groups at the 2 positions of the pyrimidine ring which are common features of all photoreactable dimers (Sutherland, 1977). Recent data (Duval and Sutherland, in preparation) show that the basic peptide antibiotic netropsin, which is known to bind in the minor groove, inhibits binding of the PRE to uvDNA. This result supports the hypothesis that the carbonyl at position 2 of the pyrimidine ring may be key moieties in the interaction between the E. coli PRE and uvDNA.

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012 CLONING THE GENE FOR ESCHERICHIA COLI PHOTOREACTIVATING ENZYME, Claud S. Rupert and Aziz Sancar, Programs in Biology, University of Texas at Dallas, Richardson, TX 75080.

The gene phr for photoreactivating enzyme (deoxyribodipyrimidine photolyase, EC 4.1.99.3) of E. coli has been inserted into the coli plasmid pMB9 by in vitro recombination. The fact that cultures can only be enriched in, and not absolutely selected for cells carrying this gene in active form, complicates isolation of such recombinant DNA molecules. Efforts to use an F' plasmid, carrying a relatively small region of the coli chromosome near phr, as the gene donor to increase the initial number of recombinant molecules, were frustrated by finding the gene is not located between gal and att λ as previously believed (1). Conjugation with several Hfr strains, deletion mapping, F' transfer and transduction data all showed it lies counterclockwise from this location, near 15.7 min, in agreement with independent work of Youngs and Smith (private communication). This is outside the regions carried by the F-primes readily available to us, while F153, which does carry it, is too large for efficient separation from cellular DNA fragments. Consequently a technique for enriching whatever recombinant molecules are present was developed from the photolyase membrane-binding assay of Madden et al. (2). DNA from coli strain ND24(F153) was UV irradiated either before or after digestion with EcoRI restriction endonuclease, and mixed with EcoRI-digested, unirradiated pMB9 plasmid DNA. After annealing and ligating, purified yeast photoreactivating enzyme was added, and the mixture filtered through a nitrocellulose membrane. The enzyme attached itself to regions of the DNA carrying photoreactivable UV damage and bound the molecules carrying such regions (i.e., those having coli DNA in them) to the membrane. The majority of uncombined pMB9 molecules passed through. The membrane was then transferred to a vial, exposed to light to repair the UV damage and release the binding, and competent uvrA recA phr cells were added for E. coli transformation (3). The cells were grown in tetracyclene medium to select those which had incorporated a pMB9 plasmid (imparting tetracyclene resistance). From the resulting cultures PHR+ cells could be isolated, after enrichment by UV irradiation and photoreactivation, without the interference from back mutations in uvrA or recA experienced without enrichment. These cells carry a recombinant plasmid which, when isolated, will transform PHR- cells to PHR+. Increased numbers of photoreactivating enzyme molecules accompany the increased numbers of gene copies.

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013 SOME PROPERTIES OF A DNA PHOTOREACTIVATING ENZYME FROM STREPTOMYCES GRISEUS

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A factor has been isolated from vegetative Streptomyces griseus cells which accomplished photoreactivation in a H. influenzae transforming system. This factor exhibits true photoreactivating activity i.e. splitting of pyrimidine dimers in UV-irradiated DNA with the constituent pyrimidines as reactionproduct. It needs blue light as a physical cofactor. From its behaviour against proteases and heat-inactivation it was concluded that this factor has a proteineous character. Therefore it can be named as a photoreactivating enzyme (PRE).

Some properties are:			
	mol. weight:	43000	(gelfiltration)
	number of protein chains:	1	(SDS-electrophoresis)
	optimum pH:	7.0	
	optimum ionic strength:	0.04	
	isoelectric point:	4.7	(isoelectric focussing)

These properties are not very different from properties found for PRE's obtained from other sources.
We believe that this PRE contains an intrinsic chromophore, adducing the following evidence:

- purified PRE has, besides protein absorption, an absorption band in the visible region, max. 445 sh. 425 nm.
- the action spectrum for photoreactivation resembles the absorption spectrum very well. Both spectra coincide with the action spectrum for PR in vivo of conidia of S. griseus (1).
- denaturation of PRE causing detachment of the chromophore is accompanied by a sharp increase of fluorescence intensity. Stabilisation of PRE by addition of UV-DNA prevents the increase of fluorescence intensity.
- in 5 different chromatographic systems co-chromatography of biological activity and chromophore fluorescence has been observed.

The presence of a chromophoric group in PRE from S. griseus is in contrast with the findings for PRE's from other sources which seem to lack such a group. However, no absolute proof can be given that our PRE really carries a chromophore.
From fluorescence and phosphorescence spectra an estimate can be made for the energy levels of excited singlet and triplet state of the chromophore. Comparison with the corresponding assumed levels of thymine dimer seems to rule out physical energy transfer either at singlet or triplet level. So another mechanism of energy transfer, e.g. via exciplex formation, has to be considered.

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014 LOW MOLECULAR WEIGHT SUBSTANCES IN CELLS THAT ENHANCE DNA-PHOTOLYASE ACTIVITY,

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One can enhance the activity of DNA photolyase frequently by assaying the enzyme in the presence of reducing agents such as 2-mercaptoethanol or glutathione. Further enhancement of activity results when either cell-free extracts of Baker's yeast or the blue-green alga, A. nidulans, is added. Such extracts are prepared by disrupting the cells, centrifuging, holding the supernatent at pH 3.0 for 3 hrs at room temperature, neutralizing, lyophilizing, and dissolving the dry powder in buffer. The substances that enhance the activity are referred to as activators, and they can be partially purified by paper chromatography and paper electrophoresis. They are obtained from both PhR^+ and PhR^- yeast cells.

Further purification of the activators is achieved by Dowex 50 chromatography and gel filtration on Sephadex G-25. One activator purified this way had a molecular weight of 450 by gel filtration and a maximum at 248-249 nm in its absorption spectrum. This peak was not reflected in the fluorescent spectrum of the material: excitation maxima at 280 and 358 nm, emission maxima at 350 and 440 nm. The substance gave a typical ninhydrin stain. These data indicate that small molecular weight substances, possibly peptides, can mediate photoreactivation in vivo. Hélène and coworkers found that the tripeptide, lysyl-tryptophyl-lysine mimics the action of DNA-photolyase when illuminated with tryptophan absorbing wavelengths. Cleaver and coworkers[2] raise the possibility that such peptides may photosensitize dimer cleavage in mammalian cells.

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Enzymology of Nucleotide Excision Repair

015 DIMER SPECIFIC ENDONUCLEASE FROM CALF THYMUS, Evelyn Waldstein and Shoshana Peller, Department of Biochemistry, University of Tel-Aviv, Tel-Aviv, Israel.

A dimer specific endonucleolytic activity from calf thymus is partially purified and characterized. The enzyme introduces single strand nicks into covalently closed circular phage PM2 DNA containing not more than several dimers per molecule. The enzyme is Mg^{2+} dependent, does not bind to DEAE Sephadex and copurifies with a nonspecific endonuclease which has a molecular weight of 80 000. It can be recovered in thymuses, kept frozen for at least two weeks. Both in crude extracts and column fractions its activity is lost on the third day after homogenization of the tissue.

016 PROPERTIES OF A UV-ENDONUCLEASE CODED FOR BY THE uvr-GENES IN E. coli. Erling Seeberg, Norw. Def. Res. Estab., Div. Toxicol., Kjeller, Norw.

The products of the $uvrA^+$, $uvrB^+$ and $uvrC^+$ genes in Escherichia coli has been partially purified by means of an in vitro complementation assay allowing each one of these products to be detected separately. The $uvrB^+$ and $uvrC^+$ gene products co-chromatographed on DEAE-cellulose and were completely resolved from the $uvrA^+$ gene product which was further purified by phosphocellulose chromatography of the nonbinding protein fraction from the DEAE-cellulose. Neither the $uvrA^+$ nor the $uvrB^+/uvrC^+$ gene products show appreciable endonuclease activity on UV-irradiated DNA when tested separately. However, these factors complement to yield an endonuclease activity specific for UV-irradiated DNA. This activity is ATP-dependent and neither GTP, ADP, nor AMP can substitute for ATP in the endonuclease reaction. The amount of breaks induced by the reconstituted endonuclease into DNA irradiated with low UV-doses is essentially the same as that induced by purified pyrimidine dimer specific endonuclease from Micrococcus luteus. Gel filtration experiments indicate that the $uvrA^+$ product has a molecular weight of about 100,000. The $uvrB^+$ product has an apparent molecular weight of about 70,000, but it is presently unclear wether this represents the size of $uvrB^+$ alone or a complex between the $uvrB^+$ and $uvrC^+$products.

017 PARTIAL PURIFICATION, ISOLATION AND CHARACTERIZATION OF THREE NUCLEASE ACTIVITIES FROM HUMAN KB CELLS WHICH CAN EXCISE THYMINE-CONTAINING PYRIMIDINE DIMERS. Kem. H. Cook and Errol C. Friedberg, Laboratory of Experimental Oncology, Department of Pathology, Stanford University, Stanford, CA 94305

Cytoplasmic extracts prepared from KB cells contain enzyme activity which preferentially renders thymine-containing pyrimidine dimers (dimers) acid soluble from UV irradiated E. coli DNA specifically incised 5' to the dimer site.[1] Fractionation of these extracts yields three such activities with distinct chromatographic properties on ion-exchange celluloses. These three activities have been partially purified and physically and enzymatically characterized. One activity (Activity B) is clearly distinguished by a smaller sedimentation coefficient (2.6) and an alkaline isoelectric point (9.0) from the remaining two activities (A and C) which have similar sedimentation characteristics (3.0 - 3.2) and isoelectric points (6.0). The three activities can also be distinguished by their response to sulfhydral and monovalent ion inhibition and divalent cation stimulation. Preliminary enzymatic characterization suggests that activities A and C are single-strand specific exonucleases. Activity B does not actively degrade native or denatured DNA and may be similar to DNase IV or may be a single-strand specific endonuclease.

These activities are all capable of excising thymine-containing dimers in a 5' → 3' direction. Thus there are multiple activities capable of performing the dimer excision step in human cells if dimer excision occurs in the manner of the prokaryote nucleotide excision model. There is, however, no direct evidence that these activities perform such a function in vivo.

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018 ACTION OF γ-ENDONUCLEASE ACTIVITIES IN EXTRACTS OF MICROCOCCUS LUTEUS, Ulrich Hagen, Gisela Schäfer and Peter Haas, Institut für Genetik und für Toxikologie von Spaltstoffen, Kernforschungszentrum Karlsruhe, D 7500 Karlsruhe 1, Federal Republic of Germany

Several protein fractions containing endonuclease activity against γ-irradiated DNA (γ-endonuclease) were isolated from M. luteus. The crude extract was chromatographed and purified on TEAE cellulose and subsequently on hydroxyapatite. Tests for endonuclease activity were performed by analysis of single-strand breaks in PM2-DNA or calf thymus DNA as well as by end group determination with DNA polymerase I or phosphatase. The protein fractions were tested against DNA samples, treated in several ways: γ-irradiation, ultraviolet irradiation or acid hydrolysis to obtain a limited number of apurinic sites in the DNA. In γ-irradiated DNA the frequency of single-strand breaks, alkali labile lesions and of endonuclease sensitive sites were determined. The relation of endonuclease sensitive sites to single-strand breaks amounts to about 2.5. It will be discussed in detail whether the various protein fractions, isolated in respect of activity against γ-irradiated DNA, contain also activity of an apurinic specific endonuclease, i.e. are able to split the alkali labile lesions induced by γ-irradiation or apurinic sites induced by acid hydrolysis.

019 ON THE NATURE OF THE HUMAN ENDONUCLEASE ACTIVITY DIRECTED AGAINST ULTRAVIOLET-IRRADIATED DNA, George W. Teebor and Mindy S. Goldstein, Department of Pathology, New York University Medical Center, New York, N.Y., 10016, Nahum J. Duker, Department of Pathology and Fels Research Institute, Temple University Health Sciences Center, Phila. Pa., 19140, and Thomas P. Brent, Department of Pharmacology, St. Jude Children's Research Hospital, Memphis, Tenn., 38101.

The endonuclease activity in human cells directed against UV (254 nm) irradiated DNA is only evident if the DNA is heavily irradiated (1,2). Thus, it is probable that this activity is not directed against the pyrimidine dimer. Endonucleases against non-dimer sites have been purified from E. coli (3,4) and calf thymus (5). The nature of this endonuclease-sensitive damage is not certain. It was reported that this lesion was not an apurinic site since such heavily irradiated DNA was not alkali-labile (4). We show that UV-irradiated PM2 DNA contains alkali-labile sites which are only detectable under the conditions of alkaline hydrolysis employed by Lindahl and Andersson (6). One of these sites is formed for every 100-150 pyrimidine dimers. This corresponds to the number of endonuclease-sensitive sites in UV-irradiated DNA acted on by E. coli endonuclease III (4) and calf thymus UV-endonuclease (5). If such irradiated DNA is allowed to stand at room temperature and neutral pH for several hours, alkali-labile sites appear which are now detectable under other conditions of alkaline hydrolysis suitable for the detection of apurinic sites (7). Thus, the human UV-endonuclease activity present in HeLa cells, WI-38 cells, lymphoblasts and skin fibroblasts, including those of Xeroderma pigmentosum individuals (1,2,7) is probably directed against an abnormal, unstable base in DNA. This is corroborative of the model proposed by Brent (7).

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Enzymology of Base Excision Repair

020 ENZYMES FOR THE REPAIR OF APURINIC/APYRIMIDINIC SITES IN HUMAN CELLS, Stuart Linn, W. Stephen Linsley, Urs Kuhnlein, Edward E. Penhoet, and Walter A. Deutsch, Department of Biochemistry, University of California, Berkeley, CA 94720.

Apurinic/apyrimidinic endodeoxyribonuclease has been purified from human placenta so as to be free of detectable DNA phosphatase, DNA N-glycosidase, and other DNases. The enzyme is found in several forms with molecular weights ranging from 27,000 to 31,000 and distinguishable catalytic properties. The enzyme acts only upon phosphodiester bonds that are adjacent to a deoxyribose lacking a base. Such lesions can be formed by treatment of the DNA with acid, alkylating agents, DNA N-glycosidase, X-rays, or O_sO_4. The incision is made so as to form a deoxyribose 5'-phosphate and a 3'-hydroxynucleotide.

When the DNase activity was studied in extracts from cultured human fibroblasts, it was noted that the lines of the D complementation group of xeroderma pigmentosum, XP5BE, XP6BE and XP7BE, had reduced activity and an abnormally high substrate K_m. Upon phosphocellulose chromatography it was observed that the D group extracts lacked a major enzyme species that had a relatively low substrate K_m. This species was present in extracts from normal cell lines, other xeroderma pigmentosum lines, an ataxia telangiectasia line and HeLa cells.

In addition to endonucleases we have also isolated from cultured human fibroblasts other proteins that interact with DNA apurinic lesions. The properties of these proteins suggest the existence of other, novel pathways for the repair of these lesions.

021 DNA GLYCOSYLASES OF ESCHERICHIA COLI, T. Lindahl, P. Karran and S. Riazuddin, Dept. of Med. Chem. Univ. of Gothenburg, 400 33 Gothenburg, Sweden.

DNA glycosylases (previously called N-glycosidases) cleave base-sugar bonds, but not phosphodiester bonds, in DNA. Two enzymes of this class have so far been purified and characterized from E. coli and evidence for the existence of additional DNA glycosylases will be presented. One enzyme of this type releases uracil from DNA containing deaminated cytosine residues or uracil residues misincorporated instead of thymine. A second glycosylase releases 3-methyladenine (and 3-ethyladenine) from alkylated DNA in vitro. Both these enzymes are small proteins which do not require Mg^{2+}, phosphate, or other cofactors for activity. The reaction products are free bases and DNA containing apurinic or apyrimidinic sites. The enzymes are highly specific for the base which they remove from DNA; thus, 5-methyluracil (thymine), 5-bromouracil, or deaminated purines are not released by the uracil-DNA glycosylase, and 7-methylguanine, 7-methyladenine, O^6-methylguanine, N^6-methyladenine, and arylalkylated purines are not released by the 3-methyladenine-DNA glycosylase. Recent studies in several laboratories on E. coli mutants defective in glycosylase activities indicate that these enzymes participate in DNA repair.

022 THE ENDONUCLEASE ACTIVITY OF EXONUCLEASE III AND THE REPAIR OF URACIL-CONTAINING DNA IN ESCHERICHIA COLI, B. Weiss, S.G. Rogers, and A.F. Taylor, Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

dUTPase mutants of E. coli (dut mutants) display fragmentation of nascent DNA, resulting in very short Okazaki pieces (1). They incorporate uracil transiently into their DNA, and its excision-repair causes the breakage. As a result, the dut mutants have increases in generation times, in recombinational and mutational frequencies, and in sensitivity to FdUrd; their growth is also inhibited by 2 mM uracil. Uracil is removed from DNA largely by uracil-DNA glycosylase, leaving apyrimidinic sites to be incised by endonucleases. Exonuclease III has an endonuclease activity specific for apurinic/apyrimidinic sites, representing 85-90% of such activity measured in crude extracts. Exonuclease III is vital for this repair; dut^-xth^- (exo III$^-$) double mutants display filamentous growth.

The endonuclease and exonuclease activities are functions of a monomeric protein (M_r-28,000); their relationship has been verified by co-purification, co-mutation, co-reversion, and co-transduction (2,3). One site on the enzyme probably catalyzes both types of reaction by recognizing either a missing base (endonuclease activity) or an unwound 3'-terminal nucleotide (exonuclease activity) and by cleaving on its 5' side. As predicted by this model, endonucleolytic cleavage produces base-free deoxyribose-5-phosphate end groups. Thus, when $d(A\text{-}T)_n$ containing occasional [5'-^{32}P]dUMP residues was treated with uracil-DNA glycosylase and exonuclease III, the ^{32}P could then be released into a Norit-nonadsorbable form either by a phosphomonoesterase or by NaOH.

Site of endonucleolytic cleavage by exonuclease III:

Nomenclature: We previously referred to the endonuclease activity of exonuclease III as "endonuclease II activity" (2,3) because it was originally assayed and purified by schemes used to define endonuclease II. The latter term, however, has been recently redefined (4).

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023 ENDONUCLEASES SPECIFIC FOR APURINIC SITES IN DNA, Walter G. Verly, Service de Biochimie de la Faculté des Sciences, Université de Liège, Belgique.

An endonuclease specific for apurinic sites in DNA was discovered in E.coli by Verly and Paquette (1970). The enzyme was purified to homogeneity (Verly and Rassart, 1975); it is a monomeric protein of 32,000 which needs Mg^{++} to be active and is rather thermolabile. The enzyme was named endonuclease VI (Gossard and Verly, 1977) to stop the confusion, found nearly everywhere in the literature, with endonuclease II, an enzyme active on alkylated sites. An endo VI preparation devoid of endo II activity was found to contain exonuclease III; since the preparation contained several proteins, we cannot decide if endo VI and exo III are the same enzyme (Gossard and Verly, 1976).
Partially depurinated DNA was repaired on incubation with endo VI, exo III, DNA polymerase I and the 4 dNTP, ligase and its coenzyme (Verly, Gossard and Crine, 1974). Endo VI hydrolyzes a phosphoester bond (which might not be the immediate neighbour of the base-free deoxyribose) on the 5' side of the apurinic site leaving 3'-OH and 5'-phosphate; exo III removes a few nucleotides in the 3'-5' direction leaving the apurinic site in the DNA molecule (preventing ligase from closing the nick); DNA pol I, starting from the 3'-OH, fills the gap produced by exo III, excises the apurinic site in a di- or trinucleotide, then catalyzes the translation in the 5'-3' direction of a nick which is finally closed by ligase (Gossard and Verly, 1976).
Endo VI is reponsible for 90% of the activity toward DNA apurinic sites in E.coli; the remaining activity depends on endonuclease IV which is more thermostable and does not need Mg^{++} (Ljungquist et al., 1976). We have isolated from Bacillus stearothermophilus an endonuclease specific for apurinic sites with properties rather similar to those of endo IV (Bibor and Verly). An endonuclease specific for apurinic sites has been purified from rat liver (Verly and Paquette, 1973) and also from plant embryos (Thibodeau and Verly, 1977). Endonucleases for apurinic sites were found in all rat tissues, in human fibroblasts, HeLa cells, DAB rat hepatoma (Hardy), in roots and leaves of higher plants (a chloroplastic enzyme was differentiated from the nucleo-cytoplasmic endonuclease), in fungi and algae (Thibodeau and Verly, 1976). In eukaryotic cells, the endonuclease specific for apurinic sites is localized mostly in chromatin; it is a non-histone protein which, when integrated in the chromatin structure, has little effect on exogenous depurinated DNA which suggests an orientation of the enzyme to work on chromatin DNA (Thibodeau and Verly, 1976).

DNA Repair Pathways

024 EXCISION-REPAIR PATHWAYS, Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, California 94305.

A variety of structural defects in DNA are repairable in most living systems by excision of the damaged section and resynthesis, utilizing the intact complementary strand as template. This process of excision-repair is initiated by an endonucleolytic cleavage of the damaged strand, generally followed by the coupled activities of DNA polymerase and 5'-exonuclease. The process is best understood in *Escherichia coli* in which DNA polymerase I and its associated 5'-exonuclease can efficiently remove lesions and synthesize a short repair patch. However, other polymerases and other exonucleases can also operate in excision-repair, sometimes producing longer patches and perhaps resulting in mutagenesis. Studies with permeabilized cells have aided the study of precursor and energy requirements for excision-repair; results with toluenized *E. coli* have shown that either pol II or pol III can substitute for pol I in repair replication (1). *In vivo* studies implicate pol III in the removal of pyrimidine dimers and repair resynthesis in UV irradiated bacteria (2, 3). In bacterial systems the availability of a large catalogue of repair-deficient mutant strains has aided the elucidation of repair pathways and the formulation of models. Detailed analysis of the alternative pathways for excision-repair in *E. coli* serves to guide the study of this process in mammalian systems.

It is likely that the excision repair pathways in human cells are at least as complex as those in *E. coli*. C. A. Smith in our laboratory has developed a system for studying details of excision-repair in isolated nuclei from human cells. WI38 nuclei exhibit UV dependent repair replication that is Mg^{++} dependent and stimulated by added ATP and deoxyribonucleoside triphosphates. This repair synthesis is not seen in nuclei from xeroderma pigmentosum (group A) cells. However, UV dependent repair synthesis is observed when the bacteriophage T4 endonuclease V is added to the system. Furthermore, the enzyme stimulates repair synthesis in WI38 nuclei. This enzyme is specific for DNA strands containing pyrimidine dimers (4). Added pancreatic DNase I, while producing a similar type of scission does not promote UV dependent repair synthesis. These results support the view that the incision step in the excision-repair sequence is defective in xeroderma (group A) and they demonstrate the utility of permeabilized mammalian cells for probing DNA repair with exogenous enzymes and substrates. Additional classes of repair deficient cell lines are needed to help clarify the excision-repair pathways in human cells.

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025 PATHWAYS OF RECOMBINATION REPAIR IN E. COLI K12, Alvin J.Clark and Michael R.Volkert
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The first evidence that recombination might play a role in the repair of damage to DNA came from studies of recA, recB and recC mutants defective in their ability to produce recombinants in standard crosses (1,2,3). Since these mutants were more sensitive to, for example, UV irradiation than wild type (1,2,4) it seemed likely that the pathways of recovery which had been blocked were pathways of repair. Then it was discovered that in uvrA or uvrB mutants repair of gaps in newly replicated DNA (5,6) was accompanied by genetic exchange (7) and that this repair was blocked by a recA mutation (6). Subsequent work shows that this repair is also blocked by recF mutations (8) and is slowed by a recL mutation (9). Although these results might be interpreted to indicate that all repair occurring in uvrA or uvrB mutants is recombinational, other results indicate that recA and recF have regulatory functions and that enzymes of nonrecombinational pathways may be under their control. These results stem partly from studies of inducible cleavage of lambda phage repressor (10) and partly from studies of inducible reactivation of phages lambda and ϕX174 (or S13) damaged by UV irradiation (11,12,13). A major question which requires an answer in theory at this point is how many pathways need to be inferred from all of these studies of the recovery and DNA metabolism in UV-irradiated mutants. An additional question is how many of these pathways involve genetic exchange and how many involve enzymes regulated coordinately with recombination enzymes.

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026 MAKING AND CORRECTING ERRORS IN DNA SYNTHESIS : IN VITRO AND SEMI-IN VIVO STUDIES OF MUTAGENESIS, Miroslav Radman, Serge Boiteux, Oliver Doubleday, Giuseppe Villani & Silvio Spadari* Department of Molecular Biology, Université Libre de Bruxelles, B 1640 Rhode-St-Genèse, Belgium.

Mutagenesis was studied by construction of presumed molecular intermediates in spontaneous and ultraviolet light-induced mutagenesis using λ and ØX174 phage DNAs and homopolymers. Phage DNAs were used for both in vitro reactions with a variety of extracts and purified DNA polymerases, and in vivo assays by transfection of E.coli. Polypyrimidine homopolymers and terminally mismatched homopolymers were used to estimate the fidelity and the 3' to 5' exonuclease ("proof-reading") activity of DNA polymerases. Mutagenesis was found to occur through two principle pathways. (i) Direct Mutagenesis involves incorporation, usually from triphosphate precursors, of the modified mispairing DNA bases e.g. tautomers and isomers of normal bases, 2-aminopurine, 5-bromouracil, deaminated cytosine, O-6-alkyl-guanine, and is greatly suppressed by DNA methylation-instructed, mismatch excision repair which eliminates errors from the newly-synthesised (unmethylated) DNA. The absence of this repair accounts for the mutator properties of E.coli uvrE (repair-deficient) and dam (strand discrimination deficient) mutants. (ii) Indirect mutagenesis is caused by non-pairing lesions, such as pyrimidine dimers, interstrand cross-links and bulky adducts (e.g. aflatoxin B1, benzo-(a)-pyrene) in parental DNA. These lesions arrest DNA synthesis and induce SOS-repair, which in E.coli is controlled by the recA, lex A and umuC genes. An extensive study of E.coli and mammalian DNA polymerases which vary greatly in their respective base selection and 3' to 5' exonuclease activities suggests that the latter, "proof-reading", activity blocks DNA synthesis at pyrimidine dimers by causing polymerase "idling" (abortive turnover of triphosphates to monophosphates). It is likely that this proof-reading activity is suppressed as of consequence of SOS-induction. Evidence will also be presented for the existence of a functional 3' to 5' exonuclease, proof-reading, activity in mammalian cells.

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027 REPAIR PATHWAYS IN MAMMALIAN SYSTEMS, James E. Cleaver, The University of California, San Francisco, Laboratory of Radiobiology, San Francisco, CA 94143

Repair pathways are commonly discussed as a triumvirate: photoreactivation (PR), excision repair and postreplication repair. My current view is that repair in cultured mammalian somatic cells consists of a multibranched set of pathways under the one title of excision repair. Different branches are involved in nucleotide, base, crosslink and strand break repair, but there may be common initial steps involving factors mediating the access of repair enzymes to damaged sites in chromatin and common final steps involving polymerases and ligases. Some features of chromatin structure, such as nucleosomes, and higher orders of structure and control, may exercise a dominant influence on the initial steps, rates and sites of repair, patch sizes etc., and some of the defects in xeroderma pigmentosum may involve defective access to damaged sites. PR appears to be functionally vestigial despite the presence of a protein with PR activity in mammalian cells, but the role of medium-dependent factors and DNA damage caused by PR light remain to be evaluated. Post-replication repair should perhaps not be considered as a discrete repair process, but rather one aspect of complex perturbations induced by damage and by repairing sites when cells undergo semiconservative DNA replication. The perturbations seem to be a consequence of damaged sites that block DNA chain growth, delayed replication around damaged sites, and excision repair breaks which block replicon initiation. The observed perturbations may therefore be the result of a coupling of excision repair events with the control of replicon synthesis; and alterations in either could change the response. Thus supposed changes in postreplication repair seen in XP and XP variant cells may be the consequence of excision repair defects; changes in control of replicon synthesis in other diseases (Bloom's) may alter radiation response even though excision repair is normal. DNA double strand exchanges have been observed at the chromosome level (sister chromatid exchanges) but single strand recombination has not been clearly demonstrated and the supposed exchange of pyrimidine dimers may actually be artefacts of end addition during recovery of DNA synthesis. Inducible, error-prone repair, analogous to bacterial SOS repair, appears to be a concept not yet forced upon us by any data for mammalian cells and the linearity of most recent mutation frequency curves implies that the systems which result in errors during replication or repair may be constitutive. Work supported by Department of Energy.

Replicative Bypass Mechanisms in Eukaryotes

028 DNA REPLICATION IN NORMAL AND DEFECTIVE HUMAN CELLS AFTER UV-IRRADIATION, A. R. Lehmann, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, England.

Normal human cells can repair their DNA after UV-irradiation by excision-repair or post-replication repair. In cells from patients with Xeroderma pigmentosum (XP), either or both of these processes is defective. Excision-defective XP cells are hypersensitive to the lethal effects of UV, XP variants are not. Cells from a sun-sensitive child, 11961, and from patients with Cockayne's syndrome (Co) are sensitive to the lethal effects of UV, but appear to have no gross defect in either excision or post-replication repair.

The overall rates of DNA synthesis, measured as incorporation of ^{3}H-thymidine into DNA at different times after UV irradiation, have been estimated in these different cell strains. In normal cells, DNA synthesis is first inhibited but then recovers at 5-8 hours after irradiation. In XP, Co and 11961 cells, the initial inhibition of DNA synthesis is similar to that in normal cells, but the subsequent recovery of normal rates of DNA synthesis does not occur. Instead the rate of synthesis gradually declines. Thus this failure to recover normal rates of DNA synthesis seems to correlate well with hypersensitivity to the lethal effects of DNA, but it does seem to be a simple consequence of a failure to excise pyrimidine dimers.

029 DNA REPLICATION HALT BY PYRIMIDINE DIMERS AND ITS RESUMPTION IN MAMMALIAN CELLS, *Rogerio Meneghini, Carlos F.M. Menck, R. Ivan Schumacher and M. Cordeiro-Stone, Depatment of Biochemistry, University of São Paulo, CP 20780, São Paulo, Brasil.*

Evidence has been obtained that the pyrimidine dimers cause a halt in the replication fork movement in mammalian cells. If UV irradiated cells are pulse-labeled with ^{3}H-bromodeoxyuridine (^{3}H-Brdu) and the extracted DNA is centrifuged in neutral CsCl density gradient, a band of density intermediate between the hybrid and the unsubstituted DNA is formed. The amount of DNA in this band is much larger in the case of irradiated than in unirradiated cells, when both are allowed to incorporate the same amount of ^{3}H-Brdu. Since the physical characteristics of this intermediate density band have been assigned to a Brdu-containing DNA in the vicinities of the replication fork, the large amount found in irradiated cells should be reflecting an accumulation of halted forks. It thus appears that this accumulation together with some gap formation accounts for the small size of the DNA synthesized after UV-irradiation. It has also been shown that some of the pyrimidine dimers are transferred from parental to daughter strands after resumption of DNA replication. Adequate protocoles have been devised in order to rule out other possible interpretation for the detection of dimers linked to DNA pulse-labeled after irradiation. It thus seems plausible that a small proportion of the dimers are coped with by a recombinational mechanism which involves an exchange of the parental strand containing the dimer with the homologous daughter strand. However the amount of dimers dealt with by this mechanism seems to be lower than 20 percent, and other bypass processes should opperate in the cell.

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030 DNA REPLICATION IN ULTRAVIOLET-IRRADIATED MAMMALIAN CELLS. Howard J. Edenberg, Department of Biochemistry, Indiana Univ. School of Med., Indianapolis, IN. 46202.

Ultraviolet light (UV) inhibits DNA synthesis in mammalian cells, and leads to a decrease in the lengths of DNA synthesized. The interpretation of these phenomena is still controversial. Two models are A) the replication apparatus skips past pyrimidine dimers (or other lesions) leaving gaps of about 1,000 nucleotides which are later filled by de novo DNA synthesis (1) and B) the replication apparatus halts (pauses for relatively long times) when it encounters lesions (2,3), and as yet unknown mechanisms eventually allow bypass. Kinetic analyses of DNA synthesis after UV irradiation are consistent with model B (2,3,4). Studies on mammalian mitochondria (which cannot excise dimers) show that replication continues only up to dimers and then stops (5); the arrested replication intermediates accumulate and remain for at least 48 h. Replication forks accumulate in UV-irradiated (6) and methyl methane sulfonate treated (7) cells also. DNA fiber autoradiography (3) yielded results predicted by model B, and not consistent with replication skipping quickly past dimers. The synthesis of DNA in shorter segments comparable to the interdimer distance is predicted by both models (1,2,3). Model A predicts that gaps will be located opposite dimers (1); however, such gaps opposite dimers have not been detected (8,9). These data are consistent with replication halting at lesions such as dimers until either the lesions are repaired or some unknown process allows bypass (model B). This analysis raises questions about the design and interpretation of experiments. Simply increasing labeling times for irradiated cells (to allow DNA synthesis to equal that in control cells) cannot avoid artifacts arising from end labeling, since the inhibition of DNA synthesis is not due to slower fork progression, but rather to long pauses of the replication forks (3,10).

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031 THE KINETICS OF POSTREPLICATION REPAIR IN MAMMALIAN CELLS AS STUDIED BY THE ALKALINE ELUTION TECHNIQUE, Raymond E. Meyn and Susan E. Fletcher, Department of Physics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The alkaline elution technique developed by Kohn et al (1) has been used to study the kinetics of postreplication repair (PRR) in Chinese hamster cells. In this method, the cells are lysed on polyvinylchloride filters and the DNA is then slowly eluted through the filters with EDTA-tetrapropylammonium hydroxide (pH 12.1). The rate of elution of the DNA increases with decreasing single-strand length. Although this technique is more qualitative than quantitative in that it does not yield molecular weight values, it does have several advantages over alkaline sucrose gradients. The validity of the alkaline elution technique for use in studies of PRR was tested by confirming several known aspects of this repair mode. For example, it has been shown previously (2), using alkaline sucrose gradients, that the size of the DNA synthesized in cells immediately after exposure to ultraviolet light (UVL) was smaller than that synthesized in unirradiated cells. However, within 6 hours after UVL, the cells began synthesizing normal-size DNA. These observations, and several others concerning PRR, have been qualitatively reproduced using alkaline elution. This technique has been used subsequently to investigate the time course of the return to normal DNA synthesis characteristics following exposure to UVL. Preliminary results show that the rates of elution of the DNA made at 2 and 4 hours after UVL were the same as that made immediately after UVL, while the rate of elution of the DNA made 6 hours after UVL was similar to that in the unirradiated control. This return of normal synthesis between 4 and 6 hours could not be inhibited by cycloheximide. One possible explanation which has been proposed to account for this phenomenon is that PRR is enhanced at late times after UVL exposure, resulting in an increased rate of repair (3). Enhancement of PRR has also been observed by D'Ambrosio and Setlow using split doses of UVL (4). In their investigation, however, significant enhancement was observed within 2 hours and it was inhibited by cycloheximide. We conclude, on the basis of our results, that if the ability to synthesize normal-size DNA at late times following UVL-irradiation represents an enhancement of PRR, then this enhancement may be significantly different from that observed by D'Ambrosio and Setlow.

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032 POSTREPLICATION DNA REPAIR (PRR) IN UV-IRRADIATED XERODERMA PIGMENTOSUM (XP) AND XP VARIANT CELLS. Yoshisada Fujiwara, Department of Radiation Biophysics, Kobe University School of Medicine, Kobe, Japan.

We have put forward a replicative repair model as mammalian PRR for uncoding lesion: branch migration at temporarily blocked growing DNA point may allow copy of the sequences opposite a lesion to circumvent it using a new strand as an alternative template, followed by branch reannealing. PRR after UV irradiation of human cells involves at least caffeine-sensitive and its resistant pathways. We studied molecular and biological significances of these two pathways in XP and XP variant strains. All the XP variant strains (10 lines) showed nearly 100% unscheduled DNA synthesis and a little higher UV sensitivity compared to normal human cells. Caffeine potentiation of UV killing in terms of the first-order Do reduction by caffeine [S(Do) = $e^{-C/Co}$: C, caffeine dose; Co, mean Do-inactivation caffeine dose] varied greatly among the XP variant strains, and its extents enabled us to divide them into three subgroups. Subgroup I (with cancers) was characterized by the most drastic potentiation (Co = 1.5 mM), Subgroup II (with cancers) by an intermediate potentiation (Co = 2.7 mM), and Subgroup III (without cancers) by no potentiation. In addition, complementation group A XP cells exhibited a 9-fold higher UV sensitivity, but no obvious caffeine potentiation. In good correspondence with the above survival data, sedimentation rate of DNA in alkaline sucrose gradient revealed that in Subgroup I strains, elongation of [^{3}H]thymidine-pulsed DNA of initially lower molecular weight synthesized after 10 J/m^2 was retarded and inhibited most drastically by 2 mM caffeine. Further, we observed intermediate defect and caffeine suppression in the Subgroup II strains, and surprisingly neither defect nor caffeine inhibition at all in the Subgroup III strains of XP variants, as found in normal cells. Thus, an interpretation may be that XP variants from Subgroups I and II patients possessing skin cancer do concomitantly manifest both defect in and caffeine-sensitive pathway of PRR, while the cells from Subgroup III patients without cancers do only the caffeine-resistant pathway. In addition to defective excision repair, Groups A and D XP strains also manifested a recognizable, but slightly defective and caffeine-sensitive PRR pathway. Moreover, of particular interest is the cellular, but not hereditary control of manifestation of either PRR pathway which is suggested by the facts that an elder sister XP variant patient with cancer falls in Subgroup I while a younger sister patient without cancer yet possesses the characteristics of Subgroup III, and that SV 40-transformed group A XP cells manifests only the caffeine-sensitive PRR pathway. Thus, our results may indicate a possible classification of at least three Subgroups of XP variants and a striking coincidence between manifestation of a defective and caffeine-sensitive PRR pathway and the incidence of skin cancers in XP variants, including such a possibility in XPs with skin cancers already or highly carcinogenic potential. [Supported by Grants from the Japan Ministry of Education, Science&Culture]

DNA Strand Break Repair in Eukaryotes

033 REPAIR OF DNA DOUBLE-STRAND BREAKS, Franklin Hutchinson, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Conn. 06520.

Our present knowledge concerning DNA double-strand breaks and their repair can be summarized as follows. (a) Double-strand breaks are definitely repaired in bacteria; repair in yeasts is fairly well documented. However, repair in mammalian cells is less well established. (b) Repair of double-strand breaks in E. coli is much reduced in recA$^-$ strains (1). (c) Double-strand break repair was not detected in E. coli wild type cells with less than 1.5 genomes/cell, suggesting the need for an intact double helix having the same base sequence (1). (d) Repair of double-strand breaks in E. coli wild type cells is limited, and not observed at gamma ray doses several times that required to decrease colony forming ability to 37%, even though single-strand break repair was still extensive. (e) Gamma rays produce 0.1 to 0.2 double-strand breaks/10^9 daltons per kilorad in oxygenated bacterial and mammalian cells, and two to three-fold less in anoxic cells. (f) Neutral sucrose gradient sedimentation cannot be used to measure native DNA sizes greater than (1 to 3) x 10^9 daltons.

Thus, sedimentation currently limits measurements of DNA double-strand breaks in mammalian cells to situations in which there are several thousand double-strand breaks per 6 x 10^{12} dalton DNA complement. This compares with about 100 breaks at, for example, the 200 rads of X-rays needed to reduce colony forming ability to 37%. There are complications - i.e., point (d) above - in extrapolating measurements made at the high level of DNA damage to levels relevant to relating DNA lesions with biologically important quantities such as colony forming ability. We are using a method of measuring DNA size by fluctuation fluorescence spectroscopy (2), which can measure the size of DNA of molecular weight 10^8 to 10^{12} daltons. The major problem probably lies in devising gentle techniques which will allow broken DNA to separate without introducing new breaks in the extremely large DNA molecules.

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034 REJOINING OF DNA STRAND BREAKS IN NONDIVIDING CELLS IRRADIATED IN SITU, J. T. Lett, P. C. Keng and C. Sun, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO. 80523.

Despite extensive investigation over the past few decades, little is actually known about the efficacy with which normal mammalian cells repair DNA strand breaks induced by ionizing radiation. Questions such as "is rejoining complete, is the rejoining process accurate and if it is inaccurate how is that inaccuracy expressed", remain unanswered. Data obtained in attempts to answer the complementary but perhaps more fundamental questions "does nuclear DNA degrade with age and do DNA repair mechanisms deteriorate with age", are the subject of controversy.

For the past five years we have attempted to address the above questions with investigations of the differentiated, non-dividing and non-replenishible cell populations of the central nervous systems of the rabbit. Those investigations are not complete (the median life-span of the rabbit is $\sim$ 7 years), but at the time of writing the following generalized picture seems to be emerging with respect to the photoreceptors of the retina:

a) DNA strand breaks accummulate with age.
b) DNA strand breaks effected by γ-irradiation are rejoined (>99.5%) throughout the life-span of the rabbit following doses which do not cause degeneration of the photoreceptor cells (<4000 rads), but the rejoining process takes several days to reach completion. Except for c), below, the subsequent fate of the rejoined breaks is not known.
c) Following a dose of 1000 rads of ^{60}Co γ-rays the "fully rejoined" DNA starts to degrade after 3-4 years.
d) Breaks induced by doses of radiation delivered at LET_∞'s of 0.3 keV/μm and 35 keV/μm are "fully rejoined" if those doses do not cause degeneration of photoreceptor cells.

035 DNA DAMAGE AND MAMMALIAN CELL KILLING,
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The innovative technique of using alkaline cell lysis and sedimentation to quantitate single-strand DNA break induction (1) led to new insights and several expectations including the possibility of explaining how DNA-interactive agents alter cell function. In some instances, qualitative correlations between changed function (e.g., cell killing) and DNA damage--and how these are modified by factors that affect them both--support the likelihood that cause-effect relations are involved. Even when such is the case, however, the situations are few (if any) where good quantitative agreement obtains between the dose dependence of altered cell function, and the dose dependence of DNA damage registration and/or incomplete repair. Examples of qualitative correlations the quantitative bases for which are not readily apparent, or do not readily support a cause-effect relationship, are: 1) lethal damage due to the exposure of cells containing BUdR to fluorescent light (2); 2) UV-induced sublethal X-ray damage (3); and 3) lethal and sublethal X-ray damage (4).

In respect to the third example, the inventory of DNA breaks was examined in the progeny of surviving cells that had received repeated doses of 1000 rads. Even after a total accumulated dose of 50,800 rads, breaks were not evident when DNA of 2 X 10^8 daltons was examined. (Per 2 X 10^8 daltons, a dose of 50,800 rads would have registered 21 single-strand and ~8.5 double-strand breaks.) From the kinetics of break production, a D_0 dose (i.e., a "one-hit" dose producing a survival reduction by a factor of 0.37), registers ~1000 single-strand and ~40 double-strand breaks per cell. Taken together, these data suggest that lethality results from: 1) the lack of repair in a target corresponding to a small fraction of the genome; or 2) an infrequent misrepair of one of the supernumerary breaks registered by a dose that produces one lethal hit. (Work supported by the U.S. department of energy.)

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036 ALKALINE ELUTION STUDIES OF DNA SINGLE-STRAND BREAKS, INTERSTRAND CROSSLINKS AND DNA-PROTEIN CROSSLINKS IN MAMMALIAN CELLS,
Kurt W. Kohn, Regina A. G. Ewig, and Warren E. Ross, Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD 20014.

The kinetics of alkaline elution of DNA from filters are affected by interstrand and DNA-protein crosslinks as well as by single-strand breaks (1, 2). Studies with a variety of DNA damaging agents have shown how these lesions can be characterized. Single-strand breakage by X-ray is complicated by the production of DNA-protein crosslinks which reduce DNA elution due to absorption of protein to the filter. DNA-protein crosslinks are distinguished from interstrand crosslinks by their reversal by proteolytic treatment (2). The characterization of DNA-protein crosslinks was facilitated by the use of trans-Pt(II)$(NH_3)_2Cl_2$ which was found to generate this type of lesion almost exclusively. The results obeyed a simple model based on random lesion distributions, and suggested that X-ray produces 1 DNA-protein crosslink per 30 single-strand breaks in L1210 cells. Single-strand breaks secondary to alkali-labile sites in cells treated with methylnitrosourea or methylmethanesulfonate were characterized by an accelerated alkaline elution kinetics, differing from the nearly 1st-order kinetics produced by X-ray (2). The effects of interstrand crosslinks were most clearly seen with psoralen plus light, which produced only this type of lesion. A unique relationship between single-strand breaks and DNA-protein links was observed in cells treated with DNA intercalating agents, suggestive of protein links localized at the break sites. The findings provide a basis for studies of the repair of various macromolecular DNA lesions in mammalian cells.

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037 REPAIR OF DNA-BREAKS INDUCED BY GAMMA RAYS AND FAST NEUTRONS IN CHINESE HAMSTER CELLS, Gunnar Ahnström and Karl-Anders Edvardsson, Wallenberg Laboratory, University of Stockholm, S-10691 Stockholm.
Fast neutrons induce a higher proportion of slow- or non-rejoining DNA-breaks than gamma rays in chinese hamster cells (1). Ritter et. al. have shown that non-rejioning breaks have the same relative LET dependence as cell killing and suggested that non-rejoining breaks cause single-hit irreversible cell death. There are however objections to this interpretation. The amount of non-rejoining breaks after massive doses of gamma or X-rays is found to be in the order of 1 per cent. Assuming that all non-rejoining breaks are double-strand breaks we obtain for a D_o-dose,(ca 140 rads) 10 double-strand breaks per diploid genom. There are also indication from experiments by Cole et. al. that non-rejoining breaks are not linearly dependent on dose. We have similar experiences. To elucidate this problem we have employed a method for determination of DNA-breaks which is more sensitive and reproducible than sedimenta-in sucrose gradients (1). V-79 hamster cells were irradiated with gamma rays or fast neutrons. Reapair times up to 30 hours were given. RESULTS: The number of strand-breaks found in unirradiated cells were doubled by a gamma dose of 120-150 rads. This corresponds to 3 single-strand breaks per 10^8 daltons. Analysing 10 cell samples for each dose we could detect a 15 per cent increase in strand-breaks over the control i.e. 20 rads. We would then be able to detect a 1 per cent level of non-rejoining breaks after a gamma dose of 2000 rads. Up to 10 hours of repair we obtained linear dose relationships for slow-rejoining breaks. 20 hours of repair gave a minimum in remaining strand-breaks and at this time no breaks could be detected below 10 krads of gamma rays or fast neutrons. At 30 hours of repair an increase in breaks compared to 20 hours was recorded. This could indicate that degradative processes had started to take over repair. Conclusions: The experiments performed by Ritter et. al. clearly show that non-rejoining breaks are important events with regard to cell killing. We feel, however, that mammalian cells,irradiated with doses relevant for studies of cell killing, are capable of rejoining in practice all strand-break damage. Mistake in the reconstruction work, in proportion to the number of slow-rejoining breaks, occur however.These mistakes may then lead to chromosome aberrations.

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Excision Repair Pathways in Prokaryotes

038 STUDIES ON ALTERNATE PATHWAYS OF DIMER EXCISION AND REPAIR RESYNTHESIS IN ESCHERICHIA COLI, Warren E. Masker and Nancy B. Kuemmerle, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, and John W. Chase, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461.
Although the basic steps involved in the excision repair of pyrimidine dimers are well established, the details of this repair mechanism are not fully understood and the enzymes responsible for some of the steps have not been identified. Apparently Escherichia coli maintains alternate pathways used to complete excision repair and can draw upon a number of enzymes with similar properties for application to the repair process. To examine the roles of specific enzymes in alternate repair pathways we have measured repair resynthesis and pyrimidine dimer excision in UV-irradiated E. coli mutants with deficiencies in DNA polymerases and in 5'-3' exonucleases. In one aspect of our study we attempted quantitative measurements of repair in vitro, using gently lysed extracts of E. coli to repair exogenous DNA irradiated and incised with a damage-specific endonuclease. This system is capable of removing dimers from damaged DNA and restoring that DNA to its original molecular weight.[1] By treating irradiated T7 DNA with this system and packaging the DNA in vitro it was also found that the biological activity of the DNA could be restored.[2] Under our assay conditions extracts from polA1 strains perform about twice as much repair resynthesis per pyrimidine dimer as do extracts from wild-type strains. Further studies on the nucleoside triphosphate dependence of repair in this system as well as investigations of the effect of mutations such as polB, uvrC and uvrD are underway. In a parallel study we examined the effect of combined mutations in recB, recC, xseA, and pol546ex (deficient in the 5'-3' exonuclease of DNA polymerase I) on UV survival and on the rate and extent of dimer excision. Dimer excision is reduced in polA546ex strains after high UV dose, but an additional deficiency in exonuclease VII (which can excise dimers in vitro) did not measurably affect in vivo dimer excision.[3] Our measurements were complicated by a high level of postirradiation DNA degradation in polA546ex strains at high UV dose. It was found that the recB21 mutation was incompatible with polA546ex mutation. However, by introducing temperature-sensitive mutations in recB and recC into polA546ex strains, conditionally lethal strains that show no serious postirradiation DNA degradation were constructed. It was found that the polA546ex recBts recCts xseA mutants show reduced survival after UV exposure as well as a reduced rate of dimer excision. We are continuing these studies with other combinations of deficiencies in exonucleases. (Research sponsored by the Department of Energy under contract with the Union Carbide Corporation.)

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039 ALTERNATIVE PATHWAYS FOR EXCISION AND RESYNTHESIS: DNA POL III ROLE? Priscilla K. Cooper and Joyce G. Hunt, Department of Biological Sciences, Stanford, Ca. 94305

Previous work has shown that E. coli polAex2 mutants deficient in the pol I 5'-exonuclease are not greatly altered in dimer excision after UV doses of 50 J/m^2 or less (1). We find that polA1 is more deficient in dimer excision than polAex2, expecially at higher doses. Excision in ts recB,CpolA1 is identical to that in polA1, so the excision deficiency is not merely an artifact of degradation (cf ref. 1). We have examined the possible participation of pol III in excision. It is not known whether any polC mutants are deficient in the 5'-exonuclease of pol III. However, since pol I polymerase seems to be more important for excision than the exonuclease, it is of interest to look for an effect of any deficiency in pol III, especially in a polA background. We compared dimer excision in the polA1polC1026 strain HS434 to that in polA1. We found HS434 to be very deficient, removing only 30% of dimers at doses of 20 J/m^2 or above. This deficiency was especially pronounced at low doses where polA1 removes dimers efficiently. Our results implicate pol III in dimer excision in vivo.

It has been proposed that error-prone repair occurs both during post-replication repair and during excision repair at sites where closely-spaced lesions give rise to overlapping double strand gaps (2). One repair pathway for excision gaps may involve the inducible repair system as judged by a common requirement for protein synthesis and the recA$^+$ lexA$^+$ genotype (3). Earlier work implicated recA$^+$ in long patch repair (4), which has hence been proposed to be a manifestation of inducible repair. In testing this hypothesis, we find that post-UV amino acid starvation or chloramphenicol reduces repair synthesis in wild type, ts recB,C and dnaB to the same extent. The repair label in long patches (defined by density shift in alkaline CsCl gradients after sonication) is specifically reduced. Repair synthesis is increased in polA (4) and can involve pol III (5), as can completion of gap filling (6). We found that post-UV protein synthesis inhibition gives a greater reduction of repair in polA than in wild type, as predicted if more lesions are repaired by the long patch pathway in polA. These results are consistent with the interpretation that long patch repair is inducible, perhaps performed by an altered pol III. We are currently examining the effects of various inducing treatments on long patch repair and testing mutants known to be altered in the expression of inducible repair functions.

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040 THE recA AND lexA GENES OF E. Coli, Peter T. Emmerson, Stephen C. West and Keith A. Powell, Department of Biochemistry, University of Newcastle upon Tyne, NE1 7RU, U.K.

Protein X, molecular weight ∿ 40 kD, a protein which is synthesised abundantly in recA$^+$ lexA$^+$ cells following inhibition of DNA synthesis has been identified as the recA$^+$ gene product (1). The dependence of protein X synthesis on functional recA$^+$ and lexA$^+$ genes can be accounted for by assuming that the lexA$^+$ gene codes for a repressor which binds to the operator of the recA$^+$ gene from whence it can normally only be removed by the co-ordinated interaction of an effector and protein X. In this model, protein X controls its own synthesis in a positive way. One possible positive role for protein X would be that of an endopeptidase which cleaves the lexA$^+$-coded repressor (2). Protein X may also cleave the λ repressor. Proteolytic cleavage of the λ repressor by temperature step-up in a tif-1(λ) lysogen does not require de novo protein synthesis (3). Since tif-1 is a temperature sensitive mutation of the recA gene (4) it is possible that protein X is modified by temperature shift-up to a form which can cleave the repressor without the aid of an effector. Another role of the recA$^+$ product may be to bind to single-stranded DNA regions generated by excision repair (2). When protein synthesis is inhibited by chloramphenicol after UV irradiation the excessive DNA degradation, normally associated with recA and lexA mutants, occurs in wild-type cells (5). Thus the recA$^+$ gene product (protein X) may have at least two roles (a) when modified by an effector it may act as an endopeptidase and (b) at high concentrations it may act as a DNA binding protein. Since protein X is induced in large quantities in the cell it can be readily isolated and its properties investigated in vitro.

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041 REPAIR OF CROSS-LINKED DNA IN E. coli. Ronald S. Cole, George H. Yoakum and Richard R. Sinden, Departments of Biochemistry and Microbiology, University of Georgia, Athens, Georgia 30602.
Removal of psoralen cross-links from DNA was examined in E. coli, permeabilized cells, and in vitro. Genetical, physical, and biochemical analysis of this reaction suggest the following mechanism. In an ATP- and Mg^{++}-dependent reaction, the uvrA and uvrB gene products make a strand cut on the 5' side of one arm of the cross-link, producing a 3'-OH terminus. DNA polymerase (5'-3' exonuclease) makes a second strand cut on the 3' side of the cross-link in the same DNA strand. The second reaction did not occur in $uvrD^-$ strains, but could be completed in vitro with DNA polymerase I acting on the product of the first reaction. Strand cutting and cross-link removal in vitro were completed by tandem reactions using first the UV-endonuclease from either M. lutens or phage T4-infected E. coli, and then DNA polymerase I of E. coli. After cross-link removal in whole cells, a single stranded region of about 700 nucleotides is then produced near the site of removed cross-links, and the latter was not detected in a $recF^-$ strain. Completion of repair can be judged by the extent of rejoining of DNA strands cut during cross-link removal. This reaction is influenced by nearly all of the genes involved in genetic recombination, particularly the recA gene. Studies on the kinetics of strand rejoining and on completion in individual cells of repair dependent on $RecA^+$, suggest that repair events involving recombination occur singly. Analysis of the molecular weight distributions of DNA during repair suggest that successive recombination events occur in a sequential or progressive fashion around the chromosome, or in clusters comprising at least 2-3% of the genome.

042 EXCISION REPAIR AND MUTAGENESIS ARE COMPLEX PROCESSES, Kendric C. Smith, David A. Youngs, Emmanuel Van der Schueren, Kenneth M. Carlson, and Neil J. Sargentini, Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305.
Excision repair has been divided into two major pathways: a growth medium-independent, $polA^+$-dependent pathway, and a growth medium, $recA^+$, $recB^+$, $lexA^+$, $polC^+$, $uvrD^+$-dependent pathway (1). The former pathway handles most of the UV-induced lesions (1), and produces short patches of repair replication (2). The latter pathway can be subdivided into three branches: a $uvrD^+$-dependent branch that is divisible into chloramphenicol (CAP) sensitive and insensitive branches, and a $uvrD^+$-independent branch that is CAP sensitive. Long patch repair replication appears to occur via this latter branch, it is $recA^+$, $lexA^+$-dependent, it requires $polB^+$, is inhibited in buffer or by CAP, and is enhanced in uvrD strains. Liquid holding recovery (LHR), an excision repair process that is observed in recA strains (3), is blocked by a uvrD mutation, but is enhanced by lexA and recB mutations. Presumably, LHR proceeds via the CAP-insensitive $uvrD^+$-dependent pathway of excision repair. A recB uvrD strain that is $recA^+lexA^+$has no detectable UV-mutability, suggesting that current theories on error-prone repair need to be revised.

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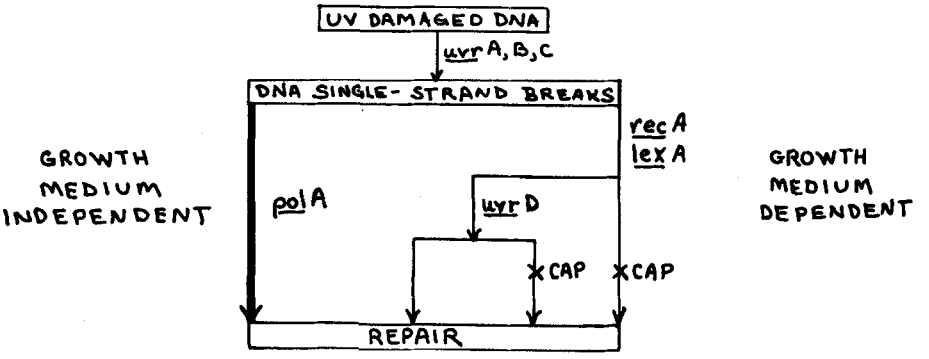

Conditioned Repair Responses

043 THE INVOLVEMENT OF DNA POLYMERASE III IN CONSTITUTIVE AND INDUCIBLE MANIFESTATIONS OF MUTAGENIC DNA REPAIR IN ESCHERICHIA COLI, B. A. Bridges and R. P. Mottershead, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, England

Incubation of E.coli WP2 in the presence of chloramphenicol (CAP) for 90 min before and 60 min after gamma irradiation had no effect on the induction of Trp^+ mutations. Bacteria that had been treated with CAP for 90 min prior to UV irradiation showed normal or near normal yields of induced mutations to streptomycin or colicin E2 resistance. Most of these mutations lost their photoreversibility (indicating "fixation") during continued incubation with CAP for a further 60 min after irradiation. It is concluded that CAP-sensitive protein synthesis is not required for mutagenic (error-prone) repair of lesions in pre-existing DNA, arguing against an inducible component in this repair.

In contrast the frequency of UV-induced mutations to Trp^+ (largely at suppressor loci) was drastically reduced by CAP pre-treatment, confirming the need for an active replication fork for UV mutagenesis at these loci. It is known from the work of others that CAP given after UV abolishes mutagenesis at these loci. We conclude that protein synthesis (consistent with a requirement for an inducible function) is necessary for mutagenic repair only in newly-replicated DNA(presumably at daughter strand gaps) and not in pre-existing DNA.

Following the demonstration that DNA polymerase III activity is required for mutagenic repair to occur following UV irradiation(1) we have studied a dnaE temperature-resistant revertant which appears to be deficient in the inducible but not in the constitutive component of mutagenic repair. The spontaneous mutation rate of the revertant is normal suggesting that base selection and proof-reading functions are normal in the absence of DNA damage. If an inducible factor is involved in inhibiting either the base selection or (perhaps more likely) the proof-reading function of polymerase III in inducible repair, the properties of the revertant are consistent with an inability of the polymerase complex to interact with the inducible factor.

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044 COMPLEXITY IN THE REGULATION OF INDUCIBLE SOS FUNCTIONS IN ESCHERICHIA COLI, Evelyn M. Witkin, Department of Biological Sciences, Douglass College, Rutgers University, New Brunswick, N. J. 08903.

In wild type E. coli, treatment with many mutagens and carcinogens results in the induction of numerous functions (SOS functions) that are repressed in intact cells (1). Expression of these functions (e.g., prophage induction, error-prone DNA repair activity, control of DNA degradation) requires the $recA^+$ $lexA^+$ genotype. Recent observations in this laboratory and others indicate that regulation of SOS functions does not depend solely upon the products of the recA and lexA genes, and that expression of these functions can be altered differentially and/or radically by mutations known to map elsewhere. The implications of these observations for models of SOS regulation will be explored.

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045 *CONSTITUTIVE AND INDUCIBLE de novo DNA SYNTHESIS PATHWAYS OF EXCISION AND POSTREPLICATION REPAIR IN E. coli*, R. Ben-Ishai, E. Pugravitsky and R. Sharon, Department of Biology, Israel Institute Technology, Haifa, Israel.

A semi in vitro system (toluenized E. coli) was used to study constitutive and inducible gap filling of excision and postreplication repair. Induction was performed in vivo by incubating cells under conditions that induce formation of protein X(1). Alternate pathways for the short and large patch gap filling steps of excision repair have been postulated. It will be shown that in toluene treated and UV irradiated E. coli only DNA polymerase I mediated short patch repair results in complete filling in of all the gaps generated at the sites of incision. On the average 13-17 nucleotides are reinserted per dimer excised and the base composition of the repaired stretches adjacent to the dimers is similar to that of total E. coli DNA. In polA mutants, DNA polymerase II and/or III mediated repair synthesis takes place. However, this synthesis results in filling in of only a small number of gaps. To test if large patch repair is inducible cells were exposed to various treatments that damage DNA or inhibit DNA synthesis. Some of these treatments enhanced the extent of rejoining of gaps whereas others inhibited it.

The gap filling step of postreplication repair was studied in induced and noninduced toluene treated uvrA mutants in which DNA synthesis on a damaged template had occurred in vivo. Induction was performed by preirradiating cells with a low dose of UV light 30 minutes before exposure to a higher dose. Filling in of gaps in daughter DNA was analyzed by alkaline sucrose gradient sedimentation, BN cellulose chromatography and incorporation of dNTP's. Gap filling in vitro was observed only in induced cells; approximately 10-15 percent of gaps were rejoined by a process that is dependent on ATP and involves dNTP incorporation. Induction of gap filling was inhibited by chloramphenicol and antipain.

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046 UV INDUCIBILITY OF P32 SYNTHESIS, H.M. KRISCH, Dept. of Molecular Biology, Univ. of Geneva, Switzerland.

The product of bacteriophage T4D gene 32 (P32) is essential for viral DNA replication, recombination, and repair. P32 binds tightly and co-operatively to single stranded DNA and catalyzes DNA denaturation and renaturation. We have previously described an additional function of P32; that of regulation of its own synthesis. Using phage mutant in gene 32 it was found that the expression of gene 32 was 5 to 10 times greater than in a wild type infection. We have also demonstrated that gene 32 expression is increased by UV irradiation and that P32 seems to be the only T4 protein which exhibits such UV inducibility. This inducibility is dependent on the function of genes 46 and 47 (exonucleases) and to a lesser extent on several other T4 genes thought to be involved in repair (e.g. P43 DNA polymerase). An infection defective in both P43 and P46 shows essentially no stimulation of the rate of P32 synthesis after irradiation. In the absence of DNA replication the parental DNA is degraded after irradiation in a dose dependent manner. The extent of P32 induction in such an infection is also proportional to dose.

It is suggested that the production of gaps during repair of irradiated DNA is responsible for the stimulation of P32 synthesis. A model is proposed in which such regions of single stranded DNA compete for P32 by binding it non specifically, thus reducing the amount of P32 free to block the expression gene 32. Because the expression of gene 32 is self-regulatory this would result in increased P32 synthesis. Additional experiments demonstrate that the rate of P32 synthesis can increase substantially in situations where new RNA synthesis is blocked and also that g32 mRNA is extremely stable. These observations as well as determination of g32 mRNA levels in various situations suggest strongly that gene 32 self-regulation operates at the level of translation, and that P32 can inhibit the translation of g32 mRNA. Such a regulatory mechanism may be particularly well suited to the important role of P32 in repair of radiation damage.

047 AN INDUCIBLE ERROR-FREE REPAIR PATHWAY INVOLVED IN THE REPAIR OF ALKYLATION DAMAGE IN E. COLI. P. Schendel, M. Defais, P. Jeggo, L. Samson and J. Cairns.

We have been studying a repair pathway of E. coli which is induced upon growth of cells in sub-lethal concentrations of alkylating mutagens. This pathway, which we call adaptation, renders the cell resistant to both the toxic and mutagenic effects of further alkylation. It is distinct from any of the previously known repair pathways of E. coli.

Adaptation is a useful tool for studying the mechanism of alkylation induced mutagenesis. The ability of this repair system to remove specific chemical lesions from the DNA of adapted cells can be correlated with the reduced mutation frequency observed in these cultures. In this way the potentially mutagenic nature of these lesions can be established. Studies of this type will be discussed.

Alkylating agents induce the lex-recA dependent SOS response. Adaptation alters the pattern of SOS induction without directly affecting the cells capacity to express SOS. This has allowed us to dissect part of the SOS response and to gather information on the nature of the signal which causes SOS induction subsequent to attack by alkylating agents.

048 SPECIALIZED TRANSDUCTION OF THE LEXA GENE OF E. COLI BY PHAGE LAMBDA. Kevin McEntee, Dept. of Biochemistry, University of Chicago, Chicago, IL 60637 (Presently: Dept. of Biochemistry, Stanford University, Stanford, CA 94305

The lexA gene of E. coli regulates expression of the recA gene as well as the induction of SOS functions following DNA damage or arrest of DNA synthesis (1). A λ phage carrying the lexA genetic region has been isolated in order to study this gene and identify its product. Genetic mapping data indicate that the ubiA gene (coding for octaprenyl transferase which is required for ubiquinone biosynthesis (2)) is 92% cotransduced with lexA- and 96% linked to the UV-resistant revertant of lexA-, tsl. The gene order in this region of the linkage map has been established by two factor P1kc transductional crosses to be

..... malF malE malK lamB ubiA lexA(tsl) dnaB.....

Bacteriophage λ has been inserted in the malF cistron of both lexA+ and lexA- strains by the method of Shimada et al (3). From these secondary site insertions, transducing phages have been isolated which carry different extents of bacterial DNA. A plaque-forming phage has been obtained (λpmalK) which carries the malE and malK genes (and presumably lamB) but not ubiA or lexA. Two independent ubiA+ transducing phages have been isolated which carry the malE, malK (lamB) and ubiA genes but not lexA or tsl. These phages are defective gal-type transducing variants (λdubiA). Three independent lexA phages have been identified among phages selected for the ability to transduce ubiA+. Approximately 30% of all phages which transduce ubiA also transduce lexA, a result which is consistent with the extremely high linkage of the two genes. Both lexA+ (1 isolate) and lexA- (2 independent isolates) transduce malE, malK (lamB), ubiA as well as tsl and spr. Both λdlexA+ and λdlexA- prevent constitutive recA protein synthesis in spr mutant strains. The lexA- phages confer sensitivity to UV irradiation to lexA+ hosts, a result which is consistent with the dominance of the lexA- mutation.

The proteins coded by these transducing phages have been analyzed by polyacrylamide gel electrophoresis. The λdubiA and λdlexA protein patterns differ by a 85,000 molecular weight protein. These results suggest that this high molecular weight protein is the product of the lexA (tsl, spr) gene.

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Genetic Defects in DNA Repair

049 XERODERMA PIGMENTOSUM, D. Bootsma, Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

Xeroderma pigmentosum (XP) is an autosomal recessive disorder in man characterized by a hypersensitivity of the skin to sunlight. Cleaver discovered in 1968 that XP cells are defective in the repair of ultraviolet light induced DNA lesions. This finding has been extended in a large number of studies involving clinical, (cyto)genetical and biochemical characteristics of the disease.

Clinically the differences in expression of the symptoms in XP patients suggest a heterogeneity which is difficult to be explained by variable exposure to environmental factors only. Of interest is the occurrence of mental abnormalities and retardation of growth in some of the patients.

The development of cancers in the skin of XP patients suggests a relationship between defective DNA repair and carcinogenesis. Therefore elucidation of the genetic defect in XP may provide clues to understanding of mechanisms of chemical and physical carcinogenesis.

Genetic studies have revealed the presence of at least 6 different complementation groups in the excision deficient class of XP patients. Whether these mutations are present in the same or in different genes has to be shown.

Gene transfer systems established in somatic cell genetics (those mediated by isolated chromosomes and micro cells) may provide new tools for the genetic analysis of XP.

Complementation analysis of a postreplicative repair defect in socalled XP variants has not yet resulted in the demonstration of genetic heterogeneity in this class of XP patients.

Biochemical and enzymological studies of XP cells have sofar not resulted in the identification of the affected primary gene product(s). The effect of the XP mutation(s) on DNA repair is clearly demonstrated. However, even within one complementation group different repair systems seem to be involved.

These observations suggest that the primary genetic defect in XP affects an as yet unknown cellular mechanism, that secondary interfers with different pathways of DNA repair. Experiments with extracts of normal and XP cells indicate that this mechanism is involved in the functions of chromatin structures in eukaryotic cells.

050 ATAXIA TELANGIECTASIA: A MODEL GENETIC SYNDROME LINKING DEFECTIVE DNA REPAIR WITH RADIOSENSITIVITY AND CANCER PRONENESS, M.C. Paterson, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, KOJ 1JO, Canada.

Humans afflicted with ataxia telangiectasia (AT), a rare autosomal recessive disorder characterized by neurovascular, immune, and hepatic dysfunctions, are predisposed to lymphoreticular malignancy and respond fatally to radiotherapy. Dermal fibroblasts from AT donors also exhibit hypersensitivity to ionizing radiation, as judged by impaired colony forming ability[1]. This has inspired numerous *in vitro* studies designed to identify the molecular basis of the disease. While their rates of sister chromatid exchange and transformation by SV40 virus are normal, AT strains display spontaneous chromosomal instability[2]. The cytogenetic and molecular properties of AT cells are in keeping with a defect in the enzymatic repair of radiogenic DNA damage: (i) X-rays produce chromosomal breaks and exchanges in excess of normal levels and the general pattern of chromosomal aberrations suggests defective repair of both one- and two-strand lesions[3]; most strains are impaired in executing DNA repair replication induced by anoxic γ-rays, apparently due to defective excision of putative base defects[4]. (AT strains are proficient both in removing lesions of the 5,6-dihydroxydihydrothymine type[5] and in rejoining single- and double-strand breaks[1,4].) Preliminary data based on the use of *Aspergillus oryzae* endonuclease S_1 as a lesion probe suggest that a class of γ radioproducts which causes gross distortions in the double helix remains in the DNA of AT cells for protracted periods. Thus, certain AT cells may be defective in a "UV-like, long patch" excision-repair process. Genetic heterogeneity in the disease is indicated by: (i) the discovery of at least two complementation groups among repair-replication-deficient strains[6] and (ii) the finding that certain AT strains, although radiosensitive in colony forming ability, execute γ-ray-induced repair replication normally[6]. Some, but not all, heterozygous strains are radiosensitive, i.e., their γ-ray survival and repair-replication levels are intermediate between those observed in normal and AT cells. This may explain why blood relatives of AT patients tend to develop cancer. It can also be estimated that $\sim$0.5% of the general population may be at increased risk to radiation exposure due to the inheritance of a single AT gene. Besides offering clues into the pathogenesis of this complex disease and elucidating basic DNA repair mechanisms in man, future laboratory studies on cultured AT cells promise to provide new insight into the interplay between environmental and genetic factors in human carcinogenesis.

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051 FANCONI'S ANEMIA: A HUMAN MUTATION POSSIBLY ASSOCIATED WITH A DEFECTIVE DNA REPAIR,

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Among many other control systems reside in DNA metabolism, we have a wealth of evidence that human cells possess excellent repair mechanisms to tolerate the damage introduced into their DNA. Studies in xeroderma pigmentosum, a human analogue of uvr^- mutant strain of bacteria, have demonstrated that its impairment was causally related to many pathological changes including cancer development. Moreover, evidence has been accumulated to indicate that human repair systems are complex related with the specific intranuclear organization as well as chemical differentiation of the genome and contrast in many respects with those modeled after the works with bacteria and their viruses. Fanconi's anemia (FA) is another rare hereditary disease which is transmited as an autosomal recessive trait. The FA homozygote is identified by the combination of growth retardation, pigment anomalies of the skin, hypogonadism, diverse congenital anomalies and progressive hypoplastic pancytopenia. The condition has won a special interest, since the FA gene predisposes the affected persons to the development of leukemia and other malignant neoplasms, and an increased chromosomal fragility has been noted in cultured lymphocytes, bone marrow cells and cultured skin fibroblasts. In cell culture assay of transformation by oncogenic virus SV40, the FA cells can be transformed much more readily than normal cells. Many studies have focussed on a possible metabolic defect, such as abnormal carbohydrate metabolism, altered level of ATP, changed stability of lysosome, etc. However, more recent studies indicate that the FA is a condition associated with the defect in a biological process relating to the repair of certain types of DNA damage. Of significant finding is that the FA cells are specifically highly susceptible to killing and chromosome breakage by bifunctional alkylating agents. In cytogenetic assay of the repair of lesions leading to chromosome breakage, pre-aberration lesions induced by bifunctional alkylating agent are not lost in the FA cells. The long lasting pre-aberration lesions and persistence of the renaturability of DNA in mitomycin C-treated FA cells indicate the lesions irreparable in the FA cells are DNA interstrand cross-links. Other works show a defect in the excision of single strand lesions only when the FA cells are treated with relatively high dose of mutagens. These lines of experimental evidence suggests that the FA cells are defective in a mechanism of the repair of overlapping damage including interstrand cross-links. A process of recombinational character might be a possible candidate. This review will attempt to cover and integrate cytogenetic and biochemical works in Fanconi's anemia cells with possible inherent defect associated with this unhappy gene. Work supported by Cancer Research Grants from the Ministry of Education, Science and Culture, Japan.

052 DNA REPAIR DEFECTS AND HUMAN DISEASE, James German, New York Blood Center, New York, N.Y. 10021.

Study of the responses made to environmentally-induced DNA damage by cells from three human genetic disorders—xeroderma, the Louis-Bar syndrome, and Fanconi's anemia—is yielding highly interesting results. Are there not additional disorders, possibly including some not usually classified as genetic, which manifest defective repair of genetic material in their pathogenesis, as either a major factor or a secondary feature? The two manifestations of disease which so far have stimulated successful searches for repair defectiveness are sensitivity to some environmental agent and spontaneous chromosome "breakage" in cultured cells; tumor proneness also has figured prominently in the matter, but because dozens of disorders manifest this, it alone is not especially helpful. Additional instances of repair defectiveness in humans very possibly will be discovered if selected additional clinical features are used as clues. Chromosome instability should remain a valuable indicator, but this sometimes may be "conditional," i.e., occult or elusive until brought out by some special treatment or condition of culture; thus, xeroderma cells display no excessive chromosome instability until treated with UV. Rare conditions viewed by some as promising candidates for DNA repair defectiveness include Bloom's syndrome, Cockayne's syndrome, dyskeratosis congenita, Rothmund's syndrome, Thomson's syndrome, Werner's syndrome, the Wiskott-Aldrich syndrome. Bloom's syndrome, the prototype of the "chromosome breakage syndromes," has failed to display DNA-repair defectiveness, despite an extensive search. Although the search should be continued, this observation of apparently normal repair in the face of dramatic chromosome instability may signal the existence of a previously unrecognized class of disorders not themselves defective in a DNA repair mechanism but in which some "proximal" defect, different and specific in each disorder, allows the accumulation of substrates upon which repair systems will be invoked to act. Such substrates might themselves result from accumulation in the cell of some metabolite, or perhaps from the malfunctioning of some enzyme concerned with an aspect of DNA metabolism other than repair. This burden would in turn make unusual demands on repair mechanisms—themselves normal and intact—including error-prone mechanisms. Conceivably many of the human genetic disorders which feature premature aging or cancer will in time be shown to do so via chronic overloading of the DNA-repair mechanisms, thereby acquiring mutations at an abnormally rapid rate. Conditions falling into this presently theoretical class would not be disorders of repair sensu stricto; but repair, specifically erroneous repair, would be the process of paramount importance in their pathogenesis, at least of their lethal "complications." Persons with such genetic disorders would, with respect to their cancer proneness, be comparable to those chronically exposed to a mutagenic environment.

053 RELATION BETWEEN DNA REPAIR, CHROMOSOME ABERRATIONS, AND SISTER CHROMATID EXCHANGES, Sheldon Wolff[1,2] and Judy Bodycote[1], Laboratory of Radiobiology[1] and Department of Anatomy[2], University of California, San Francisco, San Francisco, CA 94143

Ordinary chromosome aberrations and sister chromatid exchanges (SCEs), which are two types of cytogenetic endpoints that are currently being used to determine the effects of mutagens and carcinogens on chromosomes, differ in several aspects. A) *Ordinary chromosome aberrations* that are induced by ultraviolet light are photoreactivable (Griggs & Bender, 1973), whereas we now have found in chick embryo cells that even though thymine dimers are removed by PR light, the yield of UV-induced SCEs is unaffected. B) Aberrations induced by UV (Bartram et al., 1976) or "UV-like" (Sasaki 1973), but not "X-ray-like" (Wolff et al., 1977), mutagens are higher in XP cells than in normal cells, whereas SCEs are higher in XP cells after treatment with all three types of mutagens (Wolff et al., 1977). C) The aberration yields are related to the amount of excision repair as measured by unscheduled DNA synthesis or repair replication whereas SCE yields are not. D) Aberrations are increased by application of caffeine after treatment with UV light or chemical mutagens (Kihlman et al., 1977) but SCEs are not (Palitti & Becchetti, 1977), and in post-replication defective XP variant cells, UV-induced SCE levels are as in normal cells (de Weerd-Kastelein et al., 1977).

Taken as a whole these data indicate that although aberrations are dependent upon excision repair and post-replication repair as measured in ordinary experiments, SCEs are not. The lesions that result in SCEs do not seem to be thymine dimers or alkylation products at the N-7 position of guanine. Since SCEs are induced by long-lived lesions that disappear with time, minor photoproducts, or alkylation products, that are not measured in excision repair or post-replication repair experiments seem to be the lesions responsible for SCE formation, which is contrary to the findings for chromosome aberrations.

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Xeroderma Pigmentosum

054 EVIDENCE THAT DNA REPAIR PROCESSES PROTECT NORMAL HUMANS FROM PREMATURE SOLAR SKIN DAMAGE AND FROM PREMATURE NEURONAL DEATH, Jay H. Robbins, Alan D. Andrews and Alan N. Moshell, Dermatology Branch, NCI, NIH, Bethesda MD 20014.

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease in which all patients develop at a relatively early age excessive epidermal solar degeneration characterized by atrophy, pigmentation abnormalities, and malignancies on sunlight-exposed skin. At least 6 genetic forms of XP have been confirmed, 5 of which (designated complementation groups A,B,C,D and E) are characterized by defective excision repair of ultraviolet(UV)-induced pyrimidine dimers in their DNA. The sixth form of XP has a defect in S-phase DNA synthesis after UV-irradiation. It has not yet been possible to relate the degrees of the premature, chronic solar damage present in XP patients' skin to the degrees of impairment shown by the patients' cell strains in in vitro tests of DNA repair. Such a correlation would be obscured by the absence of quantitation of the patients' previous sun exposure. Nevertheless, since no human without clinical evidence of premature, chronic, solar skin damage has yet been shown to have abnormalities in the DNA repair processes defective in XP patients, it is probable that the normal function of each of these DNA repair processes is required to protect normal humans from such premature solar damage. Some XP patients develop neurological abnormalities (NA) which first appear in infancy, childhood, or as late as the third decade of life, usually continue to worsen, and are due to death of nerve cells. Sufficient numbers of these NA have been found in several group A patients (representing 4 kindreds) and in patients from all 5 confirmed group D kindreds, to indicate that the nucleotide sequences defining the groups A and D, respectively, determine the occurrence of the NA of XP in these patients, all of whom have very low post-UV colony-forming ability (CFA) in their skin fibroblasts. We have recently found that fibroblasts from a 17-year old black woman with XP, who is reported to us to have mental retardation, microcephaly, and short stature, have post-UV CFA below that of all previously studied strains in group C (none of whose patients has XP NA) but above that of the 4 group A kindreds and the 5 group D kindreds. We have assigned this woman's fibroblast strains to group C, since fusion of her cells with known group C strains showed no complementation, while heterokaryons formed by fusing her cells with either group A or group D cells have fully restored UV-induced unscheduled DNA synthesis. Development of further XP-type NA in this patient would indicate that her present NA are in fact related to her XP (and not simply coincidental), and it could then be concluded that the nucleotide sequence defining group C, as well as the sequences defining groups A and D, must effect a certain level of functionally adequate DNA repair to prevent premature neuronal death in normal humans.

055 THE ACUTE AND CHRONIC EFFECTS OF ULTRAVIOLET ENERGY ON MAMMALIAN SKIN, John H. Epstein, Department of Dermatology, University of California, San Francisco, CA 94143

Cutaneous epidermal cancers, basal cell epitheliomas and squamous cell carcinomas are by far the most common of all human malignancies. The primary stimuli for the formation of these tumors are the ultraviolet rays (UV) from the sun. Experimental studies in mice suggest that UV carcinogenesis starts with the first exposure. However a definitive correlation of any specific acute UV effects with cancer formation has not been accomplished as yet. Acute UV Effects: These include inhibition of DNA, RNA and protein synthesis and mitosis formation, and labilization of lysosomal membranes. Recovery and acceleration of these functions occurs 24 to 72 hours post-irradiation associated with epidermal hyperplasia. A relationship between acute DNA damage and carcinogenesis has been suggested. Pyrimidine dimer formation and repair by photoreactivation, postreplication, and excision mechanisms have been described in a variety of mammalian cell types. However the excision repair process has received the greatest emphasis in epidermal UV injury to date. The possible relationship to cancer formation was emphasized by the discovery of a defect in this enzyme system in patients with xeroderma pigmentosum (XP). XP is characterized by an inordinate susceptibility to form sunlight-induced skin cancers. Chronic Effects: In human skin chronic sun exposure results in a characteristic weather beaten, leathery appearance frequently associated with actinic keratoses and at times cancer formation, especially in light complexioned individuals. Using the hairless mouse model and repeated UV exposures it has been possible to examine the progressive epidermal changes induced by such chronic injury ranging from benign hyperplasia to premalignancy and frank invasive squamous cell cancer. Examination of the epidermal kinetics revealed a progressive increase in mitoses, and premitotic DNA synthesis ("S") in the germinative cells with a shortening of the G^2 and "S" phase. In addition, there was a progressive reduction of the transit time from the basal layer into the stratum corneum. However cell production was greater than cell loss resulting in epidermal hyperplasia. When malignancy and invasion occurred it appeared to develop from the basal cells. However as the tumors grew and became more anaplastic the whole cell mass became abnormal and the germinative cells were no longer identifiable. Profound dermal changes associated with chronic sun exposure occur in human skin. Progressive degeneration occurs in the upper dermis. Specific findings include dilated blood vessels, the accumulation of acid mucopolysaccharides and abnormal appearing fibrocytes, the loss of mature collagen (but an increase in the soluble component) and a marked increase and degeneration in elastic tissue referred to as actinic elastosis. Actinic elastosis is the most prominent and obvious dermal change due to chronic solar damage. The action spectrum for the experimental induction of elastosis falls in the UVB range. The mechanism may relate to fibrocyte damage and altered function.

056 THE RELATIONSHIP BETWEEN NEUROLOGIC DISEASE, ACUTE SUN SENSITIVITY, AND POST-ULTRAVIOLET COLONY-FORMING ABILITY IN XERODERMA PIGMENTOSUM, Alan D. Andrews, Susanna F. Barrett, and Jay H. Robbins, Dermatology Branch, NCI, NIH, Bethesda, MD 20014.

Xeroderma pigmentosum (XP) is an autosomal recessive disease in which DNA repair processes are defective. All XP patients develop premature aging of sun-exposed skin, some have histories of acute sun sensitivity, and some develop neurological abnormalities due to premature death of nerve cells (1). Sensitivity to ultraviolet radiation of 24 XP fibroblast strains was studied in vitro by measuring each strain's ability to divide and form colonies after irradiation. Such post-UV colony-forming ability (CFA) is a measure of the strain's colony-forming efficiency retained after irradiation in vitro with different doses of 254 nm UV light. Details of these procedures have been described previously (2). The most sensitive strains were derived from patients who had an early onset of neurological abnormalities, less sensitive strains were from patients with a later onset, and the most resistant strains were from patients without neurological abnormalities. The clinical histories of 13 XP patients revealed that only 7 had ever experienced acute sun sensitivity, the latter defined as skin blistering and/or severe erythema after a sun exposure which would not have produced such results in normal individuals. The fibroblast strains from 6 of these 7 patients who had histories of acute sun sensitivity had the lowest post-UV CFA of the 13 strains tested. Post-UV CFA of the seventh strain was slightly higher than that of the lowest of the 6 strains from the patients without histories of acute sun sensitivity. The other 5 strains from these latter 6 patients had the highest post-UV CFA of the 13 strains. The UV sensitivities of strains from each member of a sibling pair with XP were identical, indicating that UV sensitivity of XP strains is determined by the patient's inherited DNA repair defect. The results indicate that effective DNA repair is required to maintain the functional integrity of the human nervous system by preventing premature death of neurons and that acute sun sensitivity in XP patients may be caused by the lack of functionally adequate DNA repair in vivo.

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057 RELATIONSHIP BETWEEN DNA REPAIR DEFECTS AND SKIN CANCERS IN XERODERMA PIGMENTOSUM, Hiraku Takebe, Radiation Biology Center, Kyoto University Kyoto 606, JAPAN

More than 70 cases of xeroderma pigmentosum (XP) in Japan have been examined clinically, and the DNA repair characteristics of their cells have been identified. As reported previously by Takebe et al., more then half of the patients belonged to a "low repair" group as measured by the relative amount of unscheduled DNA synthesis (UDS) after UV irradiation. Most of the patients belonging to this group were children who developed XP symptoms early and have had neurological disorders. Twenty three cases in this group have been identified to belong the complementation group A by the cell hybridization methods.

One case, XP23OS, showed UDS level of 10 % of normal level but no neurological disorder has developed and the skin lesions were moderate. Such cases may be classified as a "classical XP" and the patients belonging to the complementation group C often show such symptoms. XP23OS, however, has been identified to belong a new complementation group, or group F. Although the cells of XP23OS showed low (10 %) UDS level, the host-cell reactivation capacity as measured by comparing survival curves of UV-irradiated herpes simplex virus was considerably higher than the cells belonging to the complementation group A, B, C and D. The apparant low DNA repair demonstrated by the relative amount of UDS may not represent the true capacity of the DNA repair. Host-cell reactivation, we believe, represents the total capacity of the excision repair of UV damage more accurately than other methods.

With the exception of XP23OS and a few other cases, XP patients in Japan are roughly divided into two groups, "severe" and "moderate", which correspond to UDS levels of less than 10 % and more than 25 % respectively. There are about 10 "variants" (Fujiwara, this meeting). Essentially all the patients belonging to "severe" group will eventually develop skin cancers by the age of 15, while approximately half of the "moderate" patients will survive without being affected by cancer. So far no patient belonging to the complementation group C, most frequent in other countries, has been found in Japan. (Supported, in part, by the Research Funds from the Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare)

Takebe, et al., Cancer Research, 37, 490-495, 1977.

DNA Repair in Lower Eukaryotes

058 DNA REPAIR IN YEAST, Robert H. Haynes, Department of Biology, York University, Toronto, Ontario, Canada. M3J 1P3.

The discovery of liquid-holding recovery and of radiosensitive mutants in the yeast Saccharomyces cerevisiae provided early indications for the existence in a simple eucaryote of processes analogous to DNA repair in bacteria. Subsequently both excision of UV-induced pyrimidine dimers and repair replication as detected by the Pettijohn-Hanawalt technique (non-conservative incorporation of labelled dBUMP in UV-irradiated ρ haploid dTMP auxotrophs - O. Goldberg and J. G. Little, personal communication 1977) have been detected. The difference between the shouldered UV survival curve of an excision proficient strain and the corresponding exponential curve of an excision-deficient mutant has been accounted for quantitatively on the basis of the decline in dimer excision efficiency with increasing dose. A nuclear endonuclease capable of hydrolysing double stranded DNA (and whose activity is stimulated by UV-irradiation of the substrate) is present, in reduced amounts, in an excision-deficient mutant (rad1). However, repair in yeast is not identical with that in bacteria, as early indicated by the absence of any repair-mediated synergism between UV and X-rays even though a strong synergism of this kind is present in several species of bacteria. The genetic control of repair is more complex: there are at least 32 loci (rad) which affect radiation sensitivity; 22 further complementation groups, 17 of which show cross-sensitivities to radiation, have been identified among mutants selected for their sensitivity to MMS. No one knows how many further loci affect sensitivity to other mutagens. Some of these loci are pleiotropic and it is unlikely that all of them exist solely for the control of DNA repair. Measurements of the sensitivity of various double rad mutants have revealed the existence of three epistatic groups of loci which affect three pathways for repair: the rad3 pathway controls the error-free excision of pyrimidine dimers; the rad51 pathway appears to control the repair of DNA strand breaks; the rad6 pathway is highly pleiotropic and controls some mode of error-prone repair. Wild-type genes rad6 and 9 are required for mutagenesis by all agents tested, including NTG and EMS. Heteroduplex repair is believed to be essential for pure mutant clone formation after UV; it is related to, but not identical with excision repair. Finally, the kinetics of UV-induced mutation differ between E.coli and yeast: for reversion of various nutritional auxotrophies, UV mutagenesis follows square-law kinetics in coli whereas in yeast such kinetics are biphasic (linear-quadratic). Mathematical analysis of the yeast reversion data indicates that there must exist a significant constitutive level of error-prone repair as well as an inducible component that can be blocked by cycloheximide treatment. Thus it appears that bacterial models for repair and mutagenesis cannot be extrapolated in a straightforward way to yeast.

059 DNA REPAIR IN DROSOPHILA, James B. Boyd, Department of Genetics, University of California, Davis, CA 95616

Putative repair-deficient mutants have been obtained by selection for an elevated sensitivity to radiation or chemical mutagens[1,2,3]. These mutants have been associated with over 20 different genetic loci, at least five of which also influence female meiosis[3,4]. This latter class of mutants, therefore, provides an opportunity for studying meiotic recombination functions in somatic cells. With the aid of procedures adopted from mammalian tissue culture, it has proven possible to analyze the major known forms of DNA repair in these mutants. -- Photorepair of uv damage has been documented by analysis of pyrimidine dimers[5,6] and through analysis of repair replication[7]. No mutants affecting this process have yet been identified. -- Excision repair of pyrimidine dimers is strongly reduced by mutation at the mei-9 locus[6]. These mutants are also deficient in repair replication following both uv and x-ray exposure[4,8]. The mei-9 mutants reduce meiotic recombination by 90%[1], and increase the sensitivity of larvae to uv, x-rays and six diverse chemical mutagens[4,8]. Mutants at this locus also increase mitotic recombination and spontaneous chromosome breakage[4]. -- Mutants at four loci have been associated with reduced postreplication repair following uv irradiation [mei-41, mus(1)101, mus(1)104, and mus(3)302][9]. Of these only the mus(1)104 mutant has no apparent effect on meiosis[4]. Caffeine produces a greater inhibition of the residual postreplication repair in the mus(1)104 mutant than it does in the others. These mutants are all sensitive to nitrogen mustard as well as methyl methanesulfonate[9,10]. The mei-41 mutants dramatically increase the frequency of spontaneous somatic chromosome breakage[4]. -- Mutants tested thus far all exhibit a normal capacity to repair single strand breaks introduced by x-ray exposure[9].

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060 PATHWAYS OF DNA REPAIR IN *SACCHAROMYCES CEREVISIAE*, Louise Prakash, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, NY 14642, and Satya Prakash, Department of Biology, University of Rochester, Rochester, NY 14627.

In the eucaryotic microorganism, the yeast *Saccharomyces cerevisiae*, there seem to be three pathways involved in the repair of UV-induced damage in DNA; all the radiation-sensitive (*rad*) mutants analyzed so far fall into three epistatic groups. About one dimer is sufficient to reduce survival to 37% in a triple mutant containing one member of each epistatic group. The first of the three groups contains mutants which all enhance UV-induced mutations compared to *RAD+* strains. In addition to the two members of this epistatic group, *RAD1* and *RAD2*, previously shown to be involved in excision of UV-induced pyrimidine dimers, we have demonstrated that mutants in seven additional genes are defective in excision-repair. The assay we have used measures the susceptibility of dimer-containing DNA to nicking by either T4 UV-endonuclease V or an activity specific for UV-irradiated DNA, partially purified from *Micrococcus luteus*. Six of the genes, *RAD3*, *RAD4*, *RAD7*, *RAD10*, *RAD14* and *RAD16* are previously identified radiation-sensitive mutants, whereas the seventh, *MMS19*, was isolated as a strain sensitive to the monofunctional alkylating agent methyl methanesulfonate (MMS) and complements all the other *rad* mutants. Like the other members of this epistatic group, *mms19* mutants show enhanced UV-induced mutations. Thus, the total number of genes involved in excision repair in yeast so far is nine.

The second epistatic group consists of genes involved in error-prone repair and include the *RAD6*, *RAD8*, *RAD9*, *RAD18*, *REV1*, *REV2* and *REV3* loci. A mutation at any one of these loci reduces UV-induced mutations at many sites, and in the case of *RAD6* and *REV3*, at all sites tested. *RAD6* and *RAD9* functions are also required for mutations induced by a wide variety of unrelated chemical agents and thus appear to be necessary for induced mutations in yeast. Two other genes, when mutant, reduce UV-induced mutations. One is *cdc8*, a cell division cycle mutant which is temperature-sensitive for DNA synthesis. However, our results suggest that the *cdc8* gene apparently falls in all three repair pathways. The other is *mms3*, which exerts its effect in diploids, where lowered UV-induced reversion is observed for all loci tested, but not in haploids, where UV-induced reversions occur at levels found in *MMS+* haploids.

The third pathway consists of genes isolated as mutants sensitive to X-rays, *RAD50*, *RAD51*, *RAD52*, *RAD53*, *RAD54*, *RAD55*, *RAD56* and *RAD57*; these mutants play a role in repair of UV-induced damage when the excision pathway is defective. These eight genes are not involved in error-prone repair of either UV- or X-ray-induced damage but many of these play an important role in error-prone repair of EMS-induced damage. The molecular nature of the repair pathways controlled by these latter two groups is currently being investigated.

061 THE REPAIR OF IONIZING RADIATION DAMAGE IN FUNGI, Michael A. Resnick, Department of Biochemistry, College of Medicine, East Tennessee State University, Johnson City, TN 37601

In yeast and other fungi the genetic control of repair has been extensively investigated and for the case of UV correlations have been made between the repair of pyrimidine dimers and specific pathways. Whereas the major lethal and recombinogenic lesions following UV treatment are pyrimidine dimers, the counterpart for ionizing radiation appears to be double-strand breaks. Unlike the situation in bacteria fungi generally possess an efficient mechanism for the repair of this damage based on biochemical and genetic evidence. This repair appears to depend on the duplicated state of the chromosome (either as homologous chromosomes or as sister chromatids) and based on cell-cycle studies does not require semi-conservative replication. Cells are most resistent in the G-2 phase of the cell cycle and this corresponds to a period of very efficient repair of double-strand breaks. The pathway for the repair of double-strand breaks is important in recombination and meiosis but apparently not in mutation.

The responses of fungi to ionizing radiation are in several ways similar to those obtained with cell lines from a variety of vertebrates. While the mechanism of inactivation is not known the data is consistent with unrepaired double-strand breaks acting in a dominant lethal fashion.

062 THE FATE OF YEAST MITOCHONDRIAL DNA AND OF MITOCHONDRIAL GENETIC MARKERS AFTER ULTRAVIOLET IRRADIATION. E. Moustacchi, M. Heude and S. Hixon,* Institut du Radium, Biologie, Bat. 110, ORSAY (91405) France and Department of Biochemistry, University of Alabama, Birmingham, Alabama, U.S.A.

It is now well established that the petite (or ρ^-) mutation in yeast results from more or less extended deletions of mitochondrial DNA (mDNA) sequences which can be accompanied by repetitions of the retained segments. A number of mitochondrial genes including drug resistant loci and a variety of loci designated mit have been mapped on mDNA and it has been shown that individual ρ^- clones can retain any of these genes.

In exponential phase cells a reduction in frequency of induced ρ^- is observed after dark liquid holding (LH) of UV treated cells. On the contrary, in stationary phase cells this frequency is enhanced. Compared to a single total dose, splitting of the UV dose leads in both cases to a dramatic increase in ρ^- accompanied by an enhancement in survival. A de novo protein synthesis which aggravates the mitochondrial damage is likely to occur between the two doses since the increase in ρ^- and in survival is prevented by incubation with cyclohemimide after the first dose.

Primary ρ^- clones analysed individually for the presence or loss of seven mitochondrial genetic markers immediately after irradiation or after LH show that a) there is a constraint in the genotypes found in ρ^- clones, the region carrying the mit140 and 0^R_{11} markers is preferentially lost, b) ρ^- clones derived from treated stationary phase cells contain less genetic information after LH whereas a rescue of markers occurs for ρ^- mutants derived from irradiated exponential phase cells. In this last case a complex dose response is observed. c) the classes with large deletions increase with dose. LH results in an increase of simultaneous loss of adjacent markers in ρ^- derived from stationary phase UV treated cells and to an increase of simultaneous retention in ρ^- derived from UV irradiated growing cells, d) a directional pattern is found in the fate of markers.

In parallel with these findings it can be shown that mDNA is extensively degraded in irradiated stationary phase cells whereas the degradation is limited and reaches a plateau during LH of exponential phase cells. In this case a slight shift to a heavier buoyant density in cesium chloride gradients is seen, indicating a possible selective degradation of A-T rich regions. Although pyrimidine dimers are not selectively removed from mDNA, the first step in the fate of the irradiated mitochondrial genome appear to be mDNA degradation in all growth stages. This step is by far predominant in stationary phase cells whereas other repair events revealed by recovery of wild type genotypes or partial rescue of markers in ρ^- take place later in irradiated exponential phase cells. Since no new synthesis of mDNA occurs during LH in non nutrient medium, these events, probably of the recombination type are likely to take place only when an energy source is added and growth results.

063 EVIDENCE OF RECOMBINATION REPAIR, RECOMBINATION-PRONE REPAIR AND INDUCED RECOMBINATION IN SIMPLE EUKARYOTES, B. S. Cox, Botany School, South Parks Road, Oxford, U.K.

UV ionising radiation and many chemical mutagens induce mitotic recombination in cells. The effect has been observed in nearly a dozen species of simple eukaryotes (fungi and protozoa). Many mutants have been isolated which are deficient in both recombination and resistance to radiation damage. These correlations are taken to infer the existence of repair processes which involve recombination. The evidence that any of these mutants are involved in a repair process will be reviewed. The evidence on which it is possible to discriminate between a repair process which involves recombination, broadly defined as the exchange of segments of DNA between homologous molecules (rec-repair) and repair processes which merely increase the likelihood of recombination (rec-prone repair) will be presented. The evidence for the existence of inducible repair systems will be reviewed.

Viral Probes of DNA Repair in Mammalian Cells

064 UV-INDUCED REVERSION OF ADENOVIRUS TEMPERATURE-SENSITIVE MUTANTS, Rufus S. Day and Chuck Ziolkowski, Nucleic Acids Section, Chemistry Branch, Carcinogenesis, DCCP, NCI, NIH, Bethesda, MD 20014.

We have measured the effect of ultraviolet light on reversion of adenovirus 5 temperature-sensitive mutants (obtained through Dr. Phil Sharp, M.I.T., from Dr. Jim Williams, Glasgow) to temperature independence in a manner similar to that developed by Cleaver[1] for SV40. This is an initial study in the detection of differences in mutability of the virus (at various genetic sites) in human cells varying in their genetic background. Adenovirus 5ts2 was purified, irradiated with 0-8000 ergs/mm^2 of 254 nm ultraviolet light, and used to infect monolayers of human cells at an m.o.i. of between 1 and 10. Infected plates were incubated at 32° until cytopathic effects were maximal (about 4 days). Cells were scraped, freeze-thawed 2 or 3 times, and titered on A498 cells[2] at 32° and 39.3°.[3] (The use of the human kidney carcinoma cell line[2] for this final titration at 39.3° obviated problems we encountered while attempting to use other human cell lines for this purpose. Unlike these latter, the A498 line both adheres well to the plastic and can crowd tightly while maintaining good viability over a 2-3 week period at 39.3°).

The reversion rate of Ad5ts2 to temperature independence increased from 10^{-6} at zero UV dose to about 10^{-4} at 8000 ergs/mm^2 delivered to the virus in experiments in which irradiated viruses were passed through the A498 line at 32°. This behavior was independent of whether or not these A498 cells received 50 ergs/mm^2 of 254 nm UV 24 hours before being infected.

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065 REPAIR OF DAMAGE FROM ULTRAVIOLET IRRADIATION IN SIMIAN VIRUS 40 (SV40) DNA DURING LYTIC INFECTION OF AFRICAN GREEN MONKEY CV-1 CELLS by Jon I. Williams and James E. Cleaver, Laboratory of Radiobiology, University of California at San Francisco, San Francisco, CA 94118.

The suitability of the DNA tumor virus Simian Virus 40 (SV40) replicating permissively in monkey CV-1 cells for DNA repair studies in mammalian cells was investigated. Several parameters of nucleotide excision repair and DNA replication of UV-irradiated viral DNA were measured during lytic infection. Loss of T4 endonuclease V-sensitive (TES) sites from viral DNA occurred in a complex, dose dependent fashion. The data suggests viral DNA damaged in vivo by ultraviolet (UV) light was repaired by enzymes, presumably from the host cell nucleotide excision repair system. Nucleotide excision repair was carefully studied in host CV-1 cells for comparative purposes. Viral replication on UV-damaged templates was followed by neutral sucrose gradient velocity sedimentation and agarose gel electrophoresis of viral DNA in Hirt supernatants from pulse and pulse-"chase" labelling experiments. Total label uptake and uptake into Form I (supercoiled) DNA was found to be greatly suppressed by UV radiation. The failure of UV irradiation to appreciably suppress label uptake into replicative intermediate (RI) or Form II (open circle) molecules is consistent with the concept that UV photoproducts selectively inhibited nascent DNA strand elongation. Label in RI or Form II molecules "chased" effectively into Form I molecules even after UV fluences of 40 J/m^2 (2.0 - 3.4 pyrimidine dimers per SV40 genome). The significance of these experiments for interpretation of host cell reactivation experiments with SV40 and viral mutagenesis will be discussed as will the adequacy of this viral system for future DNA repair experiments.

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066 PROTEASE INHIBITORS PREVENT UV ENHANCED VIRUS REACTIVATION IN E. COLI BUT NOT IN MONKEY KIDNEY CELLS, C. D. Lytle, F. L. Buchta, and J. G. Goddard, Bureau of Radiological Health, Food and Drug Administration, DHEW, Rockville, MD 20857

Treatment of CV-1 monkey kidney cells with radiation (UV or x rays) or certain chemicals results in enhanced survival of UV-irradiated herpes simplex virus. Current evidence indicates that this enhanced reactivation of UV-irradiated virus may be analogous to Weigle reactivation, an SOS function, in bacteria[1]. It has been posutlated that proteases play an important role in the regulation of SOS functions[2]. Antipain, a protease inhibitor, has been shown to inhibit UV mutagenesis, filamentous growth and induction of λ phage, providing evidence for the role of proteases in bacterial SOS functions[3]. This report provides further evidence by demonstrating that antipain (1 mM) inhibits UV-enhanced reactivation of UV-irradiated λ phage in E. coli C600. However, neither antipain nor leupeptin, another protease inhibitor, had an appreciable effect on enhanced survival of UV-irradiated herpes virus in UV-irradiated CV-1 cells, even at concentrations as high as 5 mM. Both inhibitors changed the capacity of the host cells to support viral plaque formation and caused decreased plaque size, suggesting that the compounds entered the CV-1 cells. Thus a protease inhibitor prevented UV-enhanced reactivation of a UV-irradiated virus in E. coli but not in CV-1 cells. These data provide evidence that enhanced virus reactivation in mammalian cells may not be analogous to Weigle reactivation in bacteria. Furthermore, they indicate that proteases may not have a role in radiation-enhanced virus reactivation in mammalian cells.

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067 SIMIAN VIRUS 40 AS A PROBE FOR STUDYING DNA REPAIR PATHWAYS IN MAMMALIAN CELLS. Alain R. Sarasin* and Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, California 94305.

The fate of ultraviolet-irradiated SV40 DNA in monkey kidney cells has been examined as a model to study DNA repair in eucaryotic cells. The following two types of experiments have been carried out:

Enchanced survival of UV-irradiated SV40 in carcinogen-treated cells. Treatment of host cells with low concentrations of various carcinogens such as aflatoxin B_1 , acetoxy-acetylaminofluorene, methylmethanesulfonate or ethylmethanesulfonate, strongly enhances survival of UV-irradiated SV40 (1). The carcinogen-induced enhancement is correlated with the inhibition of host cell DNA synthesis suggesting that the inhibition is an inducing agent. We have hypothesized that the carcinogen treatment induces a new recovery pathway, by-passing lesions in viral DNA to result in higher survival. By analogy with bacteria this phenomenon may represent some "SOS function" induced in eucaryotic cells by carcinogens. It remains to be determined whether the recovery process is error-prone.

DNA replication on UV-irradiated SV40 templates. UV-irradiation (100 J/m^2) of monkey (MA134) cells during a lytic infection by SV40, dramatically inhibits the synthesis of Form I (supercoiled) SV40 DNA. Using the temperature sensitive mutant ts A-58 to synchronize viral DNA replication prior to irradiation we find that the first pyrimidine dimer is a block to DNA synthesis. The size of the newly-synthesized DNA is equal to or smaller than the inter-dimer distance on "parental" DNA. After a lag period (about 40-60 min) DNA synthesis resumes, presumably passing the dimer and going on to the next one. The size of the newly-synthesized DNA increases. The replication intermediates obtained after UV-irradiation possess two unusual properties: a) The DNA is no longer in a supercoiled configuration, like during normal SV40 DNA replication, but contains at least one single strand nick. b) The pyrimidine dimers present in these intermediates seem to be insensitive to the T4 endonuclease V which normally nicks DNA strands containing pyrimidine dimers. We are mapping the newly-synthesized DNA after UV-irradiation on the SV40 genome using the restriction endonuclease Hind III. Preliminary experiments show that the newly-synthesized DNA maps essentially near the origin of SV40 DNA replication confirming the model described above. We find no evidence that DNA replication starts again after each dimer, leaving gaps opposite the dimers and replicating all of the molecule rapidly.

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068 PRODUCTION OF VIRAL STRUCTURAL ANTIGENS BY IRRADIATED ADENOVIRUS AS AN ASSAY FOR DNA REPAIR IN HUMAN FIBROBLASTS, Andrew J. Rainbow, Department of Radiology, McMaster University, Hamilton, Ontario, Canada L8S 4J9.

Several different fibroblast strains were used as hosts for the production of viral structural antigens (Vag) following infection with irradiated adenovirus. Monolayer cultures were infected with either irradiated or non-irradiated adenovirus and at an appropriate time after infection, cells were examined for the presence of Vag using immunofluorescent staining.

A reduced HCR of this viral function was detected for UV irradiated adenovirus following the infection of fibroblasts from several patients suffering from Fanconi's Anaemia (FA) (1), ataxia telangiectasia (AT) and Xeroderma Pigmentosum (XP) as compared to normal fibroblasts. A reduced HCR for gamma irradiated virus was also detected in fibroblasts from several patients with FA (1), AT, XP and Progeria (2). These results indicate some deficiency in the repair of radiation induced DNA lesions for these syndromes. By an examination of Vag production at several different times after infection with irradiated adenovirus, this technique was capable of detecting differences in the rate of DNA repair for some of the fibroblast strains.

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069 DEFECTIVE HOST-CELL REACTIVATION OF UV-IRRADIATED HERPES SIMPLEX VIRUS BY A BLOOM'S SYNDROME SKIN FIBROBLAST CELL STRAIN, Clifford A. Selsky, Department of Physiology, Harvard University School of Public Health, Boston, MA. 02115

The ability of a Bloom's Syndrome fibroblast strain designated GM1492 (obtained from IMR, Camden, New Jersey) to support the growth of ultraviolet-irradiated Herpes simplex virus type 1 strain MP was determined utilizing an infectious centers assay. The results were compared to the ability of normal human skin fibroblasts and classical xeroderma pigmentosum skin fibroblast cell strains to support the growth of the same ultraviolet-irradiated virus. The data indicate defective host-cell reactivation of UV-irradiated HSV-1 MP in this strain of Bloom's Syndrome skin fibroblasts. Clonal survival of this Bloom's strain following ultraviolet-irradiation appeared normal over the dose range studied. These discrepant results indicate the importance of extensive phenotypic characterization of cells suspected of being defective in some repair function.

Comparative Aspects of DNA Repair

070 EXCISION REPAIR IN CULTURED CELLS, Michael W. Lieberman, Department of Pathology, Washington University, St. Louis, Missouri, 63110.

The purpose of this workshop is to discuss different assays for DNA repair in cultured cells. Although excision repair in ultraviolet radiation (UV)-damaged mammalian cells is one of the best studied DNA repair processes, important discrepancies exist among published data. While there is general agreement on the broad outlines of the excision repair scheme, the actual data used to support the model have often differed quantitatively from one another and have at times even appeared inconsistant. Thus, for the excision of pyrimidine dimers, different laboratories have reported different rates of removal, and, for repair synthesis, different extents and durations have been reported after the same UV dose. Not surprisingly, in some instances rates of dimer removal and repair synthesis have not agreed. In addition, relatively little attention has been paid to removal of non-dimer UV photoproducts and their contribution to repair synthesis. Part of these discrepancies may relate to the variety of methods available to measure various aspects of the excision-repair process, inaccuracies inherent in some of them, and differences in dose or shielding due to cell overgrowth. Additional discrepancies may arise from uncertainties related to other aspects of the repair process such as patch size during the repair of different types of UV-induced damage and the relative distribution of UV photoproducts in different regions of DNA and chromatin. It is hoped that a discussion of these issues will help resolve some of the discrepancies or apparent discrepancies and that from this workshop will come some new approaches to the problem of measuring excision repair.

071 UV ENDONUCLEASE-SENSITIVE SITE LOSS IN CHINESE HAMSTER OVARY CELLS IRRADIATED WITH LOW DOSES OF FAR- AND OF NEAR-UV RADIATIONS. Richard J. Reynolds* and Paul H. M. Lohman, Medical Biological Laboratory, TNO, Rijswijk, Z. H., The Netherlands.
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A sensitive procedure for the detection and quantitation of cyclobutyl pryimidine dimers in mammalian cell DNA has been reported by Paterson, et al. (1973). Their assay relies on the specificity of endonuclease activities from Micrococcus luteus and makes use of DNA sedimentation through calibrated alkaline sucrose gradients for the detection of single-strand breaks resulting from endonucleolytic incisions. Through minor modifications that allow the isolation of larger molecular weight DNA we have further increased the sensitivity of this assay. The substrate specificity of the modified assay was verified by in vitro photoreactivation using photoreactivating enzyme purified from Streptomyces griseus. More than 95% of the UV endonuclease-sensitive sites induced either by far- or by near-UV radiations were rendered insensitive to subsequent UV endonuclease attack by prior photoreactivation treatment in vitro.

The modified UV endonuclease-sensitive site assay has proven useful for studies on UV endonuclease-sensitive site loss in Chinese hamster ovary cells in tissue culture. After the irradiation of cells with either 1.2 or 2.9 J m^{-2} at 254 nm the numbers of UV endonuclease-sensitive sites were found to decrease at slow but measurable rates for up to 30 hours after irradiation. Relative to the numbers of UV endonuclease-sensitive sites detected immediately after irradiation, reductions of 30 and 20% were found 24 hours after irradiation with doses of 1.2 and 2.9 J m^{-2} at 254 nm, respectively. When compared after the induction of similar numbers of sites, site loss was comparable both in near- and in far-UV irradiated cells.

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072 INTRODUCTION OF T4 ENDO V INTO FROZEN-THAWED MAMMALIAN CELLS FOR DETERMINATION OF REMOVAL OF UV INDUCED PHOTOPRODUCTS. A.A. van Zeeland* & C.A. Smith, Dept. of Biological Sciences, Stanfor Univ., Stanfore, CA 94305. The reduction of DNA single strand molecular weight by T4 endounuclease V has been used to evalute removal of pyrimidine dimers in bacterial systems in which the endonuclease is introduced directly into detergent permeabilized cells (1). In studies with mammalian cells, the technique has been applied to isolated DNA, where it is limited by the difficulty of similar to that used with bacteria, in which cells are made permeable to T4 endo V by freezing and thawing. After treatment with the enzyme at room temperature in the presence of EDTA, cells are lysed directly atop alkaline sucrose gradients which are centrifuged after standing for 3 hr. The radioactivity profiles of these gradients allow calculation of single-strand number average molecular weight (Mn) of the DNA of cells labelled prior or subsequent to UV irradiation. In prelabelled cells, the profiles yeald Mn's of about 2.5 x 10^8. The profiles are not altered in UV irradiated cells, frozen-thawed cells, or unirradiated cells which are frozen-thawed and exposed to T4 endo V. The profiles are altered and Mn's reduced when UV irradiated cells are frozen-thawed and exposed to adequate amounts of T4 endo V.

In V79 Chinese hamster cells the calculated number of nicks introduced by T4 endo V is a linear function of UV dose up to 5 J/m^2. Comparison of molecular weights so obtained with the number of pyrimidine dimers induced, determined by chromatographic analysis of acid hydrolyzed DNA of cells exposed to 25 and 50 J/m^2, leads to the estimate that about 50% of the dimers are endo sensitive sites in this assay. DNA isolated from frozen-thawed cells exposed to T4 endo V was shown to contain roughly the expected number of additional sites. Using this endo sensitive site assay to follow excision, V79 cells exposed to 5 J/m^2 UV showed a small but reproducible loss of sites (26% in 24 hrs). At 5.5 hr. after UV, endo sentitive sites were detected in newly-synthesized DNA, but not at 23 hr post UV, suggesting that transfer of endo sensitive sites to daughter DNA occurs only at early times post UV or that these observations result from the induction of endo sensitive sites in parental DNA of unfinished replicons. The system has also been employed to examine post-replication repair after "inducing" UV doses.

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073 REMOVAL OF T4 ENDONUCLEASE V SENSITIVE SITES AND PYRIMIDINE DIMERS FROM HUMAN CELLS EXPOSED TO LOW DOSES OF UV IRRADIATION. C. A. Smith and A. A. van Zeeland*, Dept. of Biological Sciences, Stanford University, Stanford, California 94305.

T4 endonuclease V, introduced into frozen-thawed mammalian cells (see accompaning abstract) recognizes only about half the endonuclease sensitive sites present in DNA purified from irradiated cells. To ensure that loss of these sites correlates with pyrimidine dimer removal and not changing frequencies of sites accessible to the enzyme, and to provide a positive control for the results with V79 cells, similar studies were undertaken with human cells. In WI-38 diploid fibroblasts irradiated with 5 J/m^2 UV, 33% of the endo sensitive sites were removed after 6 hr and 76% removed after 24 hr incubation post UV. To measure pyrimidine dimer removal directly, DNA from cells treated identically to those used for the assay for endo sensitive sites was purified by velocity centrifugation through CsCl of density 1.4 g/ml to a shelf of CsCl of density 1.8 g/ml. This removed contaminating small molecules and any short oligonucleotides still containing pyrimidine dimers, if present. Acid digests of the purified DNA were then analyzed by a modification of the chromatographic system of Cook and Friedberg (1) in quantities great enough to ensure reliable determination of pyrimidine dimer content at these low dimer frequencies. The results showed that 38% of the pyrimidine dimers were removed after 6 hr and 72% after 24 hr. XP12BE cells (complementation group A) showed little or no pyrimidine dimer removal in 24 hr following irradiation with 5 J/m^2.

The correspondence of these results indicates that the assay for T4 endo sensitive sites does give an accurate reflection of the removal of pyrimidine dimers and also demonstrates that those endo sensitive sites not recognized by the endonuclease in frozen-thawed cells do not represent a special class with respect to kinetics of removal.

It should be noted that the results from the assay for endo sensitive sites also demonstrates that, at least for WI-38 and V79 cells, stable nicks are not put into the DNA near most or all of the pyrimidine dimers immediately following their formation, since this would have reduced the molecular weight in irradiated cells not treated with the endonuclease.

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074 STUDIES ON THE MOLECULAR MECHANISMS OF NUCLEOTIDE EXCISION REPAIR IN UV-IRRADIATED HUMAN CELLS. Ursula K. Ehmann, Kem H. Cook, Jon I. Williams and Errol C. Friedberg, Laboratory of Experimental Oncology, Department of Pathology, Stanford University, Stanford, California 94305.

In normal human diploid cells most unscheduled DNA synthesis occurred within the first 4-5 hours after irradiation with UV light. The loss of thymine-containing pyrimidine dimers from the acid precipitable DNA of these cells did not occur until later; little, if any, taking place within the first 6 hours after irradiation. The discrepancy of the timing of these two events is the subject of our investigation. This apparently late removal of dimers is not an artifact of measuring TCA precipitates of cells which might include excised, dimer-containing oligonucleotides which are long enough to be acid precipitable. We tested this by measuring thymine dimer excision on high molecular weight DNA from which short, supposed dimer-containing excised oligonucleotides would have been excluded. The kinetics of dimer removal from such high molecular weight DNA were the same as the kinetics measured with whole cell precipitates. Furthermore, the kinetics of removal of pyrimidine dimers measured by dimer assays of isolated cellular chromatin was the same as measured in whole cell precipates. We are presently testing the hypothesis that, after endonucleolytic incision, repair replication fills in the gaps at the sites of dimers before exonucleolytic excision of the dimers actually takes place. Other possible explanations for the discrepancy between the times of unscheduled DNA synthesis and dimer removal are that the high levels of ^{3}H-thymidine prelabel necessary for measuring the dimer loss kinetics interfere with the removal of dimers from the DNA, or that unscheduled DNA synthesis is not related to the cellular process of dimer removal. The kinetics of the loss of thymine dimers from the acid-insoluble fraction of xeroderma pigmentosum variant cells is indistinguishable from that of normal cells. No significant loss of dimers was detected following UV irradiation of xeroderma pigmentosum cells from complementation groups A or D.

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Biological Consequences of DNA Damage and Repair

075 BIOLOGICAL AND BIOCHEMICAL EVIDENCE THAT DNA REPAIR PROCESSES IN NORMAL HUMAN CELLS ACT TO REDUCE THE LETHAL AND MUTAGENIC EFFECTS OF EXPOSURE TO CARCINOGENS. V. M. Maher, D. J. Dorney, R. H. Heflich, J. W. Levinson, A. Mendrala and J. J. McCormick. Carcinogenesis Laboratory, C.O.M. Michigan State University, Lansing, MI 48824.

Our studies comparing the cytotoxicity and mutagenicity of UV in cells from normal persons or from classical XP patients demonstrate that normal cells possess a remarkable capacity for excision of UV-induced damage which can reduce the potentially lethal and mutagenic effects of UV. The relative slopes of the survival and induced mutation frequency curves as a function of dose reflect the excision capacity of the different strains. When the frequency of mutations is analyzed as a function of the cytotoxic effect in these strains, it is approximately the same for all, suggesting that they handle lesions remaining unexcised in their DNA in a comparable manner. We compared the cytotoxicity of a series of reactive derivatives of chemical carcinogens in these strains to determine which show a greater cytotoxic effect in XP than in normal. Differential cell killing reflecting the UV-excision capacity of the strains was observed for N-acetoxy-2-acetylaminoflourene, -biphenyl, -phenanthrene, and -stilbene; "K-region" epoxides of benzo(a)pyrene(BP), benz(a)anthracene(BA), 7,12-dimethybenz(a)anthracene, and dibenzanthracene; two isomers of BP 7,8-diol-9,10-oxide and two 7-bromomethyl derivatives. No such differential was observed with N-methyl-N'-nitro-N-nitrosoguanidine, N-methyl-N'-nitrosourea, or methyl methanesulfonate. Each agent which caused differential survival also produced sites in DNA susceptible to the action of single-strand specific endonuclease S_1. These data suggest that each produced localized distortions in the helix which are recognized by the excision process. Exposure of normal cells to low doses of BP 4,5-oxide or BP 7,8-diol-9-10-oxide caused no decrease in survival. At higher doses, the cytotoxicity of the diol-epoxide increased sharply, that of the BP 4,5-oxide more gradually. When these compounds were tested for mutagenicity, neither induced mutations at non-toxic doses. At higher doses, the mutation frequency of the diol-epoxide increased rapidly, that of the 4,5-oxide less rapidly. However, when the frequencies induced in normal cells and in XP cells by these compounds, as well as the "K-region" epoxides of DMBA and DBA, were analyzed as a function of the cytotoxic effect, all were comparable. This suggests that although these metabolites differ in their potency and produce different carcinogen-DNA adducts, the cellular processes ultimately responsible for the cytotoxicity and mutagenicity of these agents handle them in a comparable manner. To measure excision repair, we exposed confluent cultures of normal cells to N-AcO-AAF (2.2 - 71.4uM), BP 4,5-oxide (8-280uM), BP 7,8-diol-9,10-oxide (2-70uM) for 2 hr or to UV (0.5-40 J/M^2) followed by ^{3}H-TdR-BrdUrd incorporation and alkaline CsCl gradients. Excision could be detected for N-AcO-AAF as low as 2.2uM (37% survival) but for BP4,5-oxide not until extremely toxic doses (70uM).

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076 DNA REPAIR IN DIFFERENTIATION Chev Kidson, Department of Biochemistry, University of Queensland, Brisbane 4067, Australia

There are two senses in which DNA repair systems could be related to cell differentiation: alterations in repair capacities concomitant with changing developmental state and direct dependence of differentiation on repair as such to effect or stabilize particular patterns of gene expression.

Some data have been interpreted as providing evidence for the first of these. Thus there are reports of reduced ability to repair some types of DNA damage in terminally differentiated cells such as myotubes and neurons compared with proliferating cell populations. Here, however, the cessation of DNA replication clearly constrains both the number of possible repair modes and the technical feasibility of their measurement. In neuroblastoma cells which have a capacity for reversible differentiation there is a suggestion that the induction of some repair modes is linked to the overall differentiation patterns. The anomalies seen in the nervous and immune systems in some human repair mutants suggest that there may be essential roles for certain repair enzymes in normal embryonic development quite apart from their roles in coping with extrinsically generated DNA damage.

The extensive chopping of DNA into fragments which occurs in certain ciliate protozoa during macronucleus formation constitutes a major requirement for repair related systems. Further, evidence of gene transposition during differentiation such as occurs at immunoglobulin and other loci, indicates that incision and recombination phenomena are essential for the production and stabilization of some developmental characteristics. It seems likely that currently available evidence represents only the tip of the iceberg with respect to the importance of gene movement and the associated requirement for appropriate repair systems in relation to some facets of development and differentiation. Just how ubiquitous such events are and the nature of the gene functions concerned is of great interest in relation to the consequences of mutant repair systems concerned therewith.

077 THE RELATIONSHIP BETWEEN CELLULAR AGEING AND GENETIC DEFECTS. R. Holliday, Genetics Div., National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

The growth of human diploid fibroblasts through numerous sub-cultures is invariably followed by senescence, degeneration and finally death of the whole cell population. It has been shown that the senescent phase of growth is associated with a number of genetic changes, including a rise in polyploidy and chromosome abnormalities, an increase in somatic mutation frequency, a reduction in repair capacity and a decline in the activity and fidelity of DNA polymerase. It is unlikely that these changes are the result of a gradual accumulation of recessive genetic defects throughout the lifespan, since tetraploid fibroblasts have the same longevity as diploid ones. Moreover, radiation treatment has not been shown to have any dramatic life-shortening effect. It is more likely that the accumulation of genetic damage during senescence is the final consequence of other changes which occur over a long period of fibroblast growth. One possibility is that spontaneous errors in the synthesis of proteins feedback into the major pathways of macromolecular synthesis, leading finally to a breakdown in the accuracy of information transfer between such molecules. According to this hypothesis, genetic damage can be a major cause of cell death, but it is not the underlying cause of the process of ageing.

Chemical Damage and Mutagenesis in Mammalian Systems

078 CELL KILLING AND MUTAGENESIS IN REPAIR DEFECTIVE CULTURED HUMAN CELLS, Colin F. Arlett, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9QG, England.

Cells from xeroderma pigmentosum patients are sensitive to 254 nm UV light when assayed in terms of cell killing or the induction of 6-thioguanine resistant mutants. Such cells are defective in either excision or post-replication repair(1) and thus support the concept that mutants and cancer arise as a consequence of mistakes during repair. Cells from Bloom's syndrome(2) and Cockaynes syndrome(3) are also sensitive to UV light for cell killing but have no known defects in repair, thus the existence of undefined repair process must be acknowledged. No data are available for mutagenesis with these syndromes but in cells from an unclassified sun-sensitive child(4) with similar sensitivity for cell killing and again no defects in repair processes, enhanced mutation frequencies are observed.

In the radiation-sensitive syndrome, ataxia telangiectasia (AT), all cell strains examined to date have exhibited enhanced sensitivity for cell killing by ionizing radiation(5) Some but not all of these strains are defective in an endonuclease specific for γ-ray induced base damage(6). Cells from both classes are hypomutable when assayed for 6-thioguanine resistant mutants after γ irradiation. This result may mean that these cells are lacking an error prone repair process. An alternative explanation is that γ ray mutations at the HGPRT locus represent deletion events which prove to be lethal mutations in AT cells.

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079 REPAIR OF 7-BROMOMETHYLBENZ(a)ANTHRACENE (7-BMBA) DAMAGED DNA IN MAMMALIAN CELLS, John J. Roberts, *Anthony Dipple, Frank Friedlos, *Billie A. McCaw, and Susan Young, Institute of Cancer Research, Pollards Wood Research Station, Chalfont St. Giles, Bucks., U.K., *NCI Frederick Cancer Research Center, Frederick, Md., USA.

Mammalian cells survive treatment with 7-BMBA both by excising lesions in DNA and by circumventing unexcised lesions during DNA replication by a caffeine-sensitive mechanism. There are several indications that the excision repair process(es) that remove(s) arylalkyl residues from mammalian cell DNA differs from that which removes UV irradiation induced thymine dimers. Firstly, arylalkyl residues unlike thymine dimers are excised with nearly equal efficiency from both rodent and human cells (1). Secondly an enzyme extract from M. luteus that incises DNA from UV-irradiated cells does not similarly incise DNA from 7-BMBA treated cells. Thirdly, a line of Xeroderma pigmentosum cells from complementation group C exhibited relatively different excision repair and survival responses following UV irradiation on the one hand or treatment with 7-bromomethylbenz(a)anthracene on the other. Moreover, whilst in the normal foetal lung cells and variant XP cells the ratio of hydrocarbon adenine adduct to hydrocarbon guanine adduct remaining in DNA decreased notably with excision, this ratio did not change significantly with excision in this particular XP cell line. The preferential loss of adenine damaged residues in normal and XP variant cells was similar to that observed in Chinese hamster and HeLa cells (1). This same selective excision has been observed in vitro by an endonuclease II preparation which removes this type of damage through an N-glycosidase activity (2). This suggests that 7-BMBA induced damage may be excised in mammalian cells by an N-glycosidase rather than a UV endonuclease.

The rate of DNA synthesis and the rate of elongation of DNA molecules was reduced in 7-BMBA-treated Chinese hamster cells relative to those in untreated or caffeine-only treated cells (3). Post treatment incubation in the presence of caffeine partially reversed the decreased rate of DNA synthesis in 7-BMBA treated cells but had a marked further decrease in the rate of elongation of DNA molecules. The size of nascent DNA synthesised during 3 hours under these conditions approximated to the distance between arylalkylations in the template DNA, suggesting that all adducts are circumvented by a caffeine sensitive so-called post replication repair process (3). In addition to these effects on newly-synthesised DNA, caffeine also induces the slow formation of breaks in template DNA (4). It is proposed that a combination of these effects of caffeine accounts for its ability to potentiate the cytotoxic and chromosome damaging effects of 7-BMBA.

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080 LACK OF EFFECT OF CAFFEINE POST TREATMENT ON UV AND EMS INDUCED MUTATION TO PURINE ANALOGUE RESISTANCE IN V79 CHINESE HAMSTER CELLS, Margaret Fox and S. McMillan, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.

Two different experimental protocols have been used to measure kinetics of mutation induction after EMS treatment and UV irradiation. Both 8-azaguanine and 6-thioguanine were used as selective agents. Selection of cells as minicolonies using the in situ technique resulted in curvilinear dose response relationships whereas when a respreading technique was used i.e. the selective agents were applied to single cells, linear induction kinetics were observed. A number of artefacts contributing to the non-linear response obtained with the in situ technique have now been identified.

The hypothesis that mutants arising in heterogeneous colonies would be lost as a result of metabolic co-operation when in situ selection is used, was tested by comparing in situ and replated frequencies within the same experiment after mutagenising single cells. Contrary to expectation, in situ frequencies exceeded replated frequencies. Thus, the majority of mutants must arise in homogeneous colonies, i.e. they must be the result, as are chromatid and chromosome aberrations, of damage to both strands of the DNA helix. To account for linear induction kinetics however initially single strand events must be converted to double strand events at a constant probability with increasing dose.

Post-treatment exposure to caffeine, markedly potentiated cell killing by both mutagens, but had no effect on the frequency of induced mutants in the presence of either selective agent using either protocol. In allowing a higher frequency of conversion of single strand to double strand events (by slowing post-treatment gap filling) caffeine enhances chromosome damage and cell killing. The lack of a caffeine effect on induced mutant frequencies therefore suggests that the lesions resulting in mutations differ either in their nature or mode of repair from those resulting in lethal damage.

081 CHEMICAL MODIFICATION OF RADIATION-INDUCED MUTAGENESIS AND ITS ROLE IN CARCINOGENESIS, James E. Trosko and Chia-cheng Chang, Department of Human Development, E. Lansing, Michigan 48824.

The role of DNA damage in mutation fixation and carcinogenesis is well documented.[1] Theoretically, many non-mutagenic chemicals, known to modify carcinogenesis, can modify (a) the amount of DNA damage; (b) the repair of that molecular damage; and (c) the expression of the genetic consequences of the DNA damage and its repair. Various non-mutagenic, tumor-modifying chemicals were tested for their effects on UV-induced ouabain-resistant (oua^r) mutations. Those tested were shown to modify the frequency of mutations by affecting DNA repair processes or the expression of induced mutations.

Treatment of caffeine, harmane, or norharman immediately following UV irradiation significantly reduced the cell survival. The mutation frequency was increased with caffeine treatment but reduced with harmane or norharman treatment. Fractionation of UV-irradiation always increased the survival frequencies, but the mutation frequencies were either unaffected or reduced. Treatment with cycloheximide between fractionated UV-irradiations resulted in two types of effects. Long exposure to cycloheximide (i.e., > 6 hr) always resulted in reduced survival and enhanced or unchanged mutation frequency. Exposure to cycloheximide in the short fractionation regime (i.e., 4 hr) tended to give the opposite effects. The results suggest the presence of an error-free and error-prone post replication repair mechanisms in mammalian cells.

TPA, a powerful tumor promoter, increased the recovery of UV-induced oua^r mutants. Similar effects were observed with papaverine or insulin treatments. Antipromoters, such as fluocinolone acetonide, dexamethasone, retinoic acid, caffeine and dibutyryl cAMP on the other hand reduced the recovery of UV-induced oua^r mutants when present on or after the mutation expression time. These results suggest that many induced mutations can either be repressed or derepressed by agents which can alter cyclic AMP levels. The results also support the hypothesis that tumor initiation is due to a mutagenic event, while tumor promotion is the result of an epigenetic process involving cyclic nucleotide modulation of gene expression. Research was supported by grants from the National Cancer Institute (CA 13048-05, CA 21107-01).

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082 RESPONSE OF MAMMALIAN CELLS TO CHEMICAL DAMAGE. Bernard Strauss, Kallol Bose, Manuel Altamirano-Dimas, Peter Karran and Kouichi Tatsumi. Department of Microbiology, University of Chicago, Chicago, Illinois 60637.

DNA excision repair occurs by both nucleotide excision and base excision (apurinic) pathways. Only the apurinic repair pathway is characterized by the appearance of numerous breaks in the DNA, indicating that there are different limiting steps in the two pathways. We have reconstructed the apurinic repair pathway with enzymes from a human lymphoma cell line plus T4 DNA ligase. This system repairs apurinic sites in T7 bacteriophage DNA and it appears that the limiting step is a reaction catalyzed by a 5'-3' exonuclease which removes dinucleotides and other products from incised DNA.

The two excision repair pathways operate independently and a given cell type may be efficient for one but not the other. Since single compounds may react with DNA to give lesions which are repaired by different pathways, it is not always possible to judge the nature of the pathway from the compound inducing repair. Furthermore, it is still not clear which pathway is followed in the removal of certain kinds of damage. For example, it is not known which pathway is responsible for the removal of O-6 methyl guanine adducts from DNA nor is it understood why xeroderma cells are deficient in this reaction.

Organisms need not remove lesions but rather may bypass the adducts. Benzpyrene residues are reported as absolute blocks to DNA polymerase I. However, we find that cells treated with ^{3}H-anti benzpyrene diolepoxide can replicate their DNA and that the ^{3}H is found in the light strand of the resulting hybrid DNA. We think that this represents a bypass of the lesions and we suppose that the bypass occurs by a mechanism involving branch migration and not single strand gaps. However, most of the branch migration previously observed occurs *in vitro* in bromodeoxyuridine-containing DNA.

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083 MAMMALIAN CELL MUTAGENESIS AS A BIOLOGICAL CONSEQUENCE OF DNA DAMAGE, J. Justin McCormick and Veronica M. Maher, Carcinogenesis Laboratory, College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

There is abundant evidence from studies comparing repair proficient cells with repair deficient cells that DNA excision repair processes in bacteria can decrease the cytotoxic and mutagenic effect of exposure to UV, X-rays, 4NQO, alkylating agents, polycyclic aromatic compounds, mitomycin C, etc. We have recently demonstrated that this is also true of excision repair in diploid human cells exposed to UV or to reactive metabolites of polycyclic hydrocarbons and aromatic amide carcinogens. We compared the percent survival (cloning ability) and the frequency of mutations induced by UV in normal cells and in a series of xeroderma pigmentosum strains with different capacities for excision of UV damage. A distinct shoulder was observed on the survival curve for the normally-excising cells which was absent for the XP's and the relative slopes of the XP curves reflected their capacity for excision repair. A similar relationship was found for the frequency of mutations induced in these cells. When the irradiated cells were prevented from dividing, but allowed to excise for various times post-irradiation, the lethal and mutagenic effects of UV were gradually eliminated not only in the normal cells but also in XP's with residual excision capacity. This is evidence that excision is not responsible for causing mutations. Rather, they result from unexcised lesions in DNA. When the mutation frequency was analysed as a function of the cytotoxic effect, there was no significant difference between the strains, suggesting that they all handle lesions remaining unexcised in a similar fashion. The data also indicate that the lesions responsible for cell killing and for mutation induction are the same or, if different, remain in a constant ratio in the DNA even as excision is taking place.

Evidence that in E. coli particular gene products are responsible for the induction of mutations caused by unexcised lesions comes from elegant biochemical and biological experiments using a battery of repair deficient mutants. No such series is yet available in mammalian cells. The latter may handle unexcised damage in a number of ways during DNA replication, e.g. by somehow inserting nucleotides across from non-instructive bases; by temporarily stopping replication on the damaged strand before reaching the lesion while continuing it on the opposite strand and then, by means of strand displacement, using the newly-replicated strand as a template; by physically exchanging the damaged portion of the parental strand for part of a daughter strand; or by interrupting synthesis near the lesion and re-initiating it at a subsequent site, leaving a gap in the daughter strand to be filled by de novo synthesis or by recombination. We will review the supporting data for the possible mechanisms and discuss the evidence for and against each process being involved in the mechanism(s) of mammalian cell mutagenesis. (Supported by Natl. Cancer Institute Grants CA 21247 and CA 21253.)

Environmental Mutagens and Carcinogens

084 ENVIRONMENTAL CHEMICALS CAUSING CANCER AND GENETIC BIRTH DEFECTS: DEVELOPING A STRATEGY FOR MINIMIZING HUMAN EXPOSURE, Bruce N. Ames, Department of Biochemistry, University of California, Berkeley, CA 94720.

Damage to DNA appears to be the cause of most cancer and genetic birth defects and may contribute to aging and heart disease as well. A major part of this DNA damage is caused by environmental chemicals, both natural and man made. Many more chemicals will be added to the current list of human carcinogens and mutagens. Since the late 1950s we have been exposed to a flood of chemicals--from flame retardants in our children's pajamas to pesticides accumulating in our body fat--that were not tested for carcinogenicity or mutagenicity before their use. In the past this problem has been largely ignored; even high-production chemicals, with extensive human exposure, have been produced for decades without adequate carcinogenicity or mutagenicity tests. A few of these chemicals are now being tested in animals, but for most of them the human population is serving as the test animal. Because the 20- to 30-year lag time for chemical carcinogenesis in humans is almost over, the incidence of cancer may increase steeply if too many of the thousands of new chemicals to which humans have been exposed turn out to be powerful mutagens and carcinogens. We must identify the agents that have caused the cancer and genetic birth defects of today (many of these are natural compounds present in our diet as complex mixtures) and test the many man-made chemicals that have been introduced into the environment in the last few decades. Existing animal tests and human epidemiology alone are inadequate procedures for this task for a number of reasons including time, expense, and the difficulty of dealing with complex mixtures.

Over the last 14 years we have developed a simple method for identifying chemical mutagens, and we have shown that almost all chemical carcinogens are mutagens. This test combines on a petri plate special strains of *Salmonella* bacteria (as indicators of reverse mutation) and mammalian liver homogenates (rodent or human autopsy--to provide mammalian metabolism). We have validated the test for detection of carcinogens as mutagens by testing over 300 chemicals and have reported that almost all chemical carcinogens tested are mutagenic in this test (157/175) and most "non-carcinogens" (95/108) are negative. A number of the "false positives" generated by this study appear to be explainable as consequences of statistical limitations of animal carcinogenicity tests. The test is particularly useful for the detection of mutagens/carcinogens in complex mixtures (such as cigarette smoke, water, air pollution, food, and urine). It is also useful in the development of drugs and industrial chemicals where large numbers of chemicals must be screened. Most of the major drug and chemical companies in the world are now using the test system.

We are exposed to a very large number of chemicals that are mutagens and carcinogens, many of them quite useful for society, and it is clearly impractical to ban them all, yet foolish to ignore their potential danger. We must have some way of setting priorities for regulation of these chemicals, and this requires an assessment of human risk. We have shown by the quantitative analysis of animal cancer tests that there is over a million-fold range in the strength of carcinogens, and this knowledge, combined with knowledge on human exposure, may enable an assessment of human risk to be done in a more rational manner. Because few chemicals (or mixtures) in the environment have been tested in animal cancer tests, we need additional ways of obtaining information as to the mutagenic and carcinogenic danger of chemicals. There appears to be a correlation between mutagenic potency in *Salmonella* (and other short-term tests) and carcinogenic potency. Thus it appears likely that potency in a battery of short-term tests (many good ones have now been developed) will be able to be used as an aid in human risk assessment.

Repairable Damage in DNA

085 SEROLOGIC ASSAY OF DNA BASE DAMAGE, Hazel L. Lewis and John F. Ward, Lab. of Nuclear Medicine and Radiation Biology, Univ. of Calif., Los Angeles, 90024.

We have developed assays for several ionizing radiation products, UV induced pyrimidine dimers, and ethylnitrosourea produced O^6-ethylguanosine. A phage neutralization assay for hydroxymethyluracil (HMU), an ionizing radiation product of thymine, has been used to detect product within single stranded DNA at relatively low doses. Product was also determined after enzymatic hydrolysis of DNA to demonstrate the validity of the method for quantitation within the polymer. A "competitive" method in which antiserum was absorbed in the presence of HMUdR decreased cross reaction with thymidine relative to HMUdR from a 4 x 10^2 fold to 10^4 fold difference.

An assay for 8,5' cycloadenosine, an irradiation product of 5' AMP has enabled us to quantitate product in irradiated solutions of adenosine, deoxyadenosine, and DNA. Assay of product formed in these materials was not feasible by chemical procedure. (With J.A. Raleigh, Whiteshell Nuclear Research Established Canada).

A radioimmune assay utilizing ^{125}I labeled DNA has been developed for the quantitation of UV lesions. (With L. McConlogue, UCLA). Kinetics of removal of lesions by this method agrees with published results in HeLa cell excision repair studies.

We have recently prepared antibodies to O^6ethyl guanosine. (With R. Goth-Goldstein, UC Berkeley) which are extremely sensitive and specific. There is no cross reaction with guanosine or N^7ethylguanosine.

This work was supported by USPH Grant #CA13437 and U.S. Dept. of Energy.

086 A RADIOIMMUNOASSAY FOR CARCINOGEN-DNA ADDUCTS. Poirier, M.C., Dubin, M.A. and Yuspa, S.H. National Institutes of Health, Bethesda, MD 20014

A number of chemical carcinogens interact with DNA to yield multiple reaction products, some of which are more likely to induce mutagenic or carcinogenic events. To determine whether repair processes selectively remove certain adducts, techniques which can quantitate specific reaction products and assay their removal must be developed. To this end radioimmunoassay (RIA) has been utilized to study the interactions of the carcinogen N-acetoxy-2-acetylaminofluorene (N-Ac-AAF) with DNA. Previous studies have shown that the major in vivo and in vitro adducts formed are N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-8-AAF) and 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene (dG-N^2-AAF) which occur in about 80% and 20% incidences respectively. In vivo a substantial amount of the C-8-substituted adduct is in the deacetylated form, dG-8-AF, and both C-8 adducts can be removed, presumably by DNA repair, while the N^2-derivative persists. In the present study rabbits were immunized with dG-8-AAF conjugated to bovine serum albumin. The resulting antiserum contained antibodies to both dG-8-AAF and dG-8-AF which cross-reacted to allow detection of 0.5 pmoles of total C-8 adduct by RIA using ^3H-dG-8-AAF. As little as 0.15 pmoles of dG-8-AF could be determined alone using ^3H-dG-8-AF in the RIA. The whole antiserum recognized individual nucleosides or AAF-derivatives only above 2000 pmoles, and dG-N^2-AAF above 200 pmoles. When cultured cells were exposed to 10^{-5} M N-Ac-AAF for 1 hr, 7-16 pmoles of C-8 adduct were detected per 100 µgm of DNA. This level is sufficiently high to allow for assay of specific removal of C-8 adducts with time after exposure to the carcinogen, and preparation of multiple specific antibodies should allow for the study of the removal or persistence of each reaction product.

087 ENDONUCLEASE PROBES OF NON-S-DEPENDENT CLASTOGEN-DNA INTERACTION PRODUCTS, J.E.T. Kelley and M.A Bender, Medical Research Center, Brookhaven National Laboratory, Upton, NY 11973

Characterization of the products of interaction of DNA with chemical clastogens can be used to test models of chromosome aberration production. The effects of clastogen-DNA interaction are assayed by agarose gel electrophoresis of PM2 DNA which allows strand breakage to be detected. Bleomycin (BLM), streptonigrin (STN), neocarzinostatin (NCZ), adriamycin (ADM) and 8-ethoxycaffeine (EOC) were selected for study on the basis of evidence that they could produce clastogenic effects independently of DNA synthesis, whereas most chemical clastogens appear to act in an S-dependent manner. Current models of chromosome aberration formation by non-S-dependent clastogens predict that these compounds act: 1) directly on DNA to produce strand breaks; 2) by producing structural alterations in DNA which are unstable and which generate strand breaks by secondary, nonenzymatic processes; or 3) by forming interaction products with DNA which are endonuclease-sensitive sites. Direct DNA strand scission by BLM, STN and NCZ has been reported and these compounds produce strand breaks in DNA in this assay whereas, ADM, EOC and Ara-C do not. These unnicked interaction products could contain endonuclease-sensitive sites and preliminary results indicate that ADM treated PM2 DNA is nicked by Micrococcus luteus extract (MLE). Data pertaining to EOC treated DNA assayed with MLE, partially purified Micrococcus endonucleases and S_1 nuclease will also be presented.

088 DNA BINDING PROPERTIES OF T4 ENDONUCLEASE V, P. C. Seawell, T. J. Simon and A. K. Ganesan, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

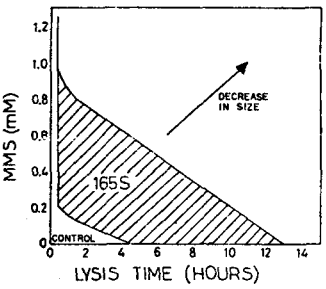
T4 endonuclease V has become an important biochemical probe for identifying and quantifying UV-induced pyrimidine dimers in DNA because it produces single strand nicks adjacent to dimers. To complement studies that have characterized the incising properties of T4 endo V, we have developed a nitrocellulose filter assay to investigate the DNA binding properties of the enzyme. In our standard incubation conditions of 3 minutes at 0°C in a 0.3ml reaction volume containing 100mM NaCl, 10mM Tris, 10mM EDTA (pH 8) and 25-200ng radioactive DNA, T4 endo V rapidly forms a specific complex with UV-irradiated DNA which can be partially stabilized with concentrated saline citrate, the stop buffer. That the binding activity we observe is due to T4 endo V is supported by two lines of evidence: (1) Mg-independent, UV-specific DNA binding activity is present in crude lysates of E. coli infected with the bacteriophages T4D (wild type), T4amN82 and T4amN82-rPT8-nd28x6 (both endo V^+), but this activity is absent in lysates of uninfected cells or cells infected with $T4v_1$ (endo V^-). (2) The binding activity co-chromatographs with Mg-independent, pyrimidine dimer-specific nicking activity through DEAE cellulose and phosphocellulose. The amount of DNA-enzyme complex retained on filters is proportional to UV dose, DNA and enzyme concentration, and concentration of the saline citrate stop buffer. Sodium cyanide added to the stop buffer reduces the rate of dissociation of the filter bound complex. Because of its specificity and simplicity, the binding assay has been used to detect T4 endo V activity in crude lysates and to monitor its purification. Furthermore, association and dissociation constants obtained from the binding assay may help elucidate the mechanism by which T4 endonuclease V recognizes and binds to pyrimidine dimers.

089 THE SENSITIVITY OF UV DAMAGED DNA TO BAL SINGLE STRAND SPECIFIC ENDODNASE, Julie Harless and Roger Hewitt, M. D. Anderson Hosp. & Tumor Inst., Houston, TX 77030

Radiation and chemical can alter DNA structure by producing DNA strand breaks, base damage, or distorting the conformation of the helix. DNA strand breakage can be measured by electrophoretic or sedimentation procedures and damage specific endoDNases are useful tools for identifying some types of base damage. We are attempting to develop a more general assay to identify structural damage, which might be recognized as distortions in helical structure. Gray, et al. (Nucleic Acid Res. 2: 1459, 1975) have characterized an extracellular DNase from Pseudomonas BAL 31, which may be a useful probe for distortion damage. Superhelical covalent circular (cc) PM2 DNA is a substrate for this enzyme, whereas relaxed ccDNA is not. Relaxed ccDNA that has been exposed to ultraviolet light or some chemicals is recognized by the BAL endoDNase as a substrate (Gray, personal communication). Using untreated supercoiled DNA and UV irradiated relaxed DNA as substrates, reaction conditions were selected which give maximum activity on irradiated DNA with minimal activity on untreated relaxed DNA. The enzyme has a pH optimum in the neutral range, an absolute requirement for calcium, and is stimulated by magnesium. Temperatures between 30 and 37C allow activity on irradiated DNA; above 40C, relaxed DNA is also a substrate. Salt concentrations above 500mM allow activity only on irradiated DNA; below this, relaxed DNA is also cleaved. Superhelical DNA shows the same requirements and optima as irradiated DNA. The efficiency of the BAL endoDNase on UV-irradiated DNA was compared to the T4 UV-endoDNase. The BAL enzyme has less than 50% of the activity of the UV-endo on the same substrate. The BAL enzyme may be less efficient in recognizing pyrimidine dimers, or it may be incising at the site of a different photoproduct.

090 APPLICATION OF ALKALINE SUCROSE GRADIENT SEDIMENTATION TO THE STUDY OF DNA DAMAGE AND ITS REPAIR IN MAMMALIAN CELLS TREATED WITH METHYLMETHANESULFONATE AND 4-NITROQUINOLINE 1-OXIDE, Ian G. Walker and Robert Pavlis, Dept of Biochem, Univ Western Ontario, London,Canada

KB cells and L cells were treated with MMS or 4NQO and the resulting damage to DNA and its repair were examined by sedimentation in an alkaline sucrose gradient. The sedimentation profiles obtained were found to be the resultant of a complex interaction between drug dosage, duration of the lysis period and the repair capacity of the cells. A systematic study of these variables was made. DNA with sedimentation coefficient of 165S is formed after various combinations of drug dosage and lysis time. 165S DNA appears to arise as a natural consequence of the shearing forces at play during the lytic process that precedes centrifugation. DNA smaller than 165S is formed when the drug-lysis time combination exceeds a critical value. The effect of these variables can be displayed in the form of a phase diagram. Both drugs produce two kinds of DNA modifications which show up as single strand breaks but affect the sedimentation profile in characteristic ways. One of these modifications which is quite alkali-labile can be studied using a 30 min lysis period. The other modification is less alkali-labile and can be studied using a long lysis period. Both KB cells and L cells can repair the former type of damage but only KB cells can repair the latter type of damage.



091 NON-ENZYMATIC DNA STRAND BREAKS INDUCED IN MAMMALIAN CELLS BY FLUORESCENT LIGHT, Matthews O. Bradley, Leonard C. Erickson, and Kurt W. Kohn, Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014
Fluorescent light is both toxic and mutagenic to V-79 Chinese hamster lung cells (Bradley, M. O., and Sharkey, N. A., Nature (1977) 266, 724-726). The effects appeared to be mainly due to an interaction of light with the cells themselves rather than to photochemical reactions with components of the medium. In this work we have attempted to correlate in a mammalian cell the mutagenicity and toxicity of fluorescent light with the DNA damage that it produces. We have found that fluorescent light (3.6 $J/m^2/sec$) induces 0.041 single-strand breaks per 10^8 daltons per hr in the DNA of cultured Chinese hamster cells. The breaks are induced at 1°C and hence are not likely to be the result of endonuclease incision. The lesions producing the breaks do not appear to be alkaline sensitive. When the cells are incubated at 37°C, the breaks are rejoined within 2 hours. At least two lesions are responsible for the observed effects. One lesion has the ability to break DNA subsequently treated with alkali but is neither toxic nor mutagenic. This lesion is produced by light of wavelength greater than approximately 350 nm. The other lesion(s) produce mutagenicity and/or toxicity, but do not necessarily produce strand breaks. These lesion(s) are produced by light of wavelength less than 350 nm. Incandescent light is neither toxic nor mutagenic to V-79 cells, however, it does produce DNA strand breaks. Further analysis of the biological and biochemical effects of various wavelengths of light may allow us to classify DNA lesions on the basis of their biological effects.

092 DETECTION OF DNA DAMAGE AND REPAIR INDUCED IN VITRO AND IN VIVO USING THE ALKALINE ELUTION ASSAY, J. Swenberg and G. Petzold, The Upjohn Company, Kalamazoo, MI 49001
Many new "short-term" tests are being reported for screening chemicals for carcinogenic and mutagenic potential. With few exceptions, these assays have been evaluated on small numbers of chemicals, most of which do not require metabolic activation. The alkaline elution assay, which measures single strand breaks, alkali-labile sites, and cross-linking in DNA, has been coupled with an S-9 metabolic activation system to evaluate the ability of over 100 chemicals to induce DNA damage in V-79 cells. Carcinogenicity data was available on 68 of these compounds. All proximate, ultimate, and non-carcinogens gave elution patterns consistent with the theory that carcinogens cause DNA damage, while non-carcinogens don't. The carcinogen HMPA, which has given negative results in most mutagenesis assays, did not cause an increase in DNA elution. When coupled with activation, HMPA induced cross-linking of DNA, resulting in dose-related decreases in DNA elution. Nine other procarcinogens failed to cause an increase in elution. Most of these same compounds have been negative in mutagenesis assays. Since one of the primary concerns in testing procarcinogens is metabolic activation, we modified the assay so that DNA damage could be measured in homogenates of 10 tissues from animals treated in vivo. Procarcinogens were found to induce the greatest extent of DNA damage in target organs for tumor induction, whereas in vivo exposure to several direct acting alkylating agents caused significant DNA damage in target and non-target tissues. DNA repair time, as measured by the return to a normal elution pattern, varied from as little as 24 hours for 4NQO to as long as 7 days with DMN.

093 DOUBLE AND SINGLE DNA STRAND BREAKAGE AND REPAIR IN ESCHERICHIA COLI MEASURED IN SUPERINFECTING PHAGE λ DNA[1], Erik Boye [2,3] and Robert E.Krisch [2,4].
DNA double-strand breaks (DSBs) are usually measured by sedimentation of chromosomal DNA in neutral sucrose density gradients. The resolution of this method is limited because of shear degradation of the DNA, speed artifacts during sedimentation, and association of DNA with other cell components. The reparability of DSBs in E.coli is disputed, mainly because of the technical difficulties cited. We have used the shear resistant superinfecting phage λ DNA to measure DSBs, single-strand breaks (SSBs), and their repair. Intracellular phage λ DNA with either a DSB, a SSB, or no breaks can be separated in neutral sucrose gradients. Thus the number of DSBs and SSBs in a λ DNA population can be assayed in the same gradient.
Lysogens of E.coli have been superinfected with ^{125}I-labelled phage λ DNA and the cells were stored in liquid nitrogen. Decays of ^{125}I were found to induce breaks at a ratio of 0.4 SSBs per DSB, and no evidence for repair of either type of break was obtained. Decays of ^{3}H incorporated into the DNA created mainly SSBs, which were rapidly repaired on incubation. Preliminary experiments with external ionizing radiation indicate that the SSB/DSB ratio is about 10. The SSBs are efficiently repaired, while the DSBs seem to remain unrepaired.

[1] Work supported by U.S. Energy Research and Development Administration.
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[3] Dept of Med Physics, The Norwegian Radium Hospital, Oslo 3, Norway (present address).
[4] Dept of Radiation Therapy, Harvard Medical School, Boston, MA 02115 (present adress).

094 VISCOELASTIC MEASUREMENT OF DNA DAMAGE, Elliott L. Uhlenhopp, Grinnell College, Grinnell, Iowa 50112.

Recent application of the technique of viscoelastometry has shown that this method is capable of measuring the molecular weight of chromosome-sized DNA molecules from simple eucaryotes. The technique should therefore be useful for detecting small amounts of DNA damage and its subsequent repair by measuring double- and single-strand molecular weights before and after irradiation. Previous experiments on mouse 3T3 cells have already revealed drastic changes in ss MW resulting from biologically-significant doses of x-rays (1). We are currently conducting viscoelastic radiation studies on the protozoan Tetrahymena (2), an organism which is sensitive to uv irradiation but remarkably resistant to x-irradiation, in an attempt to discover whether its DNA is uniquely resistant to breakage by x-rays (3), whether it possesses a particularly active repair system, or whether some other factor unique to Tetrahymena is operating.

1. E.L. Uhlenhopp "Viscoelastic Analysis of High Molecular Weight, Alkali-Denatured DNA from Mouse Cells" Biophys. J. 15, 233 (1975).
2. J.B. Williams, E.W. Fleck, L.E. Hellier and E.L. Uhlenhopp "Viscoelastic Studies on Tetrahymena Macromolecular DNA" submitted for publication.
3. K. Pasupathy, M.S. Netrawali, D.S. Pradhan, A. Sreenivasan "Repair of Radiation-Induced Strand Sessions in Nuclear and Mitochondrial DNAs in Tetrahymena pyriformis Rad. Res. 66, 147 (1976).

095 AN ACTION SPECTRUM FOR CELL KILLING AND PYRIMIDINE DIMER FORMATION IN CHINESE HAMSTER V-79 CELLS, R. H. Rothman and R. B. Setlow, Dept. Biology, Brookhaven National Laboratory, Upton, L.I., N.Y., 11973.

There is a close correlation in bacteria and phage between the absorption spectrum of DNA, the formation of pyrimidine dimers, and biological measurements such as UV survival and mutagenesis. For mammalian cells, however, the correlation between the DNA absorption spectrum, cell death, and chromosome damage is poor. These observations have been used to argue that molecules other than DNA play an important role in causing cell death and chromosomal damage. Due to the scanty amounts of data for mammalian cells, we have pursued the matter further by determining an action spectrum for pyrimidine dimer production and cell death. The data, summarized in the table below, are experessed as percentage of radioactive thymine converted to pyrimidine dimers per quantum m^{-2}, and the inverse of the quanta m^{-2} causing 10% survival, and have been normalized to 1 at 265 nm. It is clear that there is a close correlation between pyrimidine dimer production and cell death.

Wavelength (nm):	254	265	280	290	297	302	313
% Dimers/Quantum m^{-2}:	1.26	1	0.78	0.51	0.11	0.027	0.0005
1/Quanta m^{-2} 10:	0.86	1	0.67	0.44	0.09	0.021	0.0003

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096 Non-dimer UV Damage in DNA and Synthetic Polydeoxyribonucleotides, Ross S. Feldberg, Dept. Biology, Tufts University, Medford, Mass 02155

Using a DNA-binding protein isolated from human placenta and specific for some form of non-pyrimidine dimer UV damage, we have begun to explore the properties and nature of this DNA damage. This damage is introduced into T_7 DNA at low fluences (67% of the DNA is bound to protein in a filter binding assay at a fluence of 13 J/m^2) and is stable to mild heat. This non dimer damage is also introduced into poly d(A-T) at low fluences of 254 nm light. Heating poly d(A-T) to temperatures slightly below its T_m (ca. 55°C) results in a loss of the damage. Concomitant with this loss, there is a decrease in the size of the polymer upon alkaline sucrose density gradient centrifugation. These results allow us to estimate a rate of this non-dimer damage introduction into poly d(A-T) to be about 0.8 sites per 1×10^7 daltons per 100 J/m^2. Heating at alkaline pH does not result in a loss of this damage. Experiments with the triplet state sensitizers acetone and acetophenone suggest that the damage is not due to triplet state thymine photoproducts, but can be formed from triplet state adenine. This may suggest an adenine photoproduct or the involvement of an A-T excimer in the formation of a thymine photoproduct.

097 PHOTOREACTIVATION OF *Escherichia coli* IRRADIATED BY IONIZING RADIATION, Tzu-chien V. Wang and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305.

Photoreactivation has been observed in γ-irradiated cells of *uvrA recA* and *uvrB lexA* of *E.coli* K-12 (1). By comparing the results for a *uvrA recA* and a *uvrA uvrB recA phr* strain, we conclude that this photoreactivation involves the same enzyme that is responsible for the photoreactivation of UV-induced cyclobutadipyrimidines. Comparison of photoreactivable sectors in a *uvrA recA* strain after UV and ^{137}Cs γ-irradiation indicates that 10 krads of γ-irradiation produces about 0.07 Jm^{-2}-equivalents of 254 nm-induced photoreactivable damage. After 400 krads, an acid-hydrolysate of the isolated DNA revealed the presence of thymine-containing cyclobutadipyrimidines as evidenced from their chromatographic properties, and their susceptibility to UV-reversal. Thus, the photoreactivation observed after ionizing radiation in strains that are blocked in excision repair and at least partially blocked in postreplication repair is due to the production of trace amounts of cyclobutadipyrimidines.

1. M.N. Myasnik and I.I. Morozov, Int. J. Radiat. Biol., 31, 95-98, 1977.

098 REPAIR OF NEAR-ULTRAVIOLET (365nm) DAMAGE TO BACTERIOPHAGE, Rex M. Tyrrell, Inst. de Biofísica, UFRJ, Rio de Janeiro, BRAZIL.

Damage to DNA by near-ultraviolet (UV) radiation has been measured both in vivo in bacterial cells and in intact and extracted DNA of bacteriophage. Intact bacteriophage have been irradiated at 365nm and then infected into their bacterial host to test susceptibility of the damage to both phage and host-cell mediated repair systems. Near-UV damage induced in T4 is susceptible to x-gene reactivation to approximately the same extent as 254nm radiation (repair sector, approximately 0.5). V-gene reactivation acts on an even greater fraction of the near-UV damage (repair sector of 0.82 at 365nm as against 0.66 at 254nm). The host-cell mediated photoreactivation system is only slightly less effective for near-UV damage. Host-cell reactivation (as measured by comparing survival of phage λ on a uvr^+ and a uvr host) is effective against a far smaller sector of near-UV damage (0.35) than far-UV damage (0.85).Weigle reactivation of near-UV damage to phage λ does not occur and pre-irradiation of the uvr^+ host-cell with far-UV, under conditions which favour a large sector of repair of far-UV damaged phage, completely eliminates host-cell reactivation of the near-UV lethal damage. It is concluded that unless the phage damage is repaired immediately after infection, the lesions rapidly lose their susceptibility to repair with consequent loss of activity of the phage particles.

099 DNA POLYMERASE I-MEDIATED REPAIR OF 365 NM-INDUCED SINGLE-STRAND BREAKS IN THE DNA OF ESCHERICHIA COLI, Ronald D. Ley, B. Ann Sedita, and Erik Boye.

Irradiation of closed circular phage λ DNA in vivo at 365 nm results in the induction of single-strand breaks and alkali-labile lesions at rates of 1.1×10^{-14} and 0.2×10^{-14} per dalton per J/m^2, respectively. The sum of the induction rates is similar to the rate of induction of single-strand breaks plus alkali-labile lesions (1×10^{-14} per dalton per J/m^2) observed in the Escherichia coli genome. Postirradiation incubation of wild-type cells in buffer results in rapid repair of the breaks (up to 80% repaired in 10 min). No repair was observed in a DNA polymerase I-deficient mutant of E. coli.

100 UV INACTIVATION OF DNA IN SOLUTIONS CONTAINING AMINO ACIDS AND PROTEINS, Lyndon L. Larcom and William F. McNeill, Clemson University, Clemson, SC 29631

UV irradiation of DNA isolated from B. subtilis phage SPO2c1 resulted in a simple logarithmic inactivation curve. When irradiation was done in SSC the D_{37} was 114 ergs/mm^2. In 0.1 SSC or 0.04 SSC, D_{37} was 81 ergs/mm^2.

The UV sensitivity of SPO2c1 DNA in SSC was increased when irradiation was performed in solutions containing tryptophan amide, calf thymus histone type VII or β lactoglobin. For DNA in 0.1 SSC, the sensitivity was increased in solutions containing lysozyme, but was decreased in solutions of tryptophan, phenylalanine amide, tyrosine amide, or lysine.

When DNA in 0.1 SSC was irradiated in the presence of both calf thymus histone and tryptophan, the effects of these components were not additive. Instead, the observed sensitivity was significantly increased over that predicted for the mixture. A similar effect was obtained for DNA in mixtures of tryptophan and lysine and tryptophan and cysteine. These results indicate: 1) tryptophan might act to enhance a DNA-amino acid interaction which increases the sensitivity of DNA to biological inactivation by UV, 2) SPO2c1 transfection might be useful in searching for an enzyme system capable of repairing DNA crosslinked to proteins.

101 MOLECULAR MECHANISMS OF BLEOMYCIN DAMAGE TO DNA, Lawrence Povirk, Wolfgang Köhnlein and Frankiln Hutchinson, Yale University, New Haven, CT 06520

Production of single-strand breaks, double-strand breaks, alkali-labile bonds and released free bases was examined quantitatively. At low doses, formation of all lesions was directly proportional to bleomycin concentration. All four bases were released, but pyrimidines were strongly preferred. The ratio of single- to double-strand breaks was 9:1 over a wide range of bleomycin concentrations and under a variety of conditions, suggesting that the double-strand breaks are not coincidences of single-strand breaks, but are independent events, probably involving only one bleomycin molecule. Fe^{++} is apparently a cofactor in these reactions, and all bleomycin-induced lesions except the double-strand breaks could be produced by high concentrations of Fe^{++} in the absence of bleomycin. At pH 5.5, bleomycin did not break DNA, but bleomycin binding relaxed supercoiled DNA, suggesting intercalation. Bleomycin binding lengthened linear DNA by about 2.7 Å per bleomycin molecule, and dichroism (315 nm) of bleomycin bound to DNA indicated the presence of a chromophore which makes an angle of at least 60° with the helix axis, results also consistent with intercalation.

102 REPAIR RESPONSE TO BLEOMYCIN-INDUCED DAMAGE, Robb E. Moses and Steven L. Ross, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Bleomycin (BLM) is a glycopeptide antibiotic effective in the treatment of lymphomas. BLM has been reported to interact with DNA causing base loss and strand breakage. With φX174 RFI DNA we have observed two stages of reaction with BLM: creation of alkali-sensitive sites, or, in the presence of sulfhydryls, strand cleavage. Thus BLM represents an agent of interest for exploring repair capability. We have investigated the response of E. coli to BLM. We find that in the toluene-treated cell system there are two categories of response to BLM: 1) in polA+ strains we observe an increase in DNA synthesis, independent of ATP and replication, and nonconservative in nature. Replication continues at normal levels in the presence of the drug. 2) in polA- strains we observe an ATP-dependent nonconservative DNA synthesis independent of replication, but dependent on DNA polymerase III. Again replication is spared. In intact E. coli we find increased sensitivity to BLM in polA-, lig_{ts}, or recA- mutants. Toluene-treated recA- mutants do not respond to BLM with repair synthesis. Therefore the in vivo and in vitro data agree and suggest that BLM induces repair at apurinic/apyrimidinic sites, requiring strand cleavage, resynthesis and ligation. This response is of interest in that replication is spared in the face of numerous DNA strand cleavages. Supported by grants from the U.S.P.H.S. and Q543 from the Robert A. Welch Foundation.

103 BLEOMYCIN-INDUCED DNA DAMAGE, REPAIR, RECOMBINATION AND MUTATION IN SACCHAROMYCES CEREVISIAE, Carol W. Moore, Dept Biol, University of Rochester, Rochester, NY 14627

Alkaline sucrose gradient sedimentation profiles of yeast DNA from cells treated with 1, 10, 50 and 100 µg/ml bleomycin (BM) demonstrate dose-dependent induction of single-strand breaks. Killing of yeast cells by the antitumor glycopeptide is also dose-dependent. It is postulated that the DNA breaks induced in yeast are the primary cause of cell killing by bleomycins. Preliminary results suggest at least part of the induced damage is repairable.

When mutant strains of Saccharomyces containing genes conferring sensitivity to X-rays, ultraviolet light (UV), or both, were characterized for their relative sensitivities to BM and the structurally similar antibiotic, phleomycin (PM), with one exception, all *rad* mutants found very sensitive to BM and PM were sensitive to X-rays. This suggests some aspect of the repair of BM-, PM- and X-ray-induced damages may be similar. All *rad* mutants with resistance to BM and PM similar to normal (*RAD+*) strains were sensitive to UV. Since several of these mutants are defective in excision of UV-induced pyrimidine dimers from nuclear DNA, it is concluded the excision-repair system is not required for repair of BM- and PM-induced damages.

In contrast to the finding that BM was not mutagenic in the *Salmonella*/mammalian microsome mutagenicity test, BM has been found recombinogenic and mutagenic in yeast. The amount of BM-induced mutation and recombination depended on the specific genetic markers assayed. The genetic effects of the drug might be predicted if, for example, mutagenesis and recombinogenesis accompany repair replication by introducing mismatched bases opposite DNA lesions.

104 THE EFFECTS OF ACETOXYACETYLAMINOFLUORENE AND NEAR-uv ON THE INFECTIVITY OF ϕx174 RF DNA William D. Taylor, Reginald A. Deering and Chun-Lei Tsai, Biochemistry and Biophysics Department, The Pennsylvania State University.

Acetoxyacetylaminofluorene and near uv (303 nm) inactivate the transfecting activity of ϕx174 RF DNA on Ca treated E coli at carcinogen concentrations in the range 1-10 µgm/ml and doses in the range 1-2 x 10^5 ergs/mm^2, and the combined treatments have a synergistic effect. No single or double strand breaks are observed by neutral sucrose sedimentation. Alkali-labile bonds and S_1 nuclease sensitive sites are found but much less frequently than inactivating nucleotide damage. Using E coli repair mutants, it was found that the uvrA gene is partially involved in the repair of this damage.

Enzymes in DNA Repair

105 ISOLATION AND GENETIC CHARACTERIZATION OF λ p<u>uvrA</u>$^+$ AND λ p<u>uvrC</u>$^+$ TRANSDUCING PHAGES, *Jeffrey Auerbach and Paul Howard-Flanders, Yale University,* Department of Molecular Biophysics and Biochemistry, New Haven, Conn. 06520

By means of a selection procedure involving cyclic growth and irradiation, λ transducing phages, bearing the excision repair genes <u>uvrA</u>$^+$ and <u>uvrC</u>$^+$, were isolated from an unfractionated pool of λ phages carrying Eco RI fragments of the <u>E. coli</u> chromosome. The phages transduced <u>uvrA</u>$^-$ or <u>uvrC</u>$^-$ host cells to the wild type level of ultraviolet resistance and host cell reactivation. The phages are specific in their ability to restore a wild type phenotype only to host cells bearing <u>uvrA</u>$^-$ or <u>uvrC</u>$^-$ respectively. These observations suggest that the phages carry <u>uvrA</u>$^+$ and <u>uvrC</u>$^+$ rather than a suppressor mutation. Lysis defective derivatives of these phages have been isolated. We are examining the possibility that the gene amplification obtained with these phages may result in an overproduction of the ultraviolet endonuclease activity.

106 CLONING OF E. coli DNA REPAIR GENES ON PLASMIDS, W. D. Rupp, A. Sancar, W. J. Kennedy, J. Ayers and J. Griswold, Yale University School of Med., New Haven, CT 06510

We anticipate that the availability of DNA repair genes on plasmids will be useful not only for amplification of the appropriate gene products but will also facilitate studies elucidating the identity and function of these gene products, and will provide DNA for the cell-free transcription and translation of DNA repair genes. Plasmids carrying the recA gene have been reported previously and are useful for increasing the amount of recA gene product in cells. We have obtained ColE1 plasmids carrying uvr genes of E. coli since the products of these genes and their exact roles in excision repair are under active investigation. The presence of the uvrA and uvrB genes on the hybrid plasmids was confirmed by extracting the plasmid DNA and then transforming the appropriate uvr strain to UV-resistance. One plasmid carrying uvrB and the bio operon is of particular interest because of a report in the literature claiming that the bio genes could not be stably cloned using ColE1, an assertion that is contradicted by the properties of the plasmid we have studied. At present we are further characterizing the plasmids we have obtained and are also attempting to decrease the amount of DNA on the plasmids that is flanking the DNA repair genes. By reducing the "extra" DNA on these plasmids, we expect to obtain clearer results with fewer complications. (Supported by Grant No. CA 06519 from the National Cancer Inst.)

107 CHARACTERIZATION OF PHAGE λpuvrC. E.Seeberg, A.L. Steinum and O.R. Blingsmo, NDRE, Kjeller, and Genet. Dep., Univ. Oslo, Norway.

A plaque-forming bacteriophage λ carrying the uvrC gene of E. coli was isolated from in vitro constructed transducing phage stocks kindly provided by K. Murray, Edinburgh, Scotland. These phages have bacterial DNA inserted at cleavage site 3 of HindIII restriction enzyme to the left of the phage att region, in a vector deleted for about 20% of the original phage genome (Borck et al., MGG 146, 199). The λpuvrC$^+$ was selected by its ability to complement uvrC host cells in restoring viability of UV-irradiated phages. The isolated transducing phage has an intermediate UV-survival on uvrC relative to uvr$^+$ and uvrA, uvrB host cells. Lysogens of uvrC cells carrying λpuvrC$^+$ are nearly as resistant to UV as wild type cells, indicating that the uvrC$^+$ gene function is being expressed from the phage in the prophage state. UV-induction curves for λpuvrC in uvr$^+$ and uvrC lysogens show the same optimal dose for induction, but the phage yield is 20 times lower from uvrC than from uvr$^+$ lysogens. Pyrophosphate inactivation experiments show that the size of bacterial DNA present in λpuvrC is equivalent to about 10% of the phage genome. SDS gel electrophoresis followed by autoradiogarphy of radioactively labelled phage coded proteins show two protein bands of molecular weight 30,000 and 70,000 specific for the transducing phage.

108 PLASMIDS CARRYING E.COLI K12 GENES FOR EXCISION REPAIR: CONSTRUCTION AND PROPERTIES.
Cornelis A. van Sluis and Hans Pannekoek, Lab.Molec.Genetics, State Univ. of Leiden, the Netherlands.

UvrB plasmid pNP5 has been constructed by insertion of a 1.5 Mdal EcoRI fragment from λbio uvrB phage into pMB9. The expression of the uvrB gene is dependent both on the plasmid and the intracellular rho factor concentration. UvrB$^-$ bacteria carrying plasmid have a repair capacity comparable to the wild type, but no increase in UV-endonucleolytic activity could be detected in cell extracts.
A search of the Clarke-Carbon E.coli K12 colony bank, yielded plasmids pLC7-18 and 13-12 with the entire uvrC gene and several having a part of the uvrA gene (pLC44-14 and others). Complementation analysis showed that particular plasmid only increased the repair capacity of the corresponding mutant. Restriction analysis was used to localize a uvr-loci more precisely on the inserted DNA fragment. Plasmid programmed protein synthesis in minicells resulted in the formation of a number of new polypeptides, which have been investigated for a role in early steps in excision repair.

109 CLONING OF A GENE THAT CONTROLS RADIATION SENSITIVITY, CELL DIVISION AND CAPSULAR POLYSACCHARIDE SYNTHESIS, THE lon(capR) GENE Alvin Markovitz & Barbara Zehnbauer, The University of Chicago, Chicago, Ill. 60637.

Mutants in the lon(capR) gene of E.coli K-12 are pleiotropic; i.e. 1)they form nonseptate filaments(long form) after UV or nitrofurantoin treatment; 2)in the absence of such treatments they overproduce capsular polysaccharide and synthesis of 10 enzymes involved in polysaccharide production is depressed. Such clones appear mucoid. The lon gene product has been hypothesized to be a repressor. In order to identify and isolate this product it appeared necessary to clone the lon gene. An F' episome containing the lon^+ and $proC^+$ genes was obtained by crossing an Hfr strain(with $proC^+$ as an early marker)with a proC recA strain and selecting for $proC^+$ and scoring for lon^+(nonmucoid phenotype). One such clone contained an F'episome designated F'BZ105. The episome had a M.W. of 1.2×10^8 and could readily be isolated from CsCl-EtBr gradients. F'BZ 105 DNA was mixed with pHA121 DNA(a plasmid that confers tetracycline resistance(Tc^R) and colicin E1 immunity and contains pSC101 and pHA105(a 1.6 MD minicol E1)joined at EcoR1 sites),cut with EcoR1 and ligated. A lon(capR) strain was transformed with the DNA and Tc^R clones were selected followed by replica plating onto minimal medium where nonmucoid(lon^+($capR^+$)) clones could be distinguished. Three clones(out of 6000 screened)yielded plasmids which could also transform lon(capR)strains to Tc, UV, and nitrofurantoin resistance and made them nonmucoid as well. UDP-glucose dehydrogenase, an enzyme that is elevated in the mucoid recipient, was repressed in the nonmucoid transformant. Two of these plasmids, designated pBZ201 and pBZ203, when cut with EcoR1 each yielded a 6.1 MD fragment(pSC101) and a second fragment of 8MD. pBZ201 and pBZ203 have been transformed into lon strains that also produce minicells in order to identify the polypeptides specified by the 8MD fragment.

110 PHOTOREACTIVATING ENZYME FROM E.COLI,PHYSICAL & CHEMICAL PROPERTIES, R.M.Snapka & B.M. Sutherland,Univ.of Calif.,Irvine,CA 92650 & Brookhaven Nat'l Lab,Upton,NY 11973

DNA photoreactivating enzyme from E. coli has been purified to homogeneity and characterized. The apoprotein has a molecular weight of 35,200 daltons as estimated by SDS gel electrophoresis and molecular sieving in guanidinium hydrochloride. The holoenzyme has an $S^{\circ}_{20,w}$ of 3.72 S. The amino acid composition has been determined. The minimal molecular weight from amino acid analysis is 5,830 daltons (roughly one sixth of the experimental molecular weight). The enzyme is low in aromatic amino acids and the N-terminal residue is arginine. The carbohydrate composition of this enzyme has been measured by anthrone reaction and GLC analysis. Although the total carbohydrate content can vary, the same monosaccharide components are always found and always in the same relative proportions. These monosaccharides are: N-acetyl-D-glucosamine, D-mannose, D-galactose, and D-glucose. The alkaline β-elimination tests for O-glycosidic linkage to serine and threonine were negative, thus an N-acylglycosylamine linkage between the N-acetyl-D-glucosaminyl residue and an amide nitrogen of an L-asparaginyl residue is likely.

111 ULTRAVIOLET-LIGHT TRIGGERED DISAPPEARANCE OF PHOTOREACTIVATING ENZYME, Gary D. Small, Section on Biochemistry, Division of Biochemistry, Physiology & Pharmacology, The University of South Dakota School of Medicine, Vermillion, SD 57069.

UVS1 is a mutant of *Chlamydomonas reinhardi* defective in the dark-repair (presumably excision-repair) of pyrimidine dimers from nuclear DNA [Small and Greimann, Photochem. Photobiol. (1977) 25, 183-187]. All of the pyrimidine dimers in nuclear DNA can be repaired upon exposure to photoreactivating light immediately after irradiation. However, none of the dimers are photoreactivable if the cells are incubated for 24 hr in the dark in growth medium. Pyrimidine dimers induced in chloroplast DNA can be photoreactivated even after 24 hr incubation in the dark. Assay for photoreactivating enzyme in extracts prepared by sonicating whole cells shows that about 80 percent of the photoreactivating enzyme activity disappears after incubating UV-irradiated cells in the dark for 24 hr. No significant disappearance of enzyme activity occurs in the case of unirradiated cells. It is hypothesized that the residual photoreactivating enzyme activity found in incubated, irradiated cells represents chloroplast enzyme and that UV-irradiation triggers the disappearance of nuclear photoreactivating enzyme. This project was supported by NIH Grant GM 21095 from the USPHS.

112 EFFECTS OF PHAGE INFECTION ON E. COLI EXCISION REPAIR MEASURED IN VITRO, Peter Strike, Department of Genetics, University of Liverpool, Liverpool L69 3BX, England.

The effects of phage infection on the processes of excision repair in E. coli have been measured using cell free lysates and toluene treated cells. Measurement of the ATP-dependent UV specific endonuclease determined by the uvrA$^+$, uvrB$^+$ and uvrC$^+$ genes has been carried out in cell free lysates prepared from cells infected with a variety of bacteriophages. Infection with the host-cell reactivable phages lambda and T7 has little effect on the UV specific activity; it remains at uninfected levels. Infection with the non-reactivable phages T4v and T5 results in a rapid inactivation of ATP-dependent activity, which can be prevented if chloramphenicol is present during infection. Infection with the reactivable phage T3 results in the appearance of an ATP-independent UV specific endonuclease, which again is prevented if chloramphenicol is present during infection. Measurements of UV-induced DNA repair synthesis in toluene treated cells indicate that the UV endonuclease coded by the T4 v gene is capable of replacing the activities defective in uvrA$^-$, uvrB$^-$ and uvrC$^-$ mutated cells, while the ATP-independent activity seen in T3 infected cells is not. Similar studies in toluene treated uvr$^+$ cells confirm the reduced ability of infected cells to carry out DNA repair synthesis, presumably due to endonuclease inactivation.

113 UV-SPECIFIC ENDONUCLEASE IN SCHIZOPHYLLUM COMMUNE, Evelyn Waldstein, Pnina Hundert and Yigal Koltin, Tel Aviv University, Ramat Aviv, Israel.

The UV-sensitivity of Schizophyllum strains was examined. The D_{37} of wild type strains is shown to be ca. 270 J.m^{-2}. Photoreactivation and recovery after liquid holding suggest the existence of both light and dark DNA repair mechanisms in this organism. Endonuclease activity specific for UV-induced dimers was measured in crude extracts from vegetative mycelium in assays on phage PM2 DNA irradiated with low UV-doses (30 J.m^{-2}). The activity in the crude extracts is impaired and increased activity is detected after partial purification achieved by the aid of DEAE Sephadex A50. Optimal conditions for the assay of the UV-specific endonuclease are: 10 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$ and 10 mM NaCl. Dithiothreitol and ATP have no effect on its activity. Mutants sensitive to UV irradiation were isolated. The mutants retain normal photoreactivation properties, are resistant to MMS but differ in their liquid holding recovery patterns. The activity of the UV-specific endonuclease in these mutants is currently under examination.

114 DAMAGE SPECIFIC ENDONUCLEOLYTIC ACTIVITIES IN MOUSE CELLS.
Ingolf F. Nes, University of Bergen, Dept. Biochem., N-5000 Bergen, Norway.

UV damage specific endonuclease activity has been localized in the nucleus of mouse cells (plasmacytoma and L cells). When UV irradiated ØX174 RFI DNA is used as a substrate, the endonuclease recognizes one lesion per 35 thymine dimers. This fact suggests that the thymine dimer is not the lesion which is detected by the enzyme. Furthermore, the same nuclear extract also posesses endonucleolytic activities that act on depurinated DNA and osmium tetroxide treated DNA. The UV endonuclease activity and the activity which acts on osmium tetroxide treated DNA respond similarly to KCl and $MgCl_2$ and differ from that observed for the activity on depurinated DNA.
We postulate that the endonuclease activity that recognize UV and osmium tetroxide induced damages on DNA are one and the same, distinct from that which recognizes depurinated lesions on DNA. Currently, attempts are being made to separate these activities by standard protein purification technics to further substantiate this proposition.

115 Excision Repair of DNA in Human Cells. L. Hamilton, A.M. Pedrini and L. Grossman. The Johns Hopkins University, Baltimore, Maryland 21205.

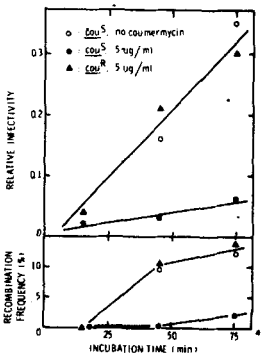
Exonuclease and DNA polymerase activities have been shown present in the nuclei of human placental cells, which after separation on phosphocellulose act sequentially to remove and replace damaged nucleotides in the DNA strand. For ultraviolet irradiated DNA this required initiation by a bacterial correndonuclease providing a break 5' to the pyrimidine dimer, and for an apurinic site in the DNA (e.g. after N-glycosidase removal of an altered base), incision by an apurinic endonuclease. Resolution of the exonuclease fraction by ion exchange and DNA cellulose affinity chromatography gave three groups of enzyme: two preferring single stranded DNA, one acting 3'→5' (DNase III like) and the other releasing nucleotides in both 3'→5' and 5'→3' directions (correxonuclease) and an exonuclease acting 5'→3' on duplex DNA (DNase IV like). In progress are experiments (1) to identify the exonucleases acting in the specific repair pathways of nucleotide removal initiated by a break in the DNA strand, and base removal which requires clean up of the ends after incision of the resulting apurinic site. For example, excision of pyrimidine dimers "in vitro" is primarily the action of exonucleases active against single stranded DNA, (2) to examine factors necessary for efficient excision of damage.

Interaction between individual exonucleases and the endogenous DNA polymerase (β) will be discussed.

116 DNA GYRASE: THE TARGET FOR NALIDIXIC ACID IN CELLULAR DNA REPLICATION, N. Patrick Higgins, Kenneth N. Kreuzer, Alan Morrison, Akio Sugino, Craig L. Peebles, Patrick Brown, and Nicholas R. Cozzarelli. The University of Chicago, Chicago, IL 60637. Nalidixic acid inhibits DNA synthesis and induces the SOS repair system and the synthesis of the recA gene product. We have purified the nalidixic acid target protein (the nalA gene product) from Escherichia coli to homogeneity. Together with a second purified protein a complex is formed that nicks and closes DNA causing the relaxation of either positive or negative supertwists in the absence of ATP; in the presence of ATP the complex introduces negative supertwists into covalently closed DNA. Novobiocin is a specific inhibitor of DNA gyrase; its effect on this complex is to inhibit supertwisting but not nicking closing. The effects of nalidixic acid and oxolinic acid are to 1) inhibit nicking and closing activity, 2) inhibit supertwisting activity, and 3) induce a site specific covalent attachment of the gyrase complex to DNA. Addition of SDS to the covalent complex results in double strand cleavage with a protein attached to each fragment of DNA. The sites of cleavage are affected by ATP and novobiocin and are mapped on ColE1 DNA.

117 INHIBITORS OF DNA GYRASE INHIBIT REPAIR AND RECOMBINATION OF UV-IRRADIATED PHAGE LAMBDA, John Hays and Sieghild Böhma, University of Md., Catonsville, Md., 21228.

Phage λ a106-19 b538 red3 contains a tandem duplication (18%). It is EDTA-sensitive, but is converted into the "single-copy" phage λ b538 red3 (length 0.84 λ^+, EDTA-resistant) by generalized recombination. Lytic infection of rec^+ E. coli yields recombinant (EDTA-resistant) phages at a frequency of 5-15%. An indirect assay (extraction of DNA from infected cells, and subsequent transfection of rec A^-B^- spheroplasts) gives the same results. When phage gene expression is blocked by phage repressor (cI857) or chloramphenicol and/or rifampin, recombination (scored by the transfection assay) is 0.1-0.2% or less. Infection with UV-irradiated phage under either blockage condition results in a time-dependent repair (increase in infectivity of DNA) and a UV dose-dependent stimulation of recombination (to 5-50%). Repair is reduced by 40% in $recA^-$ infections, 4-fold in $recB^-$, and 8-fold in $recA^-B^-$ cells. Recombination frequency is negligible in $recA^-$ and $recA^-B^-$, but normal in $recB^-$. Both coumermycin and oxolinic acid, inhibitors of different subunits of DNA gyrase, block repair and recombination (at concentrations above 5 μg/ml). Repair and recombination in a coumermycin-resistant strain are unaffected by the drug.



118 URACIL-DNA GLYCOSYLASE MUTANTS ARE MUTATORS, Bruce Duncan and Bernard Weiss, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
Spontaneous mutation to nalidixic acid or rifampicin resistance is 5-fold increased in uracil-DNA glycosylase (ung) mutants. By trp A reversion analysis we observe a 15-fold increase in the frequency of spontaneous G:C → A:T transition mutations while other transition and transversion frequencies are not affected. Duncan and Warner have previously found that uracil-containing DNA (30% U) functions normally in ung⁻ bacteria (in A:U base pairs), and the uracil is not removed from the DNA. Thus, uracil-DNA glycosylase appears to have evolved not to prevent uracil misincorporation into DNA (as A:U), but rather to edit for and repair (mutagenic) deaminated cytosine residues. This mechanism precludes the use of uracil as a "normal" DNA constituent. (Supported by NSF fellowship #SMI77-12327.)

119 METABOLISM OF URACIL-CONTAINING DNA DURING THYMINE STARVATION, Charles Garrett, Bruce K. Duncan, Daniel V. Santi and Huber R. Warner, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, and Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108
Strains of E. coli deficient in the enzymes deoxyuridine triphosphate hydrolase (dut) or uracil-DNA glycosidase (ung) have been investigated for their sensitivity to thymine starvation. Treatment of dut mutants with FdUrd, a potent inhibitor of thymidylate synthetase, resulted in a striking loss of viability, with kill rates up to three times that of the wild-type congenic strain. This effect was completely reversed by introduction of the ung mutation. In addition, ung mutants themselves were significantly more resistant than wild-type cells to FdUrd (kill rate ca. 0.6). When thymine-requiring ung strains were deprived of an exogenous source of the pyrimidine there was a variable effect, with one strain somewhat protected by the mutation and one not. Thymine starvation would be expected to result in a greatly increased intracellular ratio of dUTP to dTTP. The results described here suggest that under these conditions the incorporation of uracil residues into DNA in place of thymine and the consequent damage to DNA during their removal may be a significant factor in the loss of viability observed during thymine starvation.

120 THE ROLE OF EXONUCLEASE III IN THE REPAIR OF URACIL-CONTAINING DNA IN ESCHERICHIA COLI, Andrew F. Taylor and Bernard Weiss, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
Mutants of E. coli lacking dUTPase (dut mutants) misincorporate uracil into DNA. The uracil is removed by uracil-DNA glycosylase (the ung gene product), leaving apyrimidinic sites that are then cleaved by endonucleases. In extracts of E. coli, the cleavage of apurinic/apyrimidinic sites is catalyzed predominantly by the endonucleolytic activity of exonuclease III. The importance of exonuclease III in the repair in vivo of uracil-containing DNA is supported by the following observations: (a) dut xth (i.e., dUTPase⁻ exo III⁻) double mutants grow poorly and form filaments at or above 30°, whereas the corresponding single mutants do not; and (b) the growth defect of a dut xth double mutant can be corrected by the addition of an ung mutation. These results suggest that the dut xth double mutants grow poorly because they accumulate apyrimidinic sites through the removal of misincorporated uracil and that exonuclease III is essential for their subsequent repair.

ØX174 has a 50% reduced burst size when grown on dut mutants, and the DNA of the progeny particles contains an average of about one break per strand. When maturation of the virus is blocked by chloramphenicol, however, the RFI DNA that accumulates intracellularly in the dut mutants contains no measurable uracil or apyrimidinic sites (<1 per 20 DNA molecules). The repair is, therefore, quite rapid. (Supported by USPHS grant CA16519).

121 ENDONUCLEASES AND N-GLYCOSIDASES FOR DNA REPAIR, Mutsuo Sekiguchi, Hiroshi Hayakawa, Kenji Oeda and Kenji Shimizu, Kyushu University, Fukuoka 812, Japan

There are two classes of enzymes that act on damaged portions of DNA. Bimolecular base damages, such as pyrimidine dimers, are recognized by specific endonucleases. In vivo role of the enzymes has been shown most clearly with T4 endonuclease V, a bacteriophage T4-induced repair enzyme. The enzyme is not only capable of reactivating ultraviolet-irradiated T4, but is also active in restoring repair abilities of human xeroderma pigmentosum cells and Escherichia coli uvrA mutants when introduced into whole cells with the aid of Sendai virus (HVJ) and controlled plasmolysis, respectively. N-glycosidases, which liberate free bases from DNA, appear to be involved in repair of unimolecular base damages, such as deaminated cytosine or alkylated adenine. Uracil-DNA glycosidase releases uracil from PBS1 DNA, which contains uracil in place of thymine, whether the DNA is native or heat-denatured. The enzyme is also active on ϕX174 DNA which has been treated with sodium bisulfite to deaminate cytosine to uracil; number of the enzyme-susceptible sites, namely uracil, was proportional to the times of treatment of DNA with bisulfite. Evidence for in vivo participation of uracil-DNA glycosidase in repair of bisulfite-induced damages has been obtained with the use of Bacillus subtilis mutants defective in the enzyme activity. On the other hand, primary lesions induced by nitrous acid appear to be recognized by an enzyme other than uracil-DNA glycosidase; nitrous acid-treated DNA was not attacked by a purified preparation of uracil-DNA glycosidase, and an activity to act on nitrous acid-treated DNA was present in an extract of mutant deficient in the N-glycosidase activity.

122 THE EFFECTS OF dUTP MISINCORPORATION ON IN VITRO DNA SYNTHESIS, R.H. Grafstrom, B.Y. Tseng, and M. Goulian, University of California, San Diego, La Jolla, CA 92093

The effects of dUTP misincorporation on DNA synthesis in human lymphocytes have been investigated using an in vitro system. dUTP was found to inhibit DNA synthesis and to produce an increased amount of low molecular weight DNA fragments, which are approximately the size of Okazaki pieces but are not elongated into higher molecular weight DNA. That these fragments are produced by a repair process initiated by the action of uracil-DNA-N-glycosidase was demonstrated both by the dependence of uracil formation on DNA synthesis and also by the inhibition of uracil removal by the presence in the incorporation mixture of 6mM uracil, a known inhibitor of uracil-DNA-N-glycosidase. The high levels of deoxyuridinetriphosphatase found in our system preclude the possibility of this mechanism(uracil misincorporation and subsequent removal) being a substantial contributor to the appearance of Okazaki-size fragments found during normal DNA synthesis. (Supported by USPHS Grant CA11705 and National Research Service Award CA05364 from the National Cancer Institute and Research Grant NP102 from the American Cancer Society).

123 EXONUCLEASE III OF E. COLI AND H. INFLUENZAE: CLEAVAGE AT APURINIC/APYRIMIDINIC SITES, Stephen G. Rogers and Bernard Weiss, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Exonuclease III from E. coli or H. influenzae is a monomeric protein (M_r=28,000-29,000) with four physically inseparable catalytic activities. It is (i) a double strand-specific 3'→5' exonuclease, (ii) an endonuclease for apurinic/apyrimidinic sites in DNA, (iii) a DNA-3'-phosphatase, and (iv) an RNase H. The properties of the enzymes from the two strains are indistinguishable. In each strain, exonuclease III is the predominant endonuclease for apurinic/apyrimidinic sites. The endonuclease activity, as detected by the nicking of partially depurinated (acid-treated) ØX174 RFI DNA, cannot be separated from the exonuclease during enzyme purification or subsequently by gel filtration or electrophoresis. Both activities are affected simultaneously by mutation in each organism. The E. coli gene for exonuclease III has been cloned with phage λ and ColE1 vectors. A 30-fold increase in exonuclease III (and in its associated endonuclease activity) appears not to affect cell viability.

(Supported by American Cancer Society grant NP-126 and USPHS training grant 5 T32 CA09139).

124 ENZYMATIC DEGRADATION OF SINGLE STRANDED DNA CONTAINING SITES OF BASE LOSS BY EXTRACTS OF Escherichia coli. Thomas Bonura and Errol C. Friedberg. Laboratory of Experimental Oncology, Department of Pathology, Stanford University, Stanford, CA. 94305.

Five endonuclease activities (designated endonucleases II → VI) have been described in extracts of E. coli which can catalyze the hydrolysis of phosphodiester bonds at or near sites of base loss (apurinic/apyrimidinic sites) in double stranded DNA. However, at present, it is not firmly established that these represent 5 distinct enzymes. Furthermore, none of those activities have been shown to degrade single stranded DNA containing sites of base loss.

We have examined extracts of E. coli defective in the major apurinic endonuclease activity (endonuclease activity of exonuclease III; endonuclease VI) as well as endonuclease II and have detected an activity that catalyzes the degradation of single stranded DNA containing sites of base loss, to acid-soluble products. The activity has no requirement for divalent cation (thereby distinguishing it from endonuclease V) and is not inhibited in the presence of 5 mM EDTA (thereby further distinguishing it from endonuclease II and endonuclease VI). In the presence of EDTA no significant degradation of single stranded DNA without sites of base loss is observed. The activity is inhibited by tRNA and is 50% inactivated in the presence of 0.2 NaCl or when heated to 50°C for 10 minutes. These features distinguish it from endonuclease IV. In addition, purified endonuclease III (generously provided by Dr. S. Linn) has a very limited activity on single stranded depyrimidinated DNA. We are currently purifying this activity to determine whether it represents yet another apurinic endonuclease, possibly specific for single stranded DNA.

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125 ENZYMES INVOLVED IN BASE EXCISION REPAIR IN Micrococcus luteus. Jacques LAVAL and Josiane PIERRE, Inst.G.Roussy,94800,VILLEJUIF,FRANCE.

Enzymes that specifically remove alkylated bases or uracil residues in DNA are present in procaryotes as well as in eucaryotes. Such a DNA N-glycosidase which recognises 3-methyl Adenine has been isolated free of endonuclease for apurinic sites from M.luteus.(Laval J. Nature, 269,829-832,1977). Besides these two enzymes,we have purified and characterized from M.luteus an uracil DNA N-glycosidase and a second endonuclease for apurinic sites. Purification procedure involves phase partition.chromatography on DEAE cellulose, P-cellulose and DNA Agarose columns. This new endonuclease for apurinic sites is clearly different from the already reported by chromatographic properties, isoelectric point, sensitivity to proteases, enzymatic properties. All these enzymes are small molecules and have been obtained free of contaminating endonuclease or DNA-N-glycosidase. Mechanisms of action are reported.

This work was suported by grants from INSERM and CNRS.

126 CHROMATIN LOCALIZATION OF THE ENDONUCLEASE SPECIFIC FOR APURINIC SITES IN PHASEOLUS MULTIFLORUS EMBRYOS, Lise Thibodeau and Walter Verly, Biochimie, Université de Liège, Belgium.

Endonucleases specific for apurinic sites appear to be involved in the repair of base loss occuring spontaneously or following chemical or physical aggression of DNA. These enzymes have been found in bacteria and mammalian cells. We found endonucleases for apurinic sites in a photosynthetic alga (Anacystis nidulans), in a fungus (Agaricus campester), in leaves and roots of a higher plant (Phaseolus multiflorus). In the leaves, the chloroplastic enzyme was found to have a higher sensivity to heat than the nucleo-cytoplasmic enzyme. The nucleo-cytoplasmic endonuclease of P. multiflorus embryos has been isolated and its specificity toward apurinic sites demonstrated; it is localized almost exclusively in the nucleus. Native chromatin has a low activity on exogenous depurinated DNA, but a high activity can be demonstrated on this substrate in the non-histone fraction after dissociation of the chromatin. Reconstitution of chromatin is associated with a marked decrease of the enzyme activity on the exogenous substrate, but the activity reappears on redissociation. These results suggest that, when the enzyme is integrated in the quaternary structure of the chromatin, the active center is oriented to work on chromatin DNA which is its true substrate. Experiments, using a mammalian system, are under way to verify this hypothesis.

127 ISOLATION AND CHARACTERIZATION OF THE APURINIC ENDONUCLEASE FROM HUMAN PLACENTA. Nancy L. Shaper and Lawrence Grossman, Department of Biochemistry, The Johns Hopkins University, Baltimore, Md. 21205

The repair of apurinic sites in DNA is initiated by an incision enzyme termed the apurinic endonuclease. This enzyme has been isolated and partially purified from E. coli, rat liver, calf thymus and human placenta. We have developed an improved purification scheme for the isolation of the enzyme from human placenta using hydrophobic affinity chromatography and preparative isoelectric focusing. This scheme is relatively simple and results in a near homogeneous enzyme preparation in high yield that is free of nonspecific nucleases. Efforts are currently underway to purify the enzyme to homogeneity. Data will be presented detailing the properties of the enzyme.

128 IN VITRO REPAIR OF DEPURINATED DNA BY ENZYMES PURIFIED FROM HUMAN LYMPHOBLASTS. Kallol Bose, Peter Karran, and Bernard Strauss, University of Chicago, Chicago, Illinois 60637

A partially purified system which carries out the excision repair of DNA with apurinic sites has been obtained from the human lymphoma line Daudi. The system consists of DNA polymerase α free of nucleases and two different nucleolytic activities, one an endonucleolytic activity specific for apurinic sites in DNA and the second a 5'-3' exonuclease.

Alkali-labile lesions introduced into T7 DNA by treatment with methyl methanesulfonate were removed and the DNA repaired by the joint action of these nucleases and polymerase. The strand breaks in the DNA were then joined by incubation with T4 DNA ligase. Dinucleotides appear to be the predominant product of exonuclease action.

DNA Repair Pathways in Prokaryotes
Error Free Pathways/Recombination

129 IN VITRO SYSTEM FOR MONITORING DAMAGE TO AND REPAIR OF T7 DNA. N. B. Kuemmerle and W. E. Masker, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

A system for packaging bacteriophage T7 DNA which is dependent upon exogenously added DNA and in which recombination has been minimized has been employed to monitor the biological effects of UV radiation-induced damage to DNA. Irradiated DNA packaged in vitro into phage particles and irradiated intact phage showed almost identical survival as a function of UV dose using E. coli wild type, polA, or uvrA as host. While uvrA mutants performed less host cell reactivation, polA mutants and wild type were identical in their ability to support growth of irradiated phage or irradiated T7 DNA packaged in vitro into complete phage. A study of in vitro repair effected by T7-infected extracts of E. coli suggested that T7 DNA polymerase may substitute for E. coli polymerase I in the resynthesis step of excision repair. Treatment of UV radiation-damaged DNA with M. luteus endonuclease specific for removal of pyrimidine dimers followed by incubation of the DNA with an extract of uninfected E. coli resulted in a higher packaging efficiency than that observed in untreated DNA. T7 DNA replicated in vitro has been packaged in this in vitro packaging system. As anticipated, irradiation of template DNA results in greatly reduced synthesis, and the resultant product DNA is packaged with significantly lower efficiency than that synthesized on an unirradiated template. Efforts to examine inducible DNA repair in vitro by monitoring the biological activity of T7 DNA synthesized in vitro by extracts prepared from irradiated cells are in progress. (Research supported by the Department of Energy under contract with the Union Carbide Corporation.)

130 UVRD, UVRE AND RECL REPRESENT A SINGLE GENE, Sidney R. Kushner, John Shenherd, Gwyneth Edwards and Valerie F. Maples, Univ. of Georgia, Athens Ga. 30602

A series of isogenic strains carrying either uvrD3, uvrD101, uvrE4, uvrE100, uvrE502 or recL152 were constructed and analyzed for sensitivity to ultraviolet light increased spontaneous mutation frequency and ability to carry out host cell reactivation. All the strains showed similar levels of UV sensitivity and ability to carry out host cell reactivation. The uvrD and uvrE mutants exhibited significant increases in spontaneous mutation frequency compared to wild type or recL152 strains. All the alleles were recessive in transconjugants carrying a wild type F plasmid. Complementation analysis was carried out by transductional analysis and the formation of merodiploids. F' plasmids carrying either uvrE502 or recL152 failed to complement any of the above alleles but did complement rep-3(a closely linked marker). In transductional crosses extremely low frequencies of wild type recombinants were obtained between any of the mutations tested. These results along with experiments by Clark et al and Smirnov et al. strongly suggest that uvrD, uvrE and recL are all mutations in the same gene. It is thought that the uvrD protein serves to regulate either DNA polymerase I, DNA polymerase III or both. The variations in the observed phenotypic properties of these strains may arise from mutations at different sites within the gene. A series of strains deficient in exonuclease I, exonuclease III and exonuclease V which also carry various uvrD alleles have been constructed to test this hypothesis. (This work was supported in part by grants from the National Institute of General Medical Sciences.)

131 ARANUCLEOTIDE INHIBITION OF *E. COLI* DNA POLYMERASES: LOSS OF VIABILITY AND TEMPERATURE SENSITIVE INHIBITION OF DNA REPAIR *IN VIVO*, INHIBITION OF PURIFIED DNA POLYMERASES II AND III, AND INHIBITION OF DNA SYNTHESIS IN TOLUENIZED CELLS. Douglas W. Smith, Robert C. Tait, Sheldon S. Hendler, and William B. Helfman. Univ. Cal, San Diego, La Jolla, CA 92037.

E. coli pol$^+$ cells grow slowly at 30°C but die slowly at 43°C in the presence of 100 µg/ml arabinocytosine (araC). *E. coli polA* mutants die slowly at 30°C, more rapidly at 37°C, and most rapidly at 43°C, whereas *E. coli polA polB* mutants die twice as rapidly as *polA* mutants, *E. coli polC* mutants die at the same rate at 43°C in the presence or absence of araC, whereas *E. coli polA polC* derivatives die more rapidly at 43°C in the presence of araC. Thus, neither PolII nor PolIII alone prevent rapid araC killing, and all three DNA polymerases catalyze reactions which reduce araC killing. Alkaline sucrose gradient analysis of UV-induced DNA repair capabilities of *E. coli polA polB* mutants showed that DNA dark repair is sensitive to araC in a temperature-sensitive manner, indicating that PolIII, functioning in DNA repair, is inhibited by araC in a temperature-sensitive manner. The rate of repair is higher in *polB* cells than in *polB*$^+$ cells. Both purified PolII and PolIII are sensitive to aranocleoside triphosphate inhibition, but only at high deoxynucleoside triphosphate concentrations. PolII is more sensitive than is PolIII, and the Mn^{++}-activated reactions are more sensitive than the Mg^{++}-activated reactions. Incorporation of [α-^{32}P]araCTP in toluenized *E. coli pol* mutants showed that 50% inhibition of ATP-dependent DNA synthesis corresponds to incorporation of several hundred araCMP molecules per cell. At 5 µM and lower, araCTP inhibits ATP-dependent PolIII-mediated DNA synthesis (DNA replication), whereas at 10 µM and higher, additional inhibition and araCMP incorporation appears to be due to PolII-mediated DNA repair.

132 THE GENETIC CONTROL OF REPAIR REPLICATION AFTER UV IRRADIATION, Kenneth M. Carlson and Kendric C. Smith, Stanford University School of Medicine, Stanford, CA 94305.

The resynthesis step of short patch (growth medium-independent) excision repair after UV irradiation is performed by DNA polymerase I (1-3). Previous studies, utilizing *polA* mutants of *E.coli* K-12 also carrying a *polC*ts mutation, suggest that the resynthesis step of long patch (growth medium-dependent) excision repair after UV irradiation can utilize DNA polymerase II (i.e., *polB*) and/or III (i.e., *polC*ts) (4,5). Our repair replication data, utilizing *polA* mutants also deficient in DNA polymerase II and/or III are consistent with these results. However, we have been unable to detect any significant repair replication in a *polB* strain following the standard assay conditions for *pol*$^+$ and *polA* strains (2) (i.e., using 20 Jm^{-2} and 20 min incubation). At UV fluences >50 Jm^{-2}, however, only short-patch repair replication can be detected in a *polB* strain. Our results indicate that DNA polymerase II, but not DNA polymerase III, is responsible for the long patch repair resynthesis seen in a wild-type strain of *E.coli* K-12. Since the UV survival and the mutation frequency of a *polB* strain are similar to that for wild-type cells, DNA polymerase II-dependent long patch repair does not appear to be a major repair system after UV irradiation.

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133 DNA TURNOVER IN BUFFER-HELD ESCHERICHIA COLI AND ITS EFFECT ON REPAIR OF UV DAMAGE, M. S. Tang, T. V. Wang and M. H. Patrick, The University of Texas at Dallas, Richardson, Texas 75080

Continuous DNA degradation and resynthesis, without a net change in cellular DNA content, were observed in buffer-held, unirradiated E. coli B/r. This constant DNA turnover probably involves most of the genome and reflects random sites of DNA repair which involves the polA-dependent excision resynthesis repair pathway. Under these non-growth conditions, it appears that at any given time there is a minimum of one repair site per 6.5 x 10^6 daltons DNA, each of which is at least 160 nucleotides long.
While the amount of DNA degradation is not influenced by prior exposure to UV radiation, the synthetic activity is correlated inversely with the UV fluence. We suggest that when these sites of DNA turnover occur opposite to a cyclobutyl dipyrimidine in UV-irradiated cells, repair of this damage can be prevented. This implies that both beneficial and deleterious processes take place in irradiated, buffer-held cells, and that cell survival depends on the delicate balance between DNA turnover and repair of damage. Based on these findings, we propose a model to explain the limited repair observed during post-irradiation liquid-holding and to account for the large difference in cell survival between irradiation at low fluence rates (fluence-rate dependent recovery) and at high fluence rates followed by liquid-holding (liquid-holding recovery).

134 RADIOSENSITIZATION OF HYPOXIC E. COLI BY 8-METHOXYPSORALEN: DEPENDENCE ON DNA REPAIR CAPABILITY, J. Leslie Redpath, Department of Radiological Sciences, University of California, Irvine, CA 92717

The DNA-interactive agent 8-methoxypsoralen acts as a radiosensitizer of hypoxic E. coli carrying either uvrA, uvrB or recA mutations. Wild-type strains and polA1 or polA (5'$\longrightarrow$3' exonuclease) mutants are not radiosensitized. Thus the lesion induced in hypoxia by psoralen-plus-ionizing radiation would seem to be repaired by wild-type bacteria and polA mutants but not by those defective in uvrA, uvrB or recA genes. The genetic requirements for repair of this damage are thus different to those for repair of that induced by psoralen-plus-light (i.e. interstrand cross-links), but the same as those for the slow, medium-dependent (Type III) repair of single strand breaks. Post-irradiation incubation in the presence of acriflavine or 2,4-dinitrophenol, which are inhibitors of Type III repair, had no effect on the dose modifying effects of 8MOP in any of the strains. This would indicate that these agents did not inhibit the repair of the lesion induced by psoralen-plus-ionizing radiation and suggests the existence of another repair system with the same genetic requirements as Type III repair of single strand breaks.

135 REPAIR OF UV INDUCED DNA DAMAGE IN MYCOPLASMAS. J. A. Nowak, J. Das, A. Ghosh, U. Chaudhuri, and J. Maniloff. Depts. of Microbiology and of Radiation Biology and Biophysics. University of Rochester, Rochester, New York 14642.

The mycoplasmas are the smallest reported free living cells. This group of prokaryotes do not have cell walls: each cell is bounded by a single lipoprotein unit membrane. The repair of DNA damage in UV irradiated Mycoplasma gallisepticum A5969 (genome size 4.6 x 10^8 dal) and Acholeplasma laidlawii JA1 (genome size 1 x 10^8 dal) has been studied. A. laidlawii cells have both dark (excision) repair and photoreactivating mechanisms. In contrast M. gallisepticum has been found to have neither excision repair nor photoreactivating mechanisms for the repair of UV induced DNA damage. To our knowledge, no other prokaryote lacks both repair systems. The effect of acriflavine treatment of ultraviolet inactivation has also been investigated. Post irradiation acriflavine treatment inhibits liquid holding recovery and DNA strand breaks are not repaired. Comparison of the effects of acriflavine treatment on irradiated A. laidlawii and M. gallisepticum also indicates that, while the primary effect of acriflavine may be to inhibit exision repair, the dye also affects some other repair processes. Pre-irradiation acriflavine treatment protects A. laidlawii cells from UV inactivation.

136 PHOTODYNAMIC DAMAGE AND ITS REPAIR IN MYCOPLASMAS. J. Das, U. Chandhuri, J.A. Nowak, and J. Maniloff. Depts. Microbiology and of Radiation Biology and Biophysics, University of Rochester, Rochester, New York 14642

The mycoplasmas are a group of prokaryotes which do not have cell walls: each cell is bounded by a unit membrane. Photodynamic inactivation of Acholeplasma laidlawii and Mycoplasma gallisepticum in the presence of acriflavine have been investigated. The inactivation curve for M. gallisepticum, an organism which lacks both dark and light repair mechanisms for UV induced DNA damage, followed "single hit" kinetics. The inactivation curve for A. laidlawii, exhibited an initial shoulder after which inactivation was exponential. When held in buffer in the dark, photodynamically inactivated A. laidlawii cells can recover: the extent of recovery is dependent upon the amount of damage. No detectable repair was observed when cells were inactivated below 10% survival. A lag in the synthesis of macromolecules was observed in inactivated cells. Alkaline sucrose gradient analysis showed DNA strand breaks in photoinactivated cells. The DNA strand breaks can be repaired in cells not inactivated below 10% survival. No change in the transport of α-methyl glucoside was observed in inactivated cells compared to control cells, implying that the cell membrane is not affected by photodynamic inactivation.

137 HOST-CELL REACTIVATION OF ALKYLATED T7 BACTERIOPHAGE, Margaret D. Mamet-Bratley, Denis Lane and Barbara Karska-Wysocki, Département de Biochimie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Endonuclease for apurinic(or apyrimidinic)sites and DNA polymerase I are two enzymes thought to participate in base excision-repair of damaged DNA. Escherichia coli mutants, defective in these enzymatic activities, are now available. We have used these mutants to investigate host-cell reactivation of alkylated T7 phage. Purified T7 phage was alkylated by methyl methanesulfonate and phage survival was assayed immediately or after incubation of alkylated phage at 30°_{+} to induce depurination. Hosts for treated T7 phage were: AB1157 ($polA^{+}$,$xthA^{+}$); W3110($polA^{+}$, xth^{+}); p3478($polA^{-}$); AB3027($polA^{-}$, xth^{-}); BW2001($xthA^{-}$); RS5065 ($polAex^{-}$). Non-alkylated T7 phage plated with similar efficiency on all these strains. Phage survival, measured immediately after alkylation or following incubation at 30°, was lowest on E. coli p3478, defective in the polymerase activity of DNA polymerase I. Both strains defective in the endonuclease for apurinic sites (AB3027, BW2001) gave a significantly higher level of phage survival, as did the strain (RS5065) defective in the 5'-3' exonuclease activity of DNA polymerase I. Highest survival of alkylated T7 phage was observed on the two wild-type strains (AB1157, W3110). This shows that host-cell reactivation of alkylated phage occurs in these two strains. Thus we conclude that alkylated T7 phage is subject to repair, in a pathway involving E. coli endonuclease for apurinic sites and DNA polymerase I. (Supported by the National Research Council of Canada, La Fondation Jos. Rhéaume and l'Université de Montréal)

138 ROLE OF THE GENE mtcA IN THE RESISTANCE OF MICROCOCCUS RADIODURANS TO THE LETHAL EFFECTS OF MITOMYCIN C DAMAGE AND ALKYLATION MUTAGENESIS. B. E. B. Moseley and P. R. Tempest, Department of Microbiology, University of Edinburgh, School of Agriculture, Edinburgh, EH9 3JG, U.K.

A strain, 302, of Micrococcus radiodurans has been isolated, mutant in a gene designated mtcA, which is forty times more sensitive than the wild type to the lethal effects of both mitomycin C (MTC) and decarbamoyl MTC but which has wild type resistance to the lethal effects of ultraviolet radiation. Compared with the wild type, strain 302 has the same resistance to the lethal action of methyl methanesulphonate (MMS) but is slightly sensitive to the lethal effects of ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 7-bromomethylbenz[a]anthracene (BMBA). Alkaline sucrose gradient analyses of DNA from bacteria treated with BMBA indicate that 302 is unable to initiate excision repair of BMBA-adducts. MMS and EMS which are only slightly mutagenic in the wild type are highly mutagenic in 302 and MNNG which is very mutagenic in the wild type has this mutagenic effect enhanced fifty fold in 302. The mtcA gene product is therefore involved in the initial recognition and/or incision events in the excision repair of MTC-mono- and di-adducts and also methylated and ethylated nucleotides in DNA.

139 INCREASED DNA REPAIR PROFICIENCY IN A NEW E.coli K-12 MUTANT RESISTANT TO MITOMYCIN C, Aderson M. Aquino*, Stephen Zamenhof, and Patrice J. Zamenhof, Mol. Biol. Institute, UCLA, Los Angeles, California 90024.

A new E.coli K-12 mutant (AA101) resistant to Mitomycin C (MC) was isolated, phenotypically characterized, and the locus responsible for MC resistance (mcr) mapped around 29 minutes in the Taylor and Trotter map (Bact. Reviews 36:504, 1972). Results of the DNA repair proficiency studies on AA101 and its parent (CSH57A) are reported. The number of MC induced DNA single strand breaks present at zero time of post-treatment incubation (PTI) was 1.2 fold greater for CSH57A than for AA101. After 60 minutes of PTI that value was 7.7 fold greater for CSH57A than for AA101. During PTI CSH57A repaired 37% of the MC induced DNA single strand breaks, released 60% of the MC induced DNA cross-links, while for AA101 those figures were 90% and 87% respectively. The number of MC induced DNA cross-links per lethal hit for CSH57A and AA101 were 3.8 and 56.9 respectively. The number of MC induced DNA single strand breaks per alkylation adduct site at 0 and 60 minutes of PTI were 0.30 and 0.42 for CSH57A, and 0.27 and 0.11 respectively for AA101.

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140 THE INITIATION OF GENETIC RECOMBINATION IN E. coli (λ): recA$^+$-DEPENDENT CUTTING IN DNA MOLECULES, Peter Ross and Paul Howard-Flanders, Yale University Department of Molecular Biophysics and Biochemistry, New Haven, Conn. 06520

When undamaged λ phages infect E. coli (λ), their DNA does not replicate but forms intracellular covalent closed circles. When λ phages treated with the photocross-linking agent psoralen and 360 nm light infected E. coli uvrA$^-$ (λ), normal yields of covalent circles were recovered. When the damaged phages infected excision-proficient lysogens, however, the amount and proportion of covalent circles were reduced for up to two hours after infection. Interruptions in DNA molecules are thought to initiate recombination in bacteria. When E. coli (λ) cells containing undamaged, covalent circular λ DNA were superinfected with psoralen-damaged λ phages, the undamaged DNA was cut 'in trans'. Cutting in trans required uvrA$^+$ and recA$^+$ but did not require recB$^+$. It occurred normally in nonlysogens infected in the presence of chloramphenicol. Cutting in trans was detected in similar experiments with the temperate, P2-like phage 186, which shares no detectable homology with phage λ. In simultaneoulsy infected cells, superinfection with damaged λ phages caused cutting in trans of λ covalent circles, but not of 186 covalent circles, and vice versa. These results are of interest because they shed light on the sequence of early events in damage-induced bacterial recombination, and because cutting in trans may provide a biochemical assay for early events in recombination.

141 DAMAGE-INDUCED CUTTING in trans OF DNA MOLECULES IN recA$^+$ uvr$^+$ CRUDE EXTRACTS OF E. coli, Era Cassuto and Paul Howard-Flanders, Yale University, Dept Therapeutic Radiology, New Haven, Connecticut 06520

We have designed an in vitro system in which the cutting of crosslinked øX RF I DNA molecules by the uvr system of E. coli induces the cutting of homologous undamaged DNA during incubation with crude extracts of thermally induced E. coli (λ precA$^+$) lysogens. This reaction which has also been observed in intact E. coli lysogens infected with λ phages, is dependent on the presence of functional recA$^+$ and uvrB$^+$ gene products. Extracts from thermally induced λ precA$^+$ lysogens of E. coli proved to be substantially more active than extracts from nonlysogenic cells of the same strain. The results provide preliminary evidence for an endonuclease activity that cuts intact superhelical DNA in response to interaction with homologous damaged DNA. This system offers good prospects for the identification of proteins and enzymes involved in genetic recombination.

142 MECHANISM OF REPAIR OF DNA DOUBLE-STRAND BREAKS IN E. coli, Frank Krasin and Franklin Hutchinson, Yale University, New Haven, Connecticut, 06520

The mechanism of repair of DNA double-strand breaks in E. coli K-12 cells involves the recA gene and the presence of a duplicate genome. Evidence also indicates that cells may be capable of repairing only a small number of double-strand breaks. Genome size DNA released from spheroplasts gently lysed on top of sucrose gradients was sedimented at very low centrifuge speeds to minimize dependency of the sedimentation coefficient on speed. Results showed that breaks in DNA were produced in aerated cells linearly with gamma ray dose at 0.20 double-strand breaks per 10^9 daltons per kilorad. Cells with four to five genomes of DNA given a 16 kilorad dose showed efficient repair of double-strand breaks. Cells with about 1.3 genomes showed no repair of double-strand breaks although single-strand breaks were repaired normally. No repair of double-strand breaks was found for recombination-deficient recA$^-$ and recA$^-$ recB$^-$ cells otherwise isogenic with the repair-competent cells. DNA single-strand breaks were repaired in cells irradiated with 110 kilorads but no repair of double-strand breaks was found. Greater than 50% of cellular DNA was degraded after such high doses.

143 INFLUENCE OF Rec ON VIABILITY AND PLASMID MAINTENANCE IN E. coli K12, Stephen D. Barbour, Judith E. Miller, Glenn Ramsey and Florence N. Capaldo-Kimball, Department of Microbiology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

Rec$^-$ cultures of E. coli K12 contain viable, residually dividing, and nondividing cells. Nondividing Rec$^-$ cells do not synthesize DNA, but they contain chromosomes obtained through segregation, except for recA mutant nondividing cells which contain little or no DNA. Residually dividing and viable Rec$^-$ cells are indistinguishable from rec$^+$ cells with respect to doubling time, rates of DNA and protein synthesis, rate of oxygen uptake, and lysis time and burst size of bacteriophage T4. Rec$^-$ nondividing cells appear to be metabolically inactive and they eventually lyse. Unrepaired DNA damage may be the primary lethal event which causes cells to segregate irreversibly from the viable class into the residually dividing and then the nondividing classes. The stability of F' and R plasmids is dependent upon exonuclease V (the recB recC gene product) and influenced by the rate of expression of plasmid genes. The recA and recF gene products are not required for plasmid stability; mutations in these genes decrease plasmid instability in recB and recC mutants. This system may be a model for chromosome lethality caused by recB and recC mutations. DNA damage may result from gene expression. Repair involving exonuclease V may permit replicon survival.

144 GENE 32 PROTEIN STAINING FOR ELECTRON MICROSCOPY OF UV-INDUCED POST REPLICATION REPAIR GAPS IN ESCHERICHIA COLI, Robert C. Johnson and William F. McNeill, Medical University of South Carolina, Charleston, South Carolina 29401.

Previous studies with benzoylated naphthoylated DEAE cellulose(BN-cellulose)chromatography and dBrUrd photolysis have suggested an average UV-induced postreplication repair gap size of 10^3 to 10^4 nucleotides. These methods cannot discriminate between true gaps and double stranded DNA with single stranded ends and/or gaps in intermediate stages of filling by recombination of DNA. In this study we observe, by electron microscopy, DNA from cells that have been exposed to ultraviolet light and have then been allowed to incubate for 10 to 30 minutes. Gapped DNA is seen in these preparations as well as some single stranded end pieces. Purification by BN-cellulose column chromatography increases the frequency of sighting single strandedness. DNA obtained from BN-cellulose chromatography has a smaller proportion of gaps to single stranded end pieces. Gene 32 protein staining and ϕX174 single stranded marker DNA have allowed measurement of gap size of 800 to 5000 nucleotides in length. No complex structures suggesting recombination intermediates have been sighted. Gap size does not appear to be uniform. This research was supported by the NCI, CA18205.

145 T4-INDUCED RECOMBINATION REPAIR, Robert J. Melamede and Susan S. Wallace, New York Medical College, Valhalla, NY 10595.

The non-lethal recombination-deficient x, y and w mutants of T4 are sensitive to UV light, x-rays and a variety of chemical agents, and have been shown to function in the same repair pathway. Thus, they are in many ways analagous to the "rec" mutants of E. coli; the principal difference being that recombination is intimately linked to replication late in T4 infection. When measured by incorporation of low concentrations of ^{3}H thymidine, DNA synthesis in T4x- and y-infected cells at 15 minutes after infection is greatly reduced and sensitive to mitomycin C when compared to wild-type-infected cells. At this time, the DNA replicating complexes produced by T4x and y are also significantly smaller than those produced by wild-type T4. Chloramphenicol added at 7 minutes after infection prevents the reduction in both the DNA synthetic rate and size of replicating complexes in T4x- and y-infected cells. Interestly, there is no reduction in DNA synthesis in cells infected with T4x and y in the presence of hydroxyurea. In contrast, both the rate of DNA synthesis and the size of the DNA replicating complexes in T4w-infected cells are greater than those observed in wild-type-infected cells. Both these phenotypes are inhibited when chloramphenicol is added at 7 minutes after infection. DNA synthesis in T4w-infected cells is sensitive to hydroxyurea but not mitomycin C when compared to wild-type-infected cells. Thus, although T4x, y and w share a common phenotype with respect to repair, they clearly have distinct, perhaps sequential, roles in the DNA synthetic events associated with recombination.

146 CHARACTERIZATION OF DNA ADENINE METHYLATION (dam) DEFICIENT MUTANTS OF E. COLI K12 Martin G. Marinus, Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605.

Seven dam mutants which have reduced amounts of N6-methyladenine in DNA have been characterized. Five mutant strains have no detectable N6-methyladenine in DNA (limit of detection = 3% relative to wild type), and two strains have 25% and 50% residual N6-methyladenine relative to wild type. Compared to the wild type strain, the mutants show the following differences: (1) Morphological abnormalities (2) A 10-100 fold increase in the level of spontaneous mutability (3) A 10-20 fold increase in spontaneous induction of phage λ from lysogens (4) A small increase in sensitivity to UV light, alkylating agents and nitrofurantoin (5) A 10-100 fold increase in recombination frequency and (6) Inviability when combined with recA or recB or recC or lexA.

Inducible Repair/Mutagenesis I

147 FURTHER STUDIES OF lexA GENE FUNCTION IN E. COLI, David W. Mount, John W. Little and Susan Edmiston, Dept. of Microbiology, University of Arizona College of Medicine, Tucson, AZ 85724

DNA damage in normal E. coli strains induces a set of cellular functions known as SOS functions. This induction is blocked, however, by recA and lexA mutations. We are interested in studying the function of the lexA gene product. Previous studies have shown that lexA mutations are dominant, showing that the mutant gene codes for a diffusible product that blocks induction. This product could be a repressor. We are analyzing a suppressor of lexA mutations, termed spr. In recA^{+} spr mutants, SOS functions are again inducible, and in tif mutants, which produce an altered form of the recA protein, spr leads to very efficient constitutive expression of SOS functions. Other studies (Gudas and Mount, unpublished observations) have shown that the presence of spr results in a high, constitutive rate of synthesis of recA protein (formerly identified as protein X), corresponding to that normally observed in induced lexA^{+} recA^{+} cells. It appears that this high rate is necessary but not sufficient for induction, and that the recA protein must be modified biochemically to perform its function. Additional studies with strains which carry plasmids containing the recA or lexA genes support this argument.

148 AN E. COLI MUTANT WITH AN ALTERED INDUCIBLE REC+/LEX+ DEPENDENT DNA REPAIR PATHWAY
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The mutant DM1285, a spontaneous derivative of DM1187 (spr tif sfi) (PNAS 74:300-304), makes turbid plaques with λ^+phage, and clear plaques with λind^s phage. DM1285 maps at the lexA locus, at 90 minutes on the E. coli K-12 genetic map. In addition to having a lower spontaneous mutation rate and decreased ability to repair λ, DM1285 degrades its DNA to a greater extent than its parent, DM1187, or strain JM1(spr+tif+sfi+). After addition of nalidixic acid or mitomycin C, the mutant DM1285 also exhibits unusual kinetics of induction of protein X, which has now been shown to be the recA protein (Gudas and Mount,PNAS in press, 1977). At concentrations of mitomycin C (20-30µg/ml) which induce the recA protein in strain JM1, no recA protein is induced in strain DM1285. At similar concentrations of nalidixic acid, an increasing rate of synthesis of the recA protein is seen with time in strains JM1 and DM1187; in strain DM1285, only a short burst of recA protein synthesis occurs after nalidixic acid addition. (This burst of recA protein synthesis is observed within the first thirty minutes after nalidixic acid is added). Two dimensional gel analysis demonstrated that the pI of the recA protein in strain DM1285 was not altered, as compared to its parent, DM1187. These properties of strain DM1285 are presumably related to a less efficient induction of the rec+/lex+ dependent DNA repair system in this mutant.

149 REGULATION OF THE E. COLI RECA GENE IN VIVO.
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Following UV irradiation of cells or exposure to drugs such as nalidixic acid or mitomycin C, the recA gene is derepressed and its product (protein X) is synthesized at high levels. Derepression of the recA gene is blocked by lexA- or recA- mutations. Constitutive synthesis of recA is observed in tsl or spr strains (mutations in the lexA gene) or in tif-1 strains (recA allele). The genetic evidence suggests that lexA codes for a control element of recA and that recA+ protein is required for its own derepression. This regulatory scheme has been tested with specialized recA transducing phages and ColE1-recA plasmids. (a) The tif-1 and recA+ transducing phages make high levels of the recA protein following infection of irradiated cells. While phages carrying the lexB30, zab53, recA142 or a cold sensitive recA allele make less than 10% the wild type level of protein, spontaneous reversion of the mutation restores high levels of recA synthesis; (b) Following infection of a lexA- strain, the λprecA+ and λptif-1 phages induce little recA protein although λ coded protein synthesis is normal; (c) A ColE1-recA+ plasmid in a lexA+ strain makes high levels of recA+ protein. The recA+ plasmid suppresses the UV-sensitivity of lexA- mutations and the recA+ protein is made at high levels. In spr strains the recA protein is overproduced such that more than 10% of the cell protein is recA+. These results are consistent with negative regulation of the recA gene by lexA.

150 TRANSCRIPTIONAL REGULATION OF THE recA REGION OF E. COLI,
Ann McPartland, Linda Green and Harrison Echols, Dept. of Mol. Biol., Univ. of Calif., Berkeley, Calif. 94720

The recA gene product performs an essential function during induction of the "SOS-functions of Escherichia coli (i.e. UV-mutagenesis and DNA repair, prophage induction, inhibition of cell division). To explore the possibility that transcriptional modulation of the recA region is a feature of the SOS-induction pathway, we have compared the cellular levels of recA messenger RNA before and after ultraviolet irradiation. RNA from the recA region was identified by differential hybridization to DNA from the λsrlC and λsrlC recA transducing phages isolated by McEntee et. al.(1). Under the conditions employed very little RNA from the recA region can be detected in unirradiated cells. By 20 minutes after UV-irradiation, the fraction of RNA which is complementary to λsrlC recA DNA increases five to ten fold. Mutational lesions in recA or lexA prevent induction of the SOS-functions; mutations in these genes (recA13, lexA3) also completely abolish UV-induction of mRNA from the recA region. The abscence of UV-induced derepression in a strain bearing the missense mutation recA13 suggests that the recA product itself participates in transcriptional regulation of the recA region; this observation is in accord with recent experiments implicating the recA protein in stimulation of its own synthesis [Little and Kleid, JBC 252, 6251 (1977); McEntee, Gudas and Mount, pers. comm.]. We conclude that transcriptional derepression occurs in the recA region in response to cellular DNA damage inflicted by UV-irradiation and that the recA and lexA proteins normally control the rate of recA mRNA synthesis.

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151 ULTRAVIOLET LIGHT INDUCED RECOVERY IN E. coli OF GAMMA IRRADIATED BACTERIOPHAGE DNA, Phillip M. Achey, David C. Grier and John R. Silber, University of Florida, Gainesville, FL 32611.

Transfection of gamma-irradiated ØX 174 RFI and single-strand DNA into calcium treated E. coli which have been themselves exposed to 70 J/m^2 UV radiation (10% colony forming survival) and incubated under favorable growth conditions for 50 minutes after UV irradiation before calcium treatment leads to higher biological survival of the gamma-irradiated DNA than when the DNA is transfected into unirradiated calcium treated cells. We refer to this as UV induced recovery. The DNA was dissolved in .001 M phosphate buffer (pH 7.2) at a concentration of five micrograms/ml during gamma irradiation. Radicals resulting from gamma-radiolysis of water were scavenged by bubbling oxygen through the solution to remove the reducing species (the hydrogen atom and the solvated electron) and by addition of potassium iodide to remove the hydroxyl radical. Irradiation of the DNA under these controlled chemical environments indicates that indirect damage resulting from action of the reducing water radicals is more susceptible to repair by the UV induced recovery system than damage resulting from the hydroxyl radical, both for the single-strand and double strand forms of ØX174 DNA. The UV induced recovery system appears similar to SOS repair.

152 DOSE-RESPONSE RELATIONS FOR ULTRAVIOLET INDUCTION OF W-REACTIVATION, λ-PROPHAGE AND INHIBITION OF POST RADIATION DNA DEGRADATION IN E.coli AB1157. Donald J Fluke and Ernest C.Pollard, Duke University, Durham, N.C. 27706.

The various phenomena observed in connection with radiation induced repair are thought to be manifestations of one system, largely due to the derepression of the recA gene and the action of its gene product. If this is so, it is to be expected that the dose-response relation for induction of all these phenomena should be very closely the same. This has been shown for UV mutagenesis, induced radioresistance and inhibition of post radiation DNA degradation (prd) in strain WU 3610-89. Pollard, Person, Rader and Fluke, 1977, RADIATION RESEARCH 72 230). We here report the examination of the dose-response relations for UV induction of W-reactivation of UV damaged λ-phage, of λ-prophage in the lysogen and inhibition of prd in strain AB1157. We find that the induction of W-reactivation and inhibition of prd follow a rapidly increasing function of the $1 - e^{-D/D_o}$ form, with D_o about 4 J/m^2, while the induction of λ-prophage is more sigmoidal in character, requires more dose and shows a maximum not readily seen in the other two phenomena. We conclude that the inactivation of the λ repressors involves some additional process requiring more dose.

153 tif-1 MEDIATED RECOVERY FROM UV BLOCKAGE OF DNA SYNTHESIS IN E. coli B: A MECHANISM OF INDUCED MUTAGENESIS? Steven Sedgwick, National Institute for Medical Research, Mill Hill, London NW7 1AA, Great Britain.

The rates of uptake of ^3H-thymidine into DNA were measured several times during 2-3 hours of post-UV irradiation growth of E. coli WP2 uvrA and its mutant derivatives. After UV doses up to 2-3 J/m^2 the decrease in amount of DNA replication can be attributed to an 'all or none' effect. One portion of an irradiated population stopped synthesizing DNA whilst the remainder continued to replicate with the same exponential increase in rate as in unirradiated cells. Blockage of replication occurred with exponential one-hit kinetics within 5 mins of UV irradiation through some process requiring $recA^+$ and $lexA^+$ genes. Blockage of replication was relieved by thermal expression of the tif-1 mutation so that rates of ^3H-thymidine incorporation were restored to control levels one hour after irradiation. The decrease in the amount of ^3H-thymidine incorporation after 3 J/m^2, or more, of UV can be attributed to a decrease in the rates of DNA replication of the remaining functional replication units. tif-1 expression did not strongly influence replication ability in this dose range. It is suggested that the low UV dose mutator effect of tif-1 (Witkin, P.N.A.S. 71, 1930-1934) is caused by reactivation of blocked replication forks which normally remain inactivated in control UV irradiated cells.

154 MOLECULAR EVENTS LEADING TO INACTIVATION OF REPRESSOR MOLECULES, Cassandra L. Smith, M. Oishi, The Public Health Research Institute of the City of New York, Inc., New York, New York 10016

Different inducing agents and treatments produced distinctly different kinetic patterns of inactivation of prophage repressor molecules. The different patterns were related to differences in the initial altered states of DNA which were produced. The timing of appearance of DNA degradation was correlated with the time needed for repressor inactivation. There is evidence that the recBC-DNase has a significant role in the prophage induction process. These characteristics suggest that all inducing treatments lead to the formation of a common final predegradative DNA structure (probably involving scissions) which is acted upon by specific DNases to produce the signals for the induction of prophage. Ultraviolet light (UV) triggered inactivation of repressor molecules was reproduced in vitro in a plasmolized, permeable cell preparation. Induction without UV irradiation was produced by incubating the permeable cells in the presence of four deoxyribonucleoside triphosphates and ATP. This dNTPs-triggered induction requires a functional recBC gene product and is associated with degradation of the DNA replication fork. The role of recBC in the induction of prophage and SOS functions in general is discussed.

155 AN INDUCIBLE COMPONENT NECESSARY FOR RECOMBINATIONAL REPAIR IS A DECISIVE FACTOR IN THE HIGH RADIORESISTANCE OF MICROCOCCUS RADIODURANS, Norman E. Gentner and Mary M. Werner, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, KOJ 1JO, Canada.

The substantial resistance of M. radiodurans to ionizing radiation depends largely on recombinational repair (RR). Since RR appears to be the only caffeine-sensitive repair process, its properties can be selectively monitored by following recovery from caffeine-induced repair inhibition. No RR is observed in cells incubated under conditions restricting post-irradiation protein synthesis; an inducible component therefore appears to be necessary. RR slowly rejoins ∿20% of the γ-induced DNA strand breaks. At highly lethal γ-exposures (above about 1600 krad), however, RR is no longer observed in the majority of the cells; this failure may be a significant factor in the ultimate inactivation of M. radiodurans.

If, however, cells are first given a low ("priming") γ-exposure followed by incubation under conditions that allow protein synthesis, the resultant cells are substantially more resistant than normal to subsequent γ-exposure, even if RR of the initial "priming" damage is incomplete. In the "induced" cells, the contribution of RR can be seen even if protein synthesis is inhibited, and RR activity is now observed even at formerly highly lethal exposures. We believe that the initial exposure plus incubation supports synthesis of an inducible component(s) necessary for RR; this contributes to hyper-radioresistance because at high γ-exposures radiation damage in a gene coding for such a component (perhaps damage of a type that requires RR) no longer blocks development of this repair activity.

156 EFFECTS OF A PROTEASE INHIBITOR ON CESSATION OF RESPIRATION AND ON VIABILITY OF UV-IRRADIATED E. COLI B/r CELLS, Paul A. Swenson, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Antipain is a protease inhibitor that blocks UV-induced SOS functions in Escherichia coli (S. M. Meyn et al., Proc. Natl. Acad. Sci. U.S.A. 74, 1152, 1977), presumably by inhibiting cleavage of repressors of operons. The effects of antipain on the UV-induced cessation of respiration, a $recA^+$, $lexA^+$-dependent response, were studied in E. coli B/r cultures grown on minimal glucose medium. Following 254 nm irradiation (52 J/m^{-2}; 5% survival) a transitory cessation of respiration occurred. Addition of antipain (5 mM) caused nearly complete cessation of respiration, beginning about 60 min after UV. This response is similar to that obtained with cyclic adenosine 3',5'-monophosphate (cAMP) under the same growth and irradiation conditions (P. A. Swenson et al., J. Bacteriol. 131, 707, 1977). The rate and extent of pyrimidine dimer excision was not altered by the presence of either antipain or cAMP. The concentration of antipain used did not interfere with respiration and viability of unirradiated cultures. As is the case of cAMP treatment, antipain causes additional irradiated cells to die; however, the division rate of survivors of antipain-treated cultures, compared to untreated or cAMP-treated cultures, is reduced by 85 to 90%. The results do not support the idea that a repressor is cleaved by a protease during UV induction of an operon involved in cessation of respiration. They do suggest a role for a protease in recovery of E. coli B/r cells after UV damage to their DNA. (Research supported by the Department of Energy under contract with the Union Carbide Corporation.)

157 EFFECTS OF PROTEASE INHIBITORS ON SOME CONSEQUENCES OF DNA DAMAGE IN E. COLI. T.G. Rossman, M.S.Meyn, P. Gottlieb, & W. Troll, N.Y.U. Med. Ctr.N.Y.,N.Y. 10016. Among the consequences of damage to DNA are the expression of error-prone DNA repair and the induction of λ prophage. Induction of λ is accompanied by a proteolytic cleavage of the λ repressor (Roberts and Roberts, PNAS 72:147, 1975). We have previously shown that the competitive protease inhibitor antipain inhibits both λ prophage induction and error-prone DNA repair expression (Meyn, Rossman and Troll, PNAS 74:1152, 1977). We now report that three other protease inhibitors, of different chemical structures, also inhibit the induction of λ prophage. These are: 1) the non-competitive inhibitor TLCK, 2) the non-competitive inhibitor DFP and 3) the competitive inhibitor elastatinal. If the induction of error-prone DNA repair requires the proteolytic cleavage of a repressor, and if the cleavage of this (hypothetical) repressor involves an analogous cleavage site to that of the λ repressor, then one repressor might act as a competitive inhibitor of the cleavage of the other. To test this hypothesis, we introduced a plasmid containing the λ repressor gene cI (Bachman, Ptashne and Gilbert, PNAS 73:4174, 1976), into a tif-1 mutant, which exhibits a mutator effect at 42° due to expression of error-prone DNA repair. Tif-1 scrains carrying this plasmid (and thus containing excess λ repressor) show a 50% inhibition of the tif-1 mutator activity, assayed by reversion to the Trp+ phenotype at 42°. This result suggests that the λ repressor may compete for the same protease as that required for expression of error-prone DNA repair. Supported by: CA 19421, CA 16060 and is part of a Center Program supported by the National Institutes of Environmental Health Sciences National Institutes of Health, Grant No. ES 00260.

158 PROTEOLYTIC DEGRADATION IN NONFILAMENTOUS DERIVATIVES OF WP44$_s$ tif uvrA. Paul Kirschmeier and Evelyn M. Witkin, Douglass College, New Brunswick, NJ 08903
A temporary inhibition of cell division is one of the inducible SOS functions expressed in Escherichia coli after treatment with many DNA-damaging agents. In lon mutants after UV radiation and in tif mutants incubated at 42°C, cell division is extremely delayed, resulting in filamentous growth. Since the lon/degT gene also alters proteolytic activity(1), it has been proposed that filamentous growth may be the consequence of relatively inefficient degradation of an inducible septum-inhibiting protein(2,3,4). In that case, mutants in which filamentous growth is supressed(Sfi mutants) might be expected to show increased proteolytic activity when compared to the filamentous parent strain. We have selected nonfilamentous Sfi derivatives of the tif uvrA strain WP44$_s$ and have assayed the proteolytic activity by measuring the rate of degradation of radioactively labeled abnormal proteins produced by incorporation of puromycin or canavanine into nascent peptide chains. Many Sfi derivatives degraded puromycyl proteins 20-40% faster than WP44$_s$. When the derivatives that degraded puromycyl proteins faster were assayed with the canavanyl substrate some derivatives manifested high degradative rates while others were only as active as the filamentous parent. The role of Sfi mutations in SOS regulation will be discussed.
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159 INDUCED STABLE DNA REPLICATION AS A POSSIBLE SOS FUNCTION. Tokio Kogoma and M.J. Connaughton, Dept. Biol., Univ. New Mexico, Albuquerque,87131
The ability of the cell to continue DNA replication in the absence of protein synthesis (stable DNA replication) can be induced after treatments that result in damage in DNA or interruption of its synthesis (e.g., thymine starvation, treatments with nalidixic acid, hydroxyurea or cytosine arabinoside, or placing dnaB, dnaE, dnaG mutants at nonpermissive temperatures). Ultra-violet (UV) irradiation followed by a period of growth also induces stable DNA replication. The induction by thymine starvation, nalidixic acid or UV irradiation is found to be dependent upon the RecA+ phenotype. Induced stable DNA replication is considerably more resistant to UV irradiation than normal replication. This is also the case in a uvrA strain. The size of DNA synthesized after UV irradiation during stable DNA replication becomes progressively smaller with increasing UV dose as detected in alkaline sucrose gradient sedimentation. We suggest that during the inductive treatments a modified replication apparatus is formed which can replicate damaged DNA more efficiently than the normal replication complex.

160 STRAND-ASYMMETRICAL DNA REPAIR? Richard Bockrath, Indiana University School of Medicine, Indianapolis, Indiana 46202

Asymmetric strand specificity in DNA repair of premutational photoproducts is suggested by studies of mutation in E. coli. When UV irradiated cells are incubated under conditions unfavorable for protein synthesis there may be a large decrease in the frequency of observed mutants with negligable decrease in the overall viable titer. The decrease (mutation frequency decline, or MFD) is interpreted as enhanced excision repair of some premutational photoproducts. Potential de novo UAG suppressor mutation is very susceptible to MFD and therefore excision repair of photoproducts in tRNA genes is considered. However, potential conversion mutation, the mutation of a UAG suppressor to a UAA suppressor, is not susceptible to MFD. Data show both de novo suppressor mutation and conversion mutation to result from a photoproduct sensitive to photoreactivation. A simple model may be constructed that attributes both de novo suppression and conversion to a T-C dimer at the site of a GC to AT transition. The model has the T-C dimer in the transcribed strand of DNA to account for de novo suppressor mutation and in the non-transcribed strand of DNA to account of conversion mutation. Consequently, MFD of potential de novo suppressor mutation contrasted with the absence of MFD for potential conversion mutation suggests enhanced excision repair specifically for a dimer in the transcribed strand of a tRNA gene. Two possible mechanisms for strand-asymmetrical repair will be discussed: transcriptional asymmetry (interaction between the gene product RNA and the transcribed strand of DNA) and replicative asymmetry (possible differences in daughter strand gaps resulting in the 3' -to-5' strand and in the 5'-to-3' strand). Supported by N.I.H. grant GM 21788.

161 ULTRAVIOLET RADIATION-INDUCED MUTAGENESIS IS NOT DETECTABLE IN *uvrD recB* STRAINS OF *Escherichia coli* K-12, Neil J. Sargentini and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305.

Chloramphenicol (CAP)-sensitive pathways of postreplication repair (1) and excision repair (2,3) depend on the wild-type alleles of the *recA*, *lexA*, *uvrD*, and *recB* genes. The literature suggesting that these pathways are responsible for ultraviolet (UV) radiation-induced mutagenesis has been summarized (4). UV-induced mutagenesis is blocked by *recA* (5) or *lexA* (6) mutations. Since UV-induced mutagenesis was not totally blocked by *uvrD* or *recB* mutations, we theorized that these genes are involved in separate branches of the two error-prone repair pathways. Thus, inhibition of both the $uvrD^+$ and $recB^+$-dependent branches would be required in order to block UV-induced mutagenesis. As expected, UV-induced mutagenesis could not be detected in *uvrB uvrD recB* or *uvrD recB* strains of *Escherichia coli* K-12. Experiments are in progress to determine the ability of a *uvrD recB* strain to perform W-reactivation, W-mutagenesis, and prophage induction.

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162 ENZYMATIC PATHWAY OF ERROR-PRONE REPAIR IN UV-IRRADIATED PHAGE T4. Daniel B. Yarosh, Dept. Microbiology, University of Arizona, Tucson, Arizona 85724

The reversion frequencies of two amber mutants of phage T4 were observed after ultraviolet light treatment and subsequent growth in a permissive (su^+) host. These two mutants were strongly induced to revert under these conditions. It was found that their UV-induced reversion is abolished if the phage are defective in their ligase (gene 30) function. Preliminary results also show that UV-induced reversion is abolished if the phage-coded DNA polymerase (gene 43) is an anti-mutator or if the exonuclease coded for by gene 47 is partially defective. These results suggest that UV-induced lesions can be repaired by an error-prone pathway which employs an exonuclease (gene 47), the phage-coded DNA polymerase (gene 43), and ligase (gene 30). MMS-induced reversion of one of the amber mutants is also abolished by the ligase deficiency and the phage anti-mutator DNA polymerase (V. Johns, personal communication). This research was supported by a National Science Foundation Grant to Harris and Carol Bernstein and by a Tozer Foundation Graduate Fellowship to D.B.Y.

163 AMINO ACID SUPPORT OF NALIDIXIC ACID-INDUCED ERROR-PRONE REPAIR OF UV DAMAGE IN ESCHERICHIA COLI B/r WP2, Charles O. Doudney, Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201

The fluence-response curve for induction of trp revertants in Escherichia coli B/r WP2 thy trp is initially quadratic (N = 2) but becomes linear (N = 1) above about 24 J/m,^{2} a fluence that causes recoverable damage to all DNA replication and thus presumably leads to induction of error-prone repair in all cells (J. Bacteriol. 128, 815, 1976). Incubation with nalidixic acid, an antibiotic that prevents accumulation of DNA, also leads to an initial linear (N = 1) response to UV. This conversion began after 30 min of incubation with nalidixic acid and was completed after 60 min. Incubation without tryptophan during induction blocked the conversion, thus indicating the need for protein synthesis. When the bacteria were incubated without tryptophan after nalidixic acid induction was completed (but before UV exposure) the mutation frequency dropped drastically. The drop was prevented by supplementation with tryptophan and casein hydrolysate. Addition of chloramphenicol to the amino acid—supplemented medium caused a comparable drop. The possibility that this elimination of the induced error-prone repair capacity is the basis for "mutation frequency decline" (MFD) will be discussed.

164 PLASMID-ASSOCIATED SENSITIVITY TO ULTRAVIOLET RADIATION AND INCREASED DNA DEGRADATION IN REPAIR-DEFICIENT STRAINS OF STAPHYLOCOCCUS AUREUS, Richard V. Goering and Colleen Viola, Dept. Med. Microbiol., Creighton Univ. Sch. Med., Omaha, Neb. 68178

In gram-negative bacterial species, certain plasmids are known to influence the repair of cellular DNA, most commonly conferring some degree of protection against ultraviolet (UV) radiation on the bacterial host. We have observed that the presence of specific plasmids in certain repair-deficient strains of the gram-positive bacterium, Staphylococcus aureus, results in a marked increase in sensitivity to UV radiation with a concomitant increase in UV-induced degradation of DNA. S. aureus strains 152 and 152 rec-4 (a recA-type mutant) were transduced to carry a variety of antibiotic-resistance plasmids. Following exposure of cells to UV radiation, a comparison of plasmid and non-plasmid carrying strains revealed: (1) plasmid carriage did not influence the kinetics of cellular survival or DNA degradation in strain 152 (wild type) or strain 152 rendered phenotypically HCR$^-$ by incubation in the presence of caffeine (3.5 mg/ml), (2) plasmid carriage conferred a slight increase in UV sensitivity and DNA degradation on the recombination-deficient mutant, 152 rec-4, but was associated with a marked increase in UV sensitivity and DNA degradation when the rec$^-$ mutant was rendered phenotypically REC$^-$ HCR$^-$ by incubation in the presence of caffeine, (3) plasmid-associated sensitivity to UV was plasmid specific, thus far observed only with penicillinase plasmids pI258 and pII147, (4) the UV-sensitizing effect was not due to prophage induction. The possibility that the UV-sensitizing effect may be due to a plasmid-associated nuclease will be discussed.

165 PLASMID (pKM101)-MEDIATED REPAIR AND MUTAGENESIS, Graham C. Walker, Pamela J. Langer, William G. Shanabruch, and Patricia P. Dobson, Massachusetts Institute of Technology, Cambridge, MA 02139

The plasmid pKM101 increases i) cellular survival after UV irradiation ii) chemical and spontaneous mutagenesis iii) reactivation and mutagenesis of UV-irradiated phage in unirradiated cells. These effects are dependent on the recA$^+$ lexA$^+$ genotype in E. coli and on the recA$^+$ genotype in S. typhimurium. pKM101 plays a critical role in the Ames carcinogen detecting strains. We compare the effect of pKM101 on W-reactivation of UV-irradiated P22 in S. typhimurium and of λ in E. coli. In S. typhimurium only a very weak inducible reactivation is observed in the absence of pKM101 but a strong reactivation is observed in its presence. The inducible reactivation in pKM101 containing strains of both E. coli and S. typhimurium is independent of the presence of chloramphenicol under conditions where protein synthesis is >99% inhibited suggesting the induction involves the activation of a constitutively produced protein. A screening procedure was used to isolate mutants of pKM101 unable to enhance mutagenesis. The mutants are deficient in the other properties described above. In vitro recombinant techniques were utilized to produce smaller derivatives of pKM101 still able to enhance mutagenesis and repair. We report the results of mini cell and in vitro transcription-translation studies of plasmid-coded proteins produced by pKM101, pKM101 mutants and cloned derivatives of pKM101. The relationship of pKM101 to cellular error-prone repair will be discussed.

166 EFFECT OF PSEUDOMONAS R FACTORS ON UV-SENSITIVITY AND UV-MUTAGENESIS, Philip Lehrbach and Barry T.O. Lee, Dept Genetics, University of Melbourne, Australia 3052.
Several R plasmids (pMG1, pMG2, R931, R2, RPL11, and R1771 Hg^r) which alter the response of Pseudomonas aeruginosa (ATCC15962) to the lethal effects of ultraviolet light have been investigated. Plasmids pMG1, pMG2, R2, R931 and R1771 Hg^r increase cell survival following UV-damage in wild type host cells, *uvr*$^-$*rec*$^+$ and *pol A* mutants, but fail to alter the UV response of a *rec A* mutant of Pseudomonas aeruginosa. RPL11 decreases UV-survival in wild type hosts and to a similar degree in *uvr*$^-$ *rec*$^+$ and *pol A* mutants, but again fails to alter the UV-response of *rec A* host cells. All plasmids studied enhance the level of spontaneous and UV-induced back mutation in a variety of auxotrophic markers studied. Since the UV-protective effect of pMG1, R931, R2, pMG2 and R1771 Hg^r is dependent on the *rec A*$^+$ gene the effect of sodium arsenite, an inhibitor of *rec A*-dependent step in the repair of UV-irradiated DNA, was studied. The effect of a sublethal concentration of sodium arsenite following UV-irradiation was examined in strains trpB1, trpB1R2, trpB1pMG2, trpB1pMG1, trpB1R931 and trpB1R1771 Hg^r. The presence of sodium arsenite eliminates the increased UV-survival of trpB1pMG1, trpB1pMG2 and trpB1R931 reducing the survival to that of UV-irradiated strain trpB1 in the presence of sodium arsenite. When strains trpB1R2 and trpB1R1771 Hg^r were tested only a slight reduction in UV-survival was observed. It is concluded that in strains trpB1pMG1, trpB1pMG2 and trpB1R931 UV-protection is determined by a *rec A*-dependent arsenite-sensitive repair pathway, whereas in strains trpB1R2 and trpB1R1771 Hg^r UV-protection is determined by a *rec A*-dependent arsenite-insensitive step in DNA repair.

167 MUTAGENESIS DURING IN VITRO DNA SYNTHESIS, Thomas A. Kunkel, Lisa A. Weymouth, K.P. Gopinathan and Lawrence A. Loeb, Institute for Cancer Research, Fox Chase, Philadelphia, PA 19111
We have developed a biological assay for measuring the fidelity of DNA synthesis in vitro using a natural DNA template. The template was single-stranded ØX 174 DNA containing an amber mutation (am3 in gene E). A specific DNA fragment, obtained separately from an Hae III digest of ØX 174 RFI DNA, was hybridized to this template and served as a fixed primer terminus 83 nucleotides from the mutation. An in vitro polymerization reaction was performed using different DNA polymerases under various conditions. The copied DNA was used to infect spheroplasts or calcium-treated cells, and errors were quantitated by measuring reversion of progeny phage to wild type. Using E. coli DNA polymerase I, the reversion rate indicated an approximate error rate of 1 in 8000 under normal copying conditions. The error rate was reduced to 1 in 500 using altered nucleotide pools or when Mn^{2+} was substituted for Mg^{2+}. From a comparison of the possible codon substitutions with the effect of altering various nucleotide concentrations in the pool it is concluded that the most likely substitution is a C for a T at position 587 in the amber codon. With the avian myeloblastosis virus DNA polymerase frequent mutations were observed even under normal conditions of copying. The absolute error rate of 1 in 706 ± 300 obtained using this natural DNA template confirms our earlier findings (using synthetic polynucleotide templates) and indicates that the AMV polymerase is error prone. This assay system is currently being used to determine the accuracy of nucleotide selection by DNA polymerases-α and -β from a variety of eucaryotes.

DNA Repair in Lower Eukaryotes

168 IS THERE A UV-INDUCIBLE COMPONENT OF ERROR-PRONE REPAIR IN YEAST? Friederike Eckardt and R. H. Haynes, Department of Biology, York University, Toronto, Ontario, Canada M3J IP3.
The commonly observed 'dose-squared' induction kinetics for UV-mutagenesis in E.coli are believed to arise from the action of an inducible, error-prone repair system which attacks pre-mutational lesions in DNA (the 'one-lesion plus SOS induction' hypothesis). In haploid, repair competent strains of the yeast Saccharomyces cerevisiae mutation induction kinetics are biphasic: linear at low doses but squared at high doses. These induction curves can be fitted mathematically by a general equation for repair-mediated mutagenesis if it is assumed that there exists a significant constitutive level of error-prone repair (required for the linear kinetics) plus a UV-inducible component (required for the dose-squared kinetics). We find that if protein synthesis is blocked by incubating the cells for three days after irradiation in the presence of 20µg/ml cycloheximide, then mutation frequencies in the higher dose range are reduced and the resulting points lie along a linearly extrapolated segment of the low dose kinetics; in effect, the dose-squared component appears to be abolished. These results, together with the phenomenon of radiation induced UV-resistance (Patrick and Haynes, J.Bact.95, 1350, 1968), the demonstration of radiation induced transmissible factors capable of enhancing recombination in diploids (Fabre and Roman, PNAS 74, 1667, 1977) and the enhancement of mutagenesis concomitantly with radiation induced UV-resistance (Moustacchi, personal communication, 1977), indicate the existence of one or more modes of inducible DNA repair in yeast which in part at least are error-prone.(Supported by NRCC).

169 REGULATION BY PROTEINASES OF INTRACELLULAR ACTIVITY OF A PUTATIVE rec-NUCLEASE OF NEUROSPORA. M.J. Fraser, Dept. Biochem., McGill University, Montreal, Canada H3G 1Y6.

Two activities of Neurospora, a single-strand (ss) endonuclease and a double-strand (ds) exonuclease, have been found to be associated with a single polypeptide (now called endo-exonuclease) of molecular weight about 53,000 daltons. The enzyme degraded linear, but not covalently closed circular ds-DNA and both nuclease activities were inhibited by ATP. In vitro, endogenous proteinase(s) preferentially inactivated the ds-exonuclease function. The resulting ss-endonuclease has been shown to attack U.V.-irradiated, but not unirradiated ds-DNA. Two mutants of Neurospora which were sensitive to a wide spectrum of agents that damage DNA, uvs-3 and uvs-6, were found to be partially deficient in the endo-exonuclease activity expressed in fresh extracts of mycelia. The uvs-3 mutant was shown previously by Schroeder to have altered mitotic recombination. In extracts of wild-type log phase mycelia at least 75% of the endo-exonuclease activity was masked, but was activated in vitro by endogenous PMSF-sensitive proteinase(s) or with trypsin. The activation appeared to involve the conversion of a pronuclease to an active enzyme. Active endo-exonuclease was released from colonies growing on DNA agar and produced haloes of digestion around the colonies. The uvs-3 and uvs-6 mutants, as well as some other mutants that were NOT defective in DNA-repair (nuclease halo or nuh mutants), failed to release normal amounts of the enzyme. The pronuclease accumulated in vivo in wild-type mycelia growing in liquid culture, but was rapidly lost when the mycelia entered stationary phase, suggesting that proteolysis may have been responsible for this loss. The levels of active endo-exonuclease and pronuclease at different stages of growth of both the uvs and nuh mutants differed from each other and from the wild-type in complex ways.

170 IDENTIFYING MUTAGENIC REPAIR SYSTEMS BY ANTIMUTATOR MUTATIONS, P.J. Hastings, R.C. von Borstel and S.-K. Quah, Dept. of Genetics, Univ. of Alberta, Edmonton, Canada T6G 2E9

Antimutator mutants of yeast were sought (a) to test the hypothesis that spontaneous mutation arises from mutagenic repair of spontaneous lesions, in which case antimutators would be sensitive to mutagenic agents, and (b) to see whether there is a mutagenic repair process in yeast other than the rad18 epistasis group as identified by UV survival and mutability.

Of the ten putative antimutator strains partially characterized, 7 are sensitive to UV light. Further characterization of two of these confirms that low spontaneous mutation segregates with UV sensitivity, so that both are presumptive repair mutants.

Ant2-1, which causes a 5-fold reduction in his1-7 and lys1-1 locus reversion has no effect on suppressor production. It does not complement with rev3-1 to restore UV-resistance. It is therefore an allele of rev-3 and a member of the rad18 epistasis group.

Ant1-1, isolated in a mutator strain, is also expressed in a wild-type background, where it appears to be a general antimutator. Ant1-1 and ant2-1 show epistasis in UV-survival. However, ant1-1 and rad6-1 show an additive interaction implying that they are in different systems. Rad6 is reported to be epistatic with rev3. We are trying to resolve this contradiction.

171 SPONTANEOUS MUTATION RATE AND THE LEVEL OF ENDONUCLEASE IN SCHIZOPHYLLUM COMMUNE, Evelyn Waldstein, Yona Shneyour, Judith Stamberg and Yigal Koltin, Tel Aviv University, Ramat Aviv, Israel.

The spontaneous mutation rate in Schizophyllum is 10^{-8} - 10^{-9} in the vegetative haploid state, and 10-20 times higher following meiosis. In studies on the causes of this meiotic effect a haploid strain displaying an increased mitotic spontaneous mutation rate (10^{-7}) was detected. Its mutagenic effect appears as a recessive trait in heterokaryons with wild type strains during mitotic divisions. This mutagenic effect is also recessive in meiosis. - Endonuclease activity was tested in the mutant and a wild strain using phage PM2 DNA as substrate. Its activity in the strain displaying a low mutation rate is 3 times higher than in the strain with a high mutation rate. In heterokaryons formed between the two strains the endonucleolytic activity is equivalent to the sum of the activities of the two strains. In the fruit bodies, where meiosis occurs, the level of endonuclease is even higher than the sum found in the vegetative state and the mutation rate is low. - The mutation rate and level of endonuclease seem to be inherited thus far as a single genetic trait. Low level of endonuclease appears to be inherited along with the trait for high mutation rate that affects both mitosis and meiosis. The results suggest an interrelation between spontaneous mutagenesis and error prone processes in DNA repair occurring during normal cycles of replication in the absence of endonucleolytic activity.

172 INTERACTIONS BETWEEN RADIATION-SENSITIVE MUTATIONS IN DOUBLE MUTANT HAPLOIDS OF DICTYOSTELIUM DISCOIDEUM, R. A. Deering and D. L. Welker, Department of Biochemistry and Biophysics, The Pennsylvania State University, University Park, PA 16802.

The ability to isolate radiation sensitive mutants of the eukaryotic cellular slime mold D. discoideum and to characterize these mutations at genetic and molecular levels makes this system a useful model for the study of DNA repair in eukaryotes (Welker and Deering, J. Gen. Micro. 97, 1, 1976). Radiation sensitive mutations affecting nine complementation groups have been identified on the basis of linkage and complementation studies. These are designated radA thru radI. Mutants in the radC gene which increase sensitivity to UV but not to gamma rays have been shown to be defective in the in vivo endonucleolytic production of single strand breaks in the DNA of UV-irradiated cells. The defects associated with the other complementation groups have not yet been identified, except that radB seems to affect the rate of resealing of UV-initiated single strand breaks. Haploids carrying both the radA and radC mutations are more sensitive to UV than either of the single mutant haploids. This synergistic interaction indicates that the radA and radC gene products are involved in two different repair pathways. Haploids bearing both radA and radB have the same sensitivity as the radB single mutant, indicating that radA and radB influence the same repair pathway. Since radC appears to be in an excision pathway, these results associate radA and radB with another pathway, possibly postreplication or yet another eukaryotic mode of repair. (Supported by NIH GM-16620)

173 ANALYSIS OF EXCISION DEFICIENCY IN mei-9 MUTANTS OF DROSOPHILA melanogaster, Paul V. Harris and James B. Boyd, Dept. of Genetics, University of California, Davis, CA 95616

Cultured cells derived from embryos carrying a mei-9 mutation are mutagen sensitive and deficient in the excision of pyrimidine dimers as measured by susceptibility of uv-irradiated DNA to uv-endonuclease. In contrast, cells carrying the mei-218 mutation are proficient in dimer removal[1]. -- We have confirmed the excision deficiency in established cell cultures of the mei-9^a mutant with chromatographic analysis of pyrimidine dimers. Twenty-four hours after a dose of 15 Jm^{-2} mei-218 cells have reduced the percent thymine in dimers from 0.035 $\pm$ .002% to 0.011 $\pm$ .006%. No removal was detected in mei-9^a cells. At this dose the cells remain viable for over 36 hr. -- We are employing the carcinogen N-acetoxy-acetylaminofluorene (AAAF) to investigate the mei-9 deficiency. The highly sensitive technique of alkaline elution[2] was employed to analyze single-strand breaks in high molecular weight DNA. DNA from each of 8 cell lines investigated develop single-strand breaks following exposure to 20-25 µm AAAF for 1 hr. Cultures of mei-9 cells, however, produce 2-4-fold fewer breaks in established cell lines and 7-fold fewer breaks in primary cultures. Hydroxyurea, which promotes an accumulation of single strand gaps in uv-irradiated DNA from mammalian[3] and Drosophila[4] cells, potentiates AAAF-induced break formation in all established cell lines except mei-9. No significant potentiation occurs in mei-9 cells within the concentration range 5-200 µm. This observation may reflect a post-incision deficiency in the ability of mei-9 cells to remove modified bases.

1. Boyd et al., 1976 Genetics 84: 527-544; 2. Kohn et al., 1976 Biochemistry 15: 4629-4637; 3. Collins et al., 1977 Mutation Res. 42: 413; 4. Harris and Boyd, unpublished.

174 CHROMOSOME BREAKAGE AND REJOINING IN REPAIR DEFECTIVE MUTANTS OF DROSOPHILA.,M. Gatti, S. Pimpinelli, and B. S. Baker, Biology Dept., University of California, San Diego, USA and Istituto di Genetica, Universita di Roma, Italy.

Many recombination defective mutants and mutagen sensitive mutants of Drosophila melanogaster have been recently shown to affect DNA repair processes. To characterize the roles of these loci in the chromosomal economy of non-mutagenized cells 20 of these mutants (representing 13 loci) have been examined for effects on the frequency and type of spontaneous chromosomal aberrations in larval neuroblasts. Twelve mutants, representing 6 loci, significantly increase the frequency of chromosome breaks. The type of breaks (chromatid vs isochromatid) and the distribution of breaks along chromosomes (uniformly distributed, predominantly euchromatic, predominantly heterochromatic) were the same for all mutant alleles at a locus but differed between loci. X-ray induced aberrations have been studied in mutants at the six loci that effect spontaneous chromosome stability. These data reveal locus specific patterns of induced aberrations. In mutants at 2 loci chromatid interchanges could not be induced and these mutants are therefore defective in chromosomal rejoining. Mutants at these 6 loci have also been studied for their effects on sister chromatid exchange (SCE). None showed a significant increase above control levels in the frequency of SCEs. However, mutants at the two loci defective in chromosomal rejoining exhibited only one half the control level of SCEs. The latter observation suggests that in Drosophila the mechanisms of exchange and SCE share at least some steps in common.

175 MUTAGEN SENSITIVITY AND PATHWAYS OF DNA REPAIR IN DROSOPHILA MELANOGASTER, P. Dennis Smith, Emory University, Atlanta, Georgia 30322.

Methods have been developed which allow the isolation of mutant strains of Drosophila melanogaster which exhibit increased sensitivity to killing by mutagenic agents. Sixty-three X-linked mutations have been isolated on the basis of enhanced sensitivity to methyl methanesulfonate (Smith, 1973, Mut. Res. 20:215; Boyd et al., 1976, GEN. 84: 485; Smith, 1976, Mol. gen. Gen. 149:73). Complementation analysis has suggested the existence of at least fourteen complementation groups and recombination studies have succeeded in localizing eleven of these groups to separable genetic loci on the X chromosome. Studies of cross-sensitivity to physical and chemical mutagens indicate two general patterns. One class of mutants displays sensitivity to MMS and X ray while a second class displays a broader sensitivity, including MMS, X ray, UV and a number of chemical carcinogens and mutagens. Effects of a number of the mutants on female fertility have led to the demonstration that mutagen sensitivity and recombination deficiency can be the result of a single mutation in this organism and implicate recombination functions in DNA repair processes. Examinations of multiply-mutant strains for synergistic effects on mutagen sensitivity have allowed development of a working model for DNA repair in this lower eukaryote.

DNA Repair in Mammals

176 A POSSIBLE ROLE FOR POLY ADP-RIBOSE IN THE REPAIR OF DNA, Robert C. Benjamin and D. Michael Gill, Dept. Biology, Harvard University, Cambridge, Mass 02138

The formation of poly ADPR was studied in suspensions of HeLa or BSC cell ghosts which had been rendered permeable by gentle detergent lysis, thus avoiding nuclear damage incurred during isolation of nuclei. The incorporation of ADPR from ^{32}P-NAD was stimulated by agents that introduce cuts into the DNA, DNase I or micrococcal nuclease raised the rate of poly ADPR synthesis up to 50-fold while pre-incubation of the ghosts under conditions that allowed endogeneous nucleases to act resulted in about a 5-fold increase. Prior X-irradiation of whole cells at 0° elevated the initial rate of synthesis linearly with dose, doubling with 1200 rads. The boosted response declined at a constant rate when the irradiated cells were incubated at 35° prior to lysis, approaching control values in 4-6 minutes. Thus we assume that 1) X-irradiation generates from DNA an intermediate that stimulates poly ADPR synthesis and 2) this intermediate is inactivated by the cell and becomes unsuitable for poly ADPR synthesis. Since the inactivation is slowed by inhibitors of poly ADPR synthesis, it may involve poly ADPR synthesis. Slower inactivation occurs in lysed cells after either X-irradiation or nuclease digestion. We are examining the nature of the modification to DNA responsible for this stimulation by adding plasmid DNA to a partially purified extract of calf thymus whose poly ADPR synthesis depends on exogeneous DNA. PMB9 supercoils stimulated synthesis to some extent. Stimulation is improved slightly if the PMB9 is cut once per circle with Eco R1 and greatly if cut approximately 20 times with Hae III. Thus the system responds not so much to total DNA or to DNA of a particular sequence as to the number of DNA fragments and perhaps, therefore, to cut ends.

177 ALTERATION OF NAD METABOLISM ASSOCIATED WITH CARCINOGEN-INDUCED DNA DAMAGE, Myron K. Jacobson, Departments of Chemistry and Basic Health Sciences, North Texas State University, and Elaine L. Jacobson, Department of Biology, Texas Woman's University, Denton, Texas 76201.

The NAD content of 3T3 cells and mitogen-stimulated human lymphocytes has been measured after exposure to N-nitroso compounds that are direct-acting carcinogens, indirect-acting carcinogens, or non-carcinogens. We report that the direct-acting carcinogens cause large decreases in NAD in both 3T3 cells and human lymphocytes, that indirect-acting carcinogens cause large decreases in NAD in human lymphocytes but not in 3T3 cells, and that N-nitroso compounds that are very weak or non-carcinogens do not affect the NAD content of either cell type. This decrease in NAD levels is dependent on carcinogen concentration and time of exposure and is concurrent with damage to DNA as determined by sedimentation velocity centrifugation on alkaline sucrose gradients. NAD is the substrate for the synthesis of poly(ADP-ribose) which has been suggestec to be involved in DNA repair. This carcinogen-induced NAD depression can be prevented by the simultaneous presense of inhibitors of poly(ADP-ribose) polymerase. These data suggest a possible role of poly(ADP-ribose) in repair of carcinogen-induced DNA damage. (Supported in part by Grant No. BC-184 from The American Cancer Society and B-633 from The Robert A. Welch Foundation and Institutional Grants from NTSU and TWU.)

178 UV-ENDONUCLEASE SENSITIVE SITES IN DAUGHTER DNA FROM UV-IRRADIATED ASYNCHRONOUS AND SYNCHRONOUS CHINESE HAMSTER CELLS, S. M. D'Ambrosio and R.B. Setlow, Dept. Radiology, Ohio State Univ., Col., Ohio, 43210 and Dept. Biology, Brookhaven Nat. Lab, Upton, NY 11973. Among the several models that have been put forth to account for the process of postreplication repair in mammalian cells, one suggests that gaps are made in newly synthesized (daughter) DNA following UV-irradiation. Some controversy exsists as to how these gaps are filled-in. One mechanism suggests that gaps are filled-in by de novo synthesis, while another suggests exchange or transfer of parental DNA. Support for the latter has been derived from studies indicating the presence of UV-endonuclease sensitive-sites in daughter DNA of UV-irradiated mammalian cells. Dimers in daughter DNA could arise from: a) the transfer of parental DNA segments containing dimers; and or b) irradiation of daughter DNA during replication. In order to determine how dimers arise in daughter DNA we looked for the presence of UV-endonuclease (M. luteus) sensitive-sites in asychronous and sychronous Chinese hamster cells (low levels of excision repair) after irradiation with 10 Jm^{-2} UV. We find that the number of UV-endonuclease sensitive sites decreases (1.5 to 0.6 per 10^8D) as the time ($\frac{1}{2}$ to 6 hr) between irradiation and pulse-labeling of daughter DNA increases. Also, the number of sites in daughter DNA from cells irradiated in S-phase was 2.3 per 10^8D, while it was only 0.1 per 10^8D in cells irradiated in the G1-phase. The number of sites in parental DNA was 20 per 10^8D. We conclude from these studies that: a) few if any dimers are transfered from parental to daughter DNA; and b) the dimers that are detected in asychronous cells are probably due to irradiation of replicating daughter DNA.

179 THE MECHANISM OF POST REPLICATION REPAIR IN MAMMALIAN CELLS, Jay Doniger, Biology Dept., Brookhaven National Lab., Upton, NY 11973 and Biology Branch, National Cancer Institute, Bethesda, MD 20014.
The nature of newly replicated DNA in ultraviolet light irradiated Chinese hamster V-79 cells was determined. Alkaline sucrose sedimentation studies were used to measure the size of nascent strands and DNA fiber autoradiography was used to measure the rate and extent of fork progression. In unirradiated cells the size of pulse labeled (15' or 30') nascent strands measured in the gradients was equal to the size of growing replicons seen in the autoradiograms. In cells irradiated with 5 J/m^2 at 254 nm the mode MW of the 15' pulse labeled nascent strands is smaller than that in unirradiated cells and is equivalent to the distance between pyrimidine dimers. The rate and extent of fork progression is the same as in unirradiated cells. By 60' the average fork passed thru 5 pyrimidine dimers present in parental DNA. Most of the replicons observed initiated synthesis in the 25' interval between irradiation and addition of the pulse label. Caffeine inhibits the joining of nascent strands in irradiated cells but does not effect the rate of fork progression. Preliminary studies with human cells indicate similar results. The data support the "gapped synthesis" model of post replication repair and rule out a major role for "replicative bypass". This work was supported by the United States Department of Energy.

180 ATAXIA TELANGIECTASIA : CHARACTERIZATION OF HETEROZYGOTES, Martin F. Lavin, Philip C. Chen and Chev Kidson, Biochem. Dept., University of Queensland, Brisbane 4067 Aust.
Heterozygotes for Ataxia telangiectasia (AT) have an increased incidence of malignant tumors (Swift et al, 1976) and also a predisposition to develop autoimmunity (Friedman et al, 1977). Together these data imply that a single copy of the mutant gene can have functional consequences of fundamental and practical significance. In this context identification and characterization of heterozygotes becomes an important goal. Making use of the generally high ionizing radiosensitivity of lymphoid cells and the increased capacity of phytohemagglutinin stimulated lymphocytes for DNA repair we have developed a rapid assay for AT homozygotes using human lymphocytes. Extreme radiosensitivity of homozygotes persists in Epstein Barr virus transformed lymphoblastoid cell lines. At a carefully chosen radiation dose survival curves for homozygotes, heterozygotes and controls have a trimodal distribution, as monitored by dye exclusion. Discrimination is also demonstrable between dose response curves of cells cloned in agar. These findings clearly place heterozygotes in an intermediate position between controls and homozygotes with respect to radiosensitivity. Identification of a functional defect in heterozygotes now permits quantitative correlation between the extent of the DNA repair and the liability to mutagenesis and to carcinogenesis.
Friedman, J. M., Fialkow, P. J., Davis, S. D., Ochs, H. D. and Wedgwood, R. J. (1977) Clin. Exp. Immunol., 28, 375.
Swift, M., Sholman, L., Perry, M. and Chase, C. (1976) Cancer Res., 36, 209.

181 DNA POSTREPLICATION REPAIR INDUCED BY BENZO[a]PYRENE DIOL EPOXIDES IN MOUSE EPIDERMAL CELL CULTURES. Tim Bowden, and Stuart Yuspa. National Institutes of Health, Bethesda, MD 20014.

DNA postreplication repair after exposure of target cells to (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (isomer I), a potent mutagen and carcinogen, and (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (isomer II), a weak mutagen and carcinogen was studied in mouse epidermal cultures. At any given dose level (0.16-3.3 μM) both isomers equally inhibited DNA synthesis as measured by ^{3}H-thymidine incorporation and decreased the size of newly synthesized DNA as measured by alkaline sucrose gradients. For both isomers the decrease in size of newly synthesized was shown to be due to gaps in the newly synthesized strands. At a dose level of 0.66 μM isomer I caused 0.9 gaps per 10^{7} daltons of newly synthesized DNA. When binding of ^{14}C-isomer I to epidermal DNA was determined, 0.7 adducts were bound per 10^{7} daltons of DNA (approximately one gap for every bound adduct). Binding of ^{14}C isomer II to epidermal DNA at a given dose level was one fourth that found for isomer I. Isomer II at an equivalent level of binding (0.7 adducts per 10^{7} daltons of DNA) caused 1.9 gaps per 10^{7} daltons of newly synthesized DNA (2 gaps for every adduct). Pulse-chase experiments with both isomers indicated that with time after exposure the gaps were filled, DNA was joined and elongated. Caffeine (0.75 mM) inhibited DNA elongation in cells treated with either isomer I or II. Thus exposure to the more potent mutagen and carcinogen, isomer I, resulted in one gap in the newly synthesized DNA per DNA-bound adduct while damage by the less potent isomer II resulted in two gaps per adduct. In each case these gaps were filled by a caffeine sensitive postreplication repair process.

182 DNA BIFILARLY SUBSTITUTED WITH BROMODEOXYURIDINE IN THE FIRST ROUND OF SYNTHESIS. Kouichi Tatsumi* and Bernard Strauss, University of Chicago, Chicago, Ill. 60637.

Incubation of human lymphoblastoid cells with BrUdr for short periods produces three classes of DNA containing analog: DNA_{HL}(ρ=1.75 g/cm^3), DNA_{int}(ρ=1.71 g/cm^3) and DNA_{HH}(ρ=1.80 g/cm^3). Isolated DNA_{int} yields both DNA_{HL} and DNA_{HH} after extensive shear or after treatment with N. crassa endonuclease, indicating that DNA_{HH} originates from DNA_{int}. The transient formation of DNA_{HH} along with the observation of four-pronged DNA structures in electron micrographs of the fraction was recently presented as evidence for a model of replication repair based on the process of strand displacement and branch migration. However, crosslinking of DNA *in vivo* by treatment of cells, with trioxsalen and near UV light prevents the appearance of DNA_{HH} whereas crosslinking after lysis, has little effect. These results suggest that most DNA_{HH} is derived from *in vitro* branch migration during isolation of DNA. However, branch migration may still be involved in the "skipping past" of lesions in the DNA template without leaving gaps in daughter strands if the length of displaced strands was not long enough to be cross-linked by the method used. A large fraction of DNA_{HH} is obtained after incubation of xeroderma lymphoid cells (XPA-3) with caffeine, possibly reflecting the synthesis of DNA in smaller replicating units. Inhibition of replication repair by caffeine may result from the accumulation of unfinished replicons rather than the inhibition of gap filling. DNA_{HH}, although arising from *In vitro* reactions, can be a good probe for estimating the number of replicating forks, i.e. the number of replicons functioning.

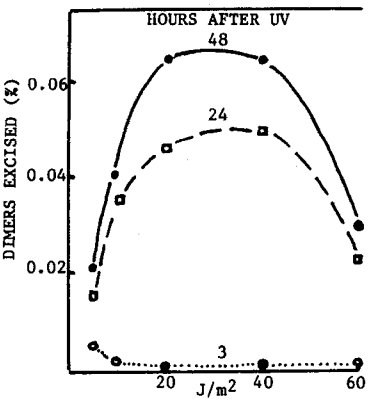
*Fellow of the Leukemia Society of America

183 INTERPRETATION OF MAMMALIAN DNA REPAIR STUDIES PERFORMED ON ALKALINE SUCROSE GRADIENTS, K.T. Wheeler, J.D. Linn, E.S. Chase, and C.T. Morton, Cancer Center, University of Rochester, Rochester, N.Y. 14642 and Brain Tumor Research Center, University of California, San Francisco, CA 94143.

Previous alkaline sucrose gradient studies on the repair of x-ray-induced DNA damage in mammalian cells indicated that the transition from DNA molecules with sedimentation coefficents ≤ 165 S to DNA species > 165 S was associated with the maintenance of their reproductive or functional integrity. However, the molecular interpretation of this transition was uncertain. To provide experimental evidence for an interpretation of this transition, we have compared: 1) the dependence of the sedimentation coefficients of these DNA species on rotor speed and radius, 2) the ability of these DNA species to renature, and 3) the ability of free radical scavengers to protect these DNA species against x-ray-induced damage. In general: 1) DNA species with sedimentation coefficients > 165 S do not exhibit a dependence on rotor speed and radius as predicted by Zimm while DNA species ≤ 165 S do, 2) DNA species > 165 S renature rapidly even under adverse conditions while species ≤ 165 S do not, and 3) free radical scavengers equally protect both sets of DNA species against x-ray damage. Thus, the data support the hypothesis that the transition from DNA species ≤ 165 S to DNA species > 165 S represents a conformation change from single-stranded random coil polymers to predominently single-stranded nonrandom coil polymers. The data also suggest that the rapid decrease to 165 S DNA species seen after low x-ray doses results from the introduction of a few single-strand breaks rather than an attack by hydroxyl free radicals on exposed radiosensitive linker sites. (Supported by CA-15203, CA-15325, CA-11051 and CA-11198)

184 PYRIMIDINE DIMER EXCISION IN HUMAN CELLS, William L. Carrier, David P. Smith and James D. Regan, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Normal human fibroblasts labeled with (^{3}H)thymidine in tissue culture excise UV-induced pyrimidine dimers from their DNA. Dimers are produced linearly at low doses of 254 nm UV light with ~0.3% of the thymine in dimers after 60 J/m^2. We have plotted the percentage of dimers excised, i.e., the difference between the amount initially formed and the amount remaining after incubation of the cells. After 3 hours very few dimers are excised. After 24 hours the amount excised is related to the dose of UV given. This pattern of excision is similar for normal human fibroblasts, WI-38, and HeLa cells. We find no difference between the rate of excision of dimers and the removal of dimer-specific, endonuclease-sensitive sites. (Research sponsored by NASA, by NCI and by DOE under contract with the Union Carbide Corp.)



185 COMPARING THE PERCENT SURVIVAL AND EXTENT OF EXCISION REPAIR OF THYMINE DIMERS FOLLOWING UV IRRADIATION OF CONFLUENT CULTURES OF HUMAN CELLS OR OF SYNCHRONIZED POPULATIONS AT VARIOUS TIMES DURING THE CELL CYCLE, Beate Konze-Thomas, Delia J. Dorney, Veronica M. Maher and J. Justin McCormick, Carcinogenesis Laboratory, C.O.M., Michigan State University, East Lansing, MI 48824, U.S.A.

To examine the effect of DNA excision repair and replication on the cytotoxicity of ultraviolet radiation (254nm) in human cells, we exposed synchronized populations of normal diploid fibroblasts to a series of low doses of UV at various times during the cell cycle (G_1, early S, middle S, early G_2) or of confluent cells in G_0 and determined the percent survival of the colony-forming ability. (80% synchrony was achieved by releasing density-inhibited cultures from confluency.) No measurable difference in percent survival could be detected between cells irradiated in G_0 and immediately released from confluency and cells released and then irradiated at various times during the cell cycle. However, if cells were irradiated in G_0 (6 J/M^2), but held in confluency for 0, 4, 8, or 16 hr before being released, the percent survival rose gradually from 20 to 25 to 45 and finally to 100. To investigate the molecular mechanisms responsible for these results, we examined the ability of cells held in confluency or of cycling cells to excise thymine dimers following irradition (40 J/M^2). Confluent cells excised 70% of the dimers during the first 6 hr post-irradiation and 90% during the first 12 hr. Preliminary results suggest that dimer excision in cycling, synchronized cells is less efficient than in confluent cells. These biochemical results correlate with the biological data and indicate that cell killing results from non-excised DNA damage. (Supported by US DHEW Grants CA 21247 & CA 21253 and by the Deutsche Forschungsgemeinschaft.)

186 EXCISION REPAIR IN MAMMALIAN CELLS, Farid E. Ahmed and R. B. Setlow, Biology Dept. Brookhaven National Laboratory, Upton, New York 11973.

Excision repair after combined treatments of UV and N-acetoxy-2-acetylaminofluorene (AAAF) was studied in repair proficient and repair deficient human fibroblasts (normal, xeroderma pigmentosum [XP] groups C, D, E, XP variants and ataxia telangiectasia). Three methods were used: a)unscheduled DNA synthesis measured radioautographically, b)photolysis of BrUra incorporated into parental DNA during repair, and c)sites sensitive to UV endonuclease from M. luteus. The three methods gave similar results (see Table). Saturation doses of 254 nm (20 Jm^{-2}) and AAAF (20 μM) were employed. Two patterns of excision repair due to combined treatments were observed: 1) total repair was additive in normal, XP variant, and ataxia cells, and AAAF did not inhibit repair of UV damage, and 2) total repair was much less than additive-usually less than observed for separate treatments- and AAAF inhibited dimer excision in XP C, D, and E. We conclude that in the 1st class of cells there are different pathways for repair of UV and AAAF lesions, and in the 2nd class the residual excision enzymes are different from those in normal cells. (Supported by U. S. Department of Energy).

Cell. line	Unscheduled synthesis[1]			Endonuclease assay[2]			BrUra photolysis[3]		
	20 Jm^{-2}	20 μM	20 Jm^{-2} + 20 μM	20 Jm^{-2}	20 Jm^{-2} + 20 μM		20 Jm^{-2}	10 μM	20 Jm^{-2} + 10 μM
Normal human	18.6	18.1	35	27.5	27.6		3.5	1.5	4.9
Ataxia	19.7	16.6	35	28.5	26.4		2.8	1.8	4.2
XP Variant	25.0	17.1	37.2	30.1	14.2		2.8	1.1	4.3
XP C	3.7	2.2	1.8	3.3	1.4		1.8	0.1	0.1
XP D	6.4	6.6	3.9	5.9	0.8		1.4	0.2	0.4
XP E	10.8	6.4	2.8	19.3	9.6		4.6	1.8	3.8

(1) Grains/Nucleus incorporated in 3hr.
(2) Sites removed in 24 hr/10^9 Daltons.
(3) A(1/M$_o$)×10^9 at highest 313 nm dose (12 hr repair).

187 A KINETIC STUDY OF UV-ENDONUCLEASE ACTIVITY IN HUMAN CELLS, Klaus Erixon and Gunnar Ahnström, University of Stockholm, S-106 91 Stockholm, Sweden

A simple and sensitive technique for detection of strand-breaks in DNA has been developed. The method has been used to follow UV-induced excision repair in intact human cells. It has been possible to study the kinetics of enzymatic reactions in cells, in which strand-breaks in DNA are produced and sealed again. Hydroxyurea, 5-fluorodeoxyuridine and 1- ⁃D-arabinofuranosyl cytosin, potent inhibitors of DNA synthesis, drastically increase the number of breaks that are observed during the repair process. This is probably due to a decreased polymerase activity, that will cause the enzymatically formed strand-breaks to stand open for longer times. - - - The initial rate of strand-break formation does not seem to be influenced by hydroxyurea, and is approximately 3.600 breaks per min in a diploid genome, after exposure to a dose of 20 J/sq.m .After 5 to 30 min, depending on the dose of UV, the number of breaks reaches a maximum and starts to decrease again. - - - Since hydroxyurea decreases the rate of repair polymerization, but does not apparently reduce excision repair induced incorporation of labeled thymidine, or the excision of pyrimidine dimers, it seems that the polymerase step is not rate-limiting. As a consequence, the endonuclease and polymerase are acting independant of each other. Under certain assumptions the time for repair of a site, i.e. the time from incision to final ligase sealing, can be estimated to be between 3 and 10 min.

Essentially no breaks are produced in Xeroderma pigmentosum cells belonging to complementation group A, and there is no enhancement by hydroxyurea. Cells from the variant typ of Xeroderma pigmentosum behave like normal cells in this respect.

188 Distribution of UV-induced DNA Repair Synthesis in Human Chromatin, Michael J. Smerdon, Thea D. Tlsty and Michael W. Lieberman, Washington University, St. Louis, Missouri 63110

Although DNA in eukaryotes is organized within the nucleus as chromatin, to date little is known about how this organization affects DNA repair. A method was developed to analyze the distribution of ultraviolet radiation (UV)-induced DNA repair synthesis in the chromatin of cultured human diploid fibroblasts (IMR-90). Analysis of the distribution of repair synthesis occurring immediately after UV damage (12 J/m^2) showed that the number of newly inserted nucleotides per unit DNA is 2-2.5 x greater in staphylococcal nuclease-sensitive regions of the genome than in resistant regions. Electrophoresis revealed a corresponding decrease in the amount of repair synthesis found in core particle DNA. Repair synthesis which occurred many hours after damage (12-29 hr) was much more uniformly distributed between staphylococcal nuclease-sensitive and -resistant regions and showed no decrease in repair synthesis in core particle DNA. Digestion studies with DNAse I, which recognizes different aspects of chromatin structure, indicated a slight preference of repair synthesis for regions sensitive to this enzyme; however, no marked difference between the distribution of repair synthesis occurring at early times and late times was observed. Whether the observed preferential repair synthesis is reflective of nonuniform distribution of damage, ease of access to uniformly distributed damage by a single repair process, or different repair processes acting in different regions of chromatin is currently under investigation.

189 REPAIR OF DNA DAMAGE INDUCED BY BENZO(a)PYRENE AND ITS PROXIMATE METABOLITES IN HUMAN ALVEOLAR TUMOR CELLS. Joyce Remsen, Kunio Shinohara and Peter Cerutti. University of Florida, Gainesville, Florida 32610.

Repair of benzo(a)pyrene (B(a)P) induced DNA damage was studied in epitheloid human alveolar tumor cells A549. Firstly, the excision of covalent guanine-B(a)P adducts was measured by chromatographic techniques in cells which had been exposed to B(a)P for a prolonged period. Within 24 hours 0.6 guanine-B(a)P adducts per 10^6 deoxynucleotides had been removed from high molecular weight DNA at an initial damage level of 1 adduct per 10^6 deoxynucleotides. Guanine-B(a)P adducts were evenly distributed between staphylococcal nuclease digestible and resistant DNA of isolated nuclei and the kinetics of product removal was the same for both fractions. Secondly, the effect of the treatment of cells with the proximate diastereomeric metabolites 7,8-dihydroxy-9,10-epoxy-benzo(a)pyrene I and II on the integrity of parent DNA strands and on daughter strand synthesis was investigated by the alkaline elution procedure. At an initial level of guanine-B(a)P adducts of 4 to 7 per 10^6 deoxynucleotides, parent DNA was fragmented within the first 3 hours to molecular weights below 10^9 daltons and then gradually reconstituted over a period of 20 to 30 hours. Treatment with 7,8-dihydroxy-9,10-epoxy-benzo(a)pyrene also led to an increase in the fraction of short daughter strand fragments formed within a period of 1.5 hours. The fraction of short daughter strands returned to control values within 15 to 20 hours of incubation. However, the total amount of DNA synthesized within 30 min. decreased steadily during 30 hours incubation. The relationship between these different facets of the repair of benzo(a)pyrene-induced DNA damage in A549 will be discussed. This work was supported by grants from N.I.G.M.S. and U.S. E.R.D.A.

190 NONUNIFORM DISTRIBUTION OF DNA REPAIR IN CHROMATIN. William J. Bodell, Department of Molecular Biology, University of California, Berkeley, CA 94720.

The purpose of this study was to examine the distribution of DNA repair in chromatin. For the repair measurements fragment cultures of late pregnant mammary tissue were incubated with MMS (3 mM) and HU (5 mM) for 3 hr. The fragments were then pulse-labeled with ^{3}H-Tdr (10 µCi/ml) for 2 hr. "Repair-labeled" nuclei (125 µg DNA) isolated from the treated cultures were incubated in 5 mM Tris. 0.1 mM $CaCl_2$ pH=7.9 with 2.5 µg micrococcal nuclease at 37°C, and the release of acid soluble (A.S.) radioactivity, and A.S. nucleotides was measured. After 5 min. of incubation 57% of the total radioactivity due to DNA repair has been converted to an A.S. form while 23% of the total DNA in chromatin is A.S. After 30 min. of digestion 74% of the total radioactivity is A.S. while 40% of the DNA is A.S. Similar results are obtained by digesting "repair-labeled" purified chromatin with 2.5 or 5.0 µg micro. nucl. Digestion of "repair-labeled" purified DNA showed that the release of A.S. radioactivity closely parallels A. S. nucleotides. This indicates that the above results are not due to preferential digestion of repaired patches, but that the repair patches are located in a chromatin subfraction which is preferentially digested by micro. nucl. The above results indicate a nonuniform distribution of DNA repair in chromatin. The DNA in chromatin digested during the first 30 min. of incubation with micro. nucl. appears to be the primary site for DNA repair synthesis in the chromatin of MMS treated cultures. Polyacrylamide gel analysis of DNA fragments produced by micro. nucl. digestion indicates that the "repair-labeled" DNA may be located in the linker portion of the nucleosome.

Supported by grant to B. Singer.

191 UV - REACTIVATION OF HERPES SIMPLEX VIRUS IS MUTAGENIC AND INDUCIBLE IN MAMMALIAN CELLS, William C. Summers and Uma Bandyopadhvay Das Gupta, Yale University School of Medicine, New Haven, Conn. 06510.

We have studied the processes of UV-reactivation (UVR) and mutagenesis in mammalian cells using HSV-1 as the experimental probe. The survival of plaque-forming ability was measured along with the frequency of forward mutation to the TK^- (thymidine kinase deficient) viral phenotype. These studies suggest that UVR and mutagenesis are in some way inducible, and may be tightly coupled phemena. Experimental support for these conclusions are: 1. Growth of both irradiated and non-irradiated viruses on UV-irradiated Vero cells resulted in increased frequency of mutation to the TK^- phenotype. 2. A delay of about 17 hours between irradiation of the host and virus infection resulted in maximum mutagenesis as well as UVR. The time course of this induction was similar for mutagenesis and UVR. 3. The development of the UV-reactivation capacity of irradiated cells was prevented by treatment of the irradiated cells with the protein synthesis inhibitor cycloheximide immediately after irradiation.

Hereditary Diseases and DNA Repair

192 AUTORADIOGRAPHIC EVIDENCE FOR CAFFEINE INHIBITED REPAIR IN XERODERMA PIGMENTOSUM CELLS D.F. Minka, Indiana U., Indpls, IN 46202, J. Nath , W. Virginia U., Morgantown, WV

Autoradiography was used to compare the effects of caffeine and UV light on the S phase of excision repair competent(XP4BE) and excision repair deficient(XP12BE) xeroderma pigmentosum(XP) fibroblast cultures. Both cell lines were synchronized with 5-fluoro-2'-deoxyuridine(FUdR). Removal of FUdR was considered the beginning of the S phase. Synchronized cultures were divided into two groups, with and without caffeine and irradiated with UV light at hourly intervals. When the cells entered G_2, caffeine was removed and cultures were labeled with ^{3}H-TdR at various times. Autoradiography was done and the number of grains per nucleus was determined. XP4BE cells, previously determined as excision repair competent showed no increase in ^{3}H-TdR incorporation during the G_2 period. XP12BE cells which have been reported as replication repair deficient, however, had a statistically significant increase in ^{3}H-TdR incorporation during the G_2 period. This increased incorporation was greatest when cultures were irradiated early in S and labeled immediately after the removal of caffeine. These results are consistent with other reports indicating gaps are produced by S phase replication past dimers and provide additional evidence (using another technique, audioradiography) for the presence of a caffeine inhibited repair system. This repair mechanism is capable of reconstituting these secondary UV lesions (gaps). (Supported by NIC Grant T01CA05170-10 and in part by PHS Grant GMP5021054 and T32 DE 7043-01.)

193 DECREASED REPAIR OF GAMMA-RAY DAMAGED DNA IN XERODERMA PIGMENTOSUM FIBROBLASTS. Andrew J. Rainbow and Margaret M. Howes, Departments of Radiology and Biology, McMaster University, Hamilton, Ontario, Canada L8S 4J9. A sensitive host-cell reactivation (HCR) technique for irradiated adenovirus type 2 was used to examine the DNA repair ability of fibroblasts from several Xeroderma Pigmentosum (XP) patients. Adenovirus stocks were irradiated at dry ice temperature (-75°C) using cobalt-60 gamma rays. Cultures were infected with either irradiated or non-irradiated adenovirus type 2 and at 48 hours after infection, cells were examined for the presence of viral structural antigens (Vag) using immunofluorescent staining. 5 XP fibroblast strains, one from each of the different complementation groups A,B,C,D and Variant, showed a reduced HCR of this viral function as compared to fibroblasts from 4 apparently normal individuals. These results indicate some deficiency in the repair of gamma ray induced DNA damage in XP. These results may be attributed to the known repair deficiencies of XP for UV damage and suggest that some part of the DNA damage induced under these conditions of gamma irradiation mimics UV damage.
(Supported by the National Cancer Institute of Canada.)

194 COUPLING OF EXCISION REPAIR TO INHIBITION OF DNA REPLICATION IN NORMAL AND XERODERMA PIGMENTOSUM (XP) HUMAN CELLS, Sang D. Park and James E. Cleaver, Laboratory of Radiobiology, University of California, San Francisco, CA 94143
UV irradiation of normal and repair defective XP cells elicits at least three phenomena: (1) rate of DNA synthesis per cell is depressed and then gradually recovers. (2) the molecular weight (MW) of newly synthesized DNA drops to a minimum soon after irradiation and recovers exponentially, more rapidly than the rate of DNA synthesis in normal cells, but at the same rate in excision defective XP cells. (3) the low MW of newly synthesized DNA in UV irradiated cells increases to parental size during subsequent growth as a linear function of time more rapidly than either process (1) or (2). Only process (3) corresponds to "post-replication repair" and this appears to be a minor aspect of the overall response. These results suggest that both DNA damage and breaks involved in excision repair interferes with DNA replication. The major response in normal cells (i.e., a reduced rate of DNA synthesis per cell) is caused by excision breaks that prevent initiation of DNA replication; minor responses (2 and 3) involve transient blocks to DNA chain growth in those replicons active in DNA synthesis when irradiated, and from which cells recover either through excision, bypass or new replication origins. Hypotheses involving de novo synthesis of repair enzymes seem unnecessary in these explanations, and normal XP and XP variants seem to have the same rate of post replication repair but greater initial depressions in MWs. Work supported by the Department of Energy and SNU-AID.

195 DIFFERENTIAL CYTOTOXICITY OF DNA DAMAGING AGENTS BETWEEN NORMAL AND XERODERMA PIGMENTOSUM CELLS. A. D. Burrell, M. A. Howard, J. J. Andersen. General Products Division, H60/282, IBM Corp. San Jose, CA. 95193
Increased cytotoxicity as a consequence of unrepaired DNA damage has been demonstrated previously in DNA repair deficient xeroderma pigmentosum cells. We have developed a method which facilitates comparison of cytotoxic effects of chemicals between repair proficient VA13 human lung fibroblasts and repair deficient SV40 transformants of XP12RO xeroderma pigmentosum fibroblasts. In this method, nearly confluent cells are overlaid with medium containing 1% agar and the test compound diffuses from a central well. After appropriate incubation cell viability is examined by addition of a further agar overlay containing 0.01% neutral red. Zones of killing of equal diameter can be identified in both cell lines after 24 hours exposure to all toxic compounds. An increase in the diameter of killing in the XP cells is observed only after 48 hours and only after exposure to DNA damaging agents, but not x-ray mimetic compounds. This suggests that the additional toxicity is related to unrepaired DNA damage in the XP cells and requires 48 hours for expression. This method allows rapid identification of uv-mimetic compounds. Among these compounds are three pesticides which have been reported to cause "long-patch" repair in human cells, 9-amino acridine which causes differential growth inhibition between normal and uvr⁻ mutants of E.coli, and three carcinogenic aromatic amines. In the Salmonella mutation test these aromatic amines are positive only when activated by exogenous microsomal enzymes. In our test 2-aminoanthracene requires such activation for differential killing, but benzidine and 2-aminofluorene do not.

196 A COMPARISON OF THE RESPONSE OF ARRESTED HDF POPULATIONS TO UV AND X-RAYS, G.J. Kantor and D.R. Hull. Dept. of Biol. Sci., Wright State University, Dayton, Ohio 45435

Cultured populations of human diploid fibroblasts arrested with respect to division (incubation in 0.5% fetal calf serum medium) can be maintained for long periods (> 1 yr) without loss of cells. Irradiation with reasonably low doses of UV (254 nm) causes cell loss, the extent being dose dependent. Most of the cell loss (~ 90%) occurs between 48 and 72 hrs post-irradiation. DNA repair-deficient cells (XP12BE) are more sensitive than DNA repair-proficient cells (WI-38). Results to be presented show that arrested populations of XP4BE, an XP variant strain capable of DNA excision repair, are as resistant to UV as WI-38. This is interpreted as further evidence that the sensitive target in this assay is DNA and that the increased sensitivity of classical XP is due to a lack of DNA repair. In contrast, exposure to X-ray doses of up to 16,000 r, a dose that destroys proliferative capacity, has no effect on maintenance of arrested HDF populations for periods of up to 50 days post-irradiation. An HDF strain deficient in repair of X-ray induced DNA damage (ataxia telangiectasia, CRL 1343, Se Pan) exhibits no extra sensitivity. These results demonstrate a significantly different biological response to molecular damage caused by UV compared to ionizing radiation. Whereas UV-induced DNA damage affects maintenance, ionizing radiation-induced DNA or other molecular damage does not. Preliminary results suggest that the molecular event responsible for cell loss is an effect on m-RNA transcription. (Supported by NIH Grant CA-16477).

197 EFFECTS OF LIQUID HOLDING ON CELL KILLING AND MUTATION INDUCTION IN NORMAL AND REPAIR-DEFICIENT HUMAN CELL STRAINS. J.W.I.M. Simons, Dep. of Radiation Genetics and Chemical Mutagenesis. Leiden, The Netherlands.

Cultures of human diploid fibroblasts become stationary when they reach confluence. This condition can be used as a "liquid holding (LH) technique" to study excision repair after mutagenic treatments.

The change in cell number over a seven-day LH-period usually was less than twenty percent and the frequencies of labeled cells after continuous labeling with tritiated thymidine usually were below ten percent. This indicates that LH-experiments can be performed over seven days.

During LH, the survival of UV-irradiated cells increased not only in wild-type cells but also, although to a smaller extent, in cells derived from Xeroderma pigmentosum (XP) patients (complementation groups A and D). After 7 days LH, increase in survival in XP cells (compl. gr. D) was correlated with complete loss of induced mutants. Similar experiments on the effect of LH in wild type cells indicated a loss of mutants for exposures up to 5 J/m^2 and the same or higher mutant frequencies for high exposures (8 and 10 J/m^2.

198 EXCHANGE BETWEEN PARENTAL AND DAUGHTER DNA STRANDS IN NORMAL XERODERMA PIGMENTOSUM AND BLOOM'S SYNDROME FIBROBLASTS. Raymond Waters and James D. Regan, Biology Division ORNL, Oak Ridge, TN 37830. James German, New York Blood Center, New York, N. Y. 10021. Increased exchanges between sister chromatids (SCEs) occur spontaneously in cells from patients with Bloom's syndrome (BS), in normal cells treated with mutagens, and the increase in SCE in normal cells after UV is higher in excision deficient XP cells receiving the same UV dose. In an attempt to correlate the microscopic observations for normal, XP and BS cells with molecular events, two approaches were utilized. The first measured the exchange of UV-induced pyrimidine dimers from parental to daughter DNA; the second measured the exchange between DNA density labeled with ^3H BrdUrd for less than one round of replication. Thus spontaneous and UV-induced exchanges are seen as hybrid DNA banding at densities heavier than semi-conservatively replicated heavy/light DNA. A UV endonuclease assay showed 5% of dimers induced were seen in daughter strands. Hybrid DNA obtained from a nuclear cell fraction was double stranded DNA of 10^7 daltons and UV increased the spontaneous amount in normal, XP and BS cells. However, the amount of hybrid DNA only accounts for 10% of the dimers estimated to be in daughter DNA by the UV endonuclease assay. This discrepancy may be due to an inability of the endonuclease assay to distinguish between truly exchanged dimers and any induced in replicons initiated prior to UV. Finally, a significant increase was seen in the spontaneous amount of hybrid DNA in BS cells when compared to that in normal and XP cells. This observation at the molecular level may reflect an increase in steps which are eventually responsible for the increased number of exchanges seen cytologically in BS cells.

199 AN INVESTIGATION OF DNA REPAIR POTENTIAL IN BLOOM'S SYNDROME. R. Tice[1], J.M Rary[2], M.A Bender[1], [1]Medical Dept, Brookhaven Natl Lab, Upton, NY 11973, [2]School of Med, Johns Hopkins Hosp, Baltimore, MD 21205

Bloom's syndrome (BS), a rare human autosomally recessive condition, is characterized cytogenetically by the increased 'spontaneous' occurrence of both chromosomal aberrations and sister chromatid exchanges (SCEs). On the basis of increased chromosomal aberration frequency, BS has been generally considered to be a suspected DNA repair deficiency syndrome along with Fanconi's anemia and ataxia telangiectasia. We have recently demonstrated the presence of a diffusable substance released by BS fibroblast cells capable of eliciting an increase in SCEs in control cell populations (presented at the Annual Meeting, American Society of Human Genetics, San Diego, 1977). Based on this observation we suggest that BS cells might possibly suffer from the endogeneous production of an agent capable of damaging DNA and are not DNA repair deficient per se other than as an already stressed cell population. We have examined this possibility by comparing the DNA repair potential of BS cell populations with normal cell populations using a variety of techniques, including ultraviolet-induced unscheduled DNA synthesis, G_2 chromosomal aberration induction by ionizing radiation and cellular sensitivity to a bifunctional alkylating agent, Mitomycin C. The results of these studies indicate that BS cells are, if anything, less sensitive to these agents than normal cells. The possible significance of these findings will be presented.

200 ETHYL METHANESULPHONATE SENSITIVITY OF BLOOM'S SYNDROME LYMPHOCYTES, Alena B. Krepinsky, John A. Heddle, and James German[1], York University, Downsview, Ontario, Canada M3J 1P3 and [1]The New York Blood Center, New York, N.Y.

Bloom's syndrome blood lymphocytes show a greater increase (in absolute numbers) of sister-chromatid exchanges (SCEs) after ethyl methanesulphonate (EMS) treatment than do heterozygous (bl/+) or control (+/+) lymphocytes. Our micronuleus-test data also suggest an increase in sensitivity to EMS of bl/bl lymphocytes, but they permit less firm conclusions in this case than do the SCE studies.

Untreated bl/bl lymphocytes characteristically show many more SCEs (mean >70 per cell) than do bl/+ or +/+ cells. Some persons with Bloom's syndrome have circulating in their untreated blood two populations of PHA-responding lymphocytes: one with the characteristically high SCE frequency and one with a low frequency, as found in +/+ cells (mean <10 per cell). After EMS treatment of blood from such persons, only their "high" population of cells shows an abnormally great sensitivity (i.e., responds with an unusually great number of SCEs); the response to EMS in their "low" population is similar to that made by bl/+ and +/+ lymphocytes. The ratio of "high" to "low" cells after several days of culture is not influenced by the EMS treatment. (Supported by grants from the National Cancer Institute of Canada, the National Research Council of Canada, and [1]the American Cancer Society.)

201 UNSCHEDULED DNA SYNTHESIS AND DNA SINGLE-STRAND BREAKAGE REPAIR IN BLOOM'S SYNDROME CELLS, Russell A. Vincent, Jr., Jack Rary, Helen L. Braid, and Robert C. Johnson, Medical University of South Carolina, Charleston, South Carolina 29403.

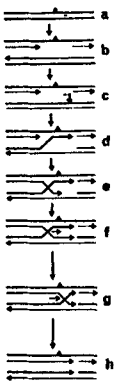
Bloom's syndrome (BS) is a rare, autosomal recessive disorder. BS patients manifest small stature, telangectatic skin lesions, transient sun sensitivity, immunological disturbances, and disposition to malignancy. Cells from BS patients are sensitive to ultraviolet light (UV) and exhibit increased numbers of exchanges between sister chromatids and corresponding regions of homologous chromosomes, suggesting that BS cells may be defective for a DNA repair process involving DNA synthesis. A repair defect in BS may also involve the excision of thymine dimers and the restitution of single-strand breaks in DNA. Thus, we have compared the dose response and time course of UV-induced unscheduled DNA synthesis (UDS) and the time course of DNA single-strand breakage repair in BS cultures GM-811, 1492, 1493, 1629, and 2520 and three non-mutant controls (including GM-179 and 316). BS cells were at least as proficient at UDS as the non-mutant controls, and generally exhibited slightly increased rates and extents of UDS over the controls. No differences in the kinetics of ligation of single-strand breaks in DNA induced by 30 kR of x-rays were detectable by assay on alkaline sucrose gradients.

Supported by grants from the NIH (AM-10956-11), the State of South Carolina (GR-38), and the American Cancer Society (NP-236).

202 DEVELOPMENT AND CHARACTERIZATION OF LYMPHOBLASTOID CELL LINES (LCLS) FROM "CHROMOSOME BREAKAGE SYNDROMES" AND RELATED GENETIC DISORDERS, E. Henderson, and J German, Temple Univ. School of Medicine, Phila., Pa. 19140 and New York Blood Center, NY, NY 10021

LCLs have been established from individuals with "chromosome breakage syndromes" or related disorders by transformation of isolated blood lymphocytes with Epstein-Barr virus (EBV). As compared to cells from heterozygous and control persons, cells from Bloom's syndrome (BS), Fanconi's anemia, and the Louis-Bar syndrome transformed less efficiently. Baseline characterization of the LCLs, now in progress, already shows the following. Some but not all lines established from BS exhibit increased sister-chromatid exchange (SCE). This corresponds to observations of cultures of PHA-stimulated BS lymphocytes, in which cells with an abnormally increased amount of SCE may co-exist with cells with a normal amount. In Fanconi's anemia, sensitivity to mitomycin C is demonstrable, by abnormally great depression in colony froming ability in agar. In Cockayne's syndrome, blood lymphocytes exposed to EBV transformed with the efficiency of controls; however, sensitivity to UV-radiation is demonstrable, again by depressed colony forming ability. Because LCLs from these syndromes apparently will proliferate in culture indefinitely and because a characteristic abnormal phenotype is maintained in vitro, LCLs should facilitate study of the fundamental metabolic or regulatory disturbance underlying each syndrome.

203 A MODEL FOR POSTREPLICATION REPAIR OF UV DAMAGE IN MAMMALIAN CELLS, Martin F. Lavin, Biochem. Dept., University of Queensland, Brisbane 4067 Australia.



A model is presented for bypass of UV induced damage in DNA during replication (Fig. 1). The mechanism proposed provides an alternative explanation to existing models and is entirely consistent with available data on postreplication repair in mammalian cells. A number of steps are proposed; (a) UV irradiation gives rise to damage in DNA. (b) A gap in newly synthesized DNA is formed opposite the damaged site. (c) A nick is introduced into the complementary parental strand at a point opposite the gap. (d) The nicked strand switches to pair with the other parental chain by strand migration. (e) Reciprocal crossing over and DNA synthesis follows. (f) The linkage point is free to migrate. (g) Movement of the linkage point to the right leads to restoration of the DNA strands to their original positions. (h) The overall effect is the filling in of the gap opposite the damaged strand. The intermediate structures described in the model are consistent with data from density label experiments, electron microscopy and enzymological studies. The model provides an explanation for the low level of recombination repair observed in mammalian cells.

204 REPAIR DEFICIENCY IN N-METHYL-N'-NITRO-N-NITROSOGUANIDINE TREATED ATAXIA TELANGIECTASIA (AT) FIBROBLASTS, Dominic A. Scudiero, Chemistry Branch, NCI, Bethesda, Md. 20014. Fibroblasts derived from patients with the autosomal recessive disease AT are unusually sensitive to ionizing radiation, exhibiting increased chromosomal aberations, depressed levels of DNA repair synthesis, and enhanced cell lethality. Utilizing the benzoylated naphthoylated DEAE cellulose method for quantitating DNA repair synthesis (Scudiero et. al. Mut. Res. 29, 473,1975) we have measured repair synthesis in log phase skin fibroblasts derived from 5 normal and 5 AT patients after treatment with ultraviolet light (UV) and alkylating agents. We find similar levels of repair synthesis following treatment with methyl methanesulfonate (MMS) in all AT and normal cell lines tested. Although UV induces 6 times more repair synthesis than MMS, we find equal amounts of UV-induced repair in all ten lines. However, some AT cell lines show significantly less repair synthesis than normal lines after treatment with N-methyl-N'-Nitro-N-Nitrosoguanidine(MNNG). We observe a repair pattern similar to that reported for ionizing radiation-treated AT cells (Paterson et.al. Res. In Photobiol. Castellani, ed, 207, 1977): 2 AT lines (AT5BI, CRL 1347) treated with MNNG have specific activities of repair not significantly different from the values obtained for 5 normal lines, and 3 AT lines (AT3BI, CRL 1312, CRL 1343), previously shown to yield low levels of repair after treatment with ionizing radiation, show 20-35 % of the repair synthesis found in normal cell lines. In all of the normal and AT lines, most MNNG- and MMS-induced repair synthesis occurs within 3 hours after treatment. DNA synthesis immediately after MNNG-treatment is diminished equally in all normal and AT lines tested.

205 UNUSUAL X-RAY SENSITIVITY OF CELLS FROM D-DELETION RETINOBLASTOMA AND FROM OTHER PATIENTS WITH ABNORMALITIES OF CHROMOSOME 13, John B. Little, John Nove and Ralph R. Weichselbaum, Harvard University, School of Public Health, Boston, MA. 02115

Retinoblastoma is a rare malignant eye tumor. Some patients with this tumor bear a deletion in the long arm of chromosome 13 in many of their somatic cells; this deletion systematically includes the 1-4 region. These patients have an increased risk for developing tumors at other sites, both distant and within the radiation field following radiotherapy. We recently reported that diploid skin fibroblasts from a D-deletion retinoblastoma patient were abnormally sensitive to X-rays by colony formation (Nature 266: 726, 1977).

Preliminary data indicate that fibroblasts from another D-deletion retinoblastoma and from a patient with a coloboma (rare eye lesion also associated with a deletion in chromosome 13) are also abnormally X-ray sensitive. On the other hand, cells from a patient with no eye lesions but with a large terminal deletion of the long arm of this chromosome (not including the 1-4 region) were normally X-ray sensitive. Two other X-ray sensitive cell strains have been identified, one from a patient with trisomy 13 and another from a patient with multiple cancers including bilateral retinoblastomas who was part of a familial cancer syndrome. Cells from a ring chromosome 13 and from six other cases of retinoblastoma were normal in their X-ray sensitivity.

These results will be analyzed in terms of their possible implications that a gene or genes involved in the control of X-ray sensitivity might be located at a specific region on chromosome 13.

206 PRENATAL DETECTION OF THE FANCONI'S ANEMIA GENE BY CYTOGENETIC METHODS, A. D. Auerbach, D. Warburton*, and R. S. K. Chaganti, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 (*Columbia U., New York, NY 10032)

Fibroblasts derived from individuals who are homozygous as well as those heterozygous for the Fanconi's anemia (FA) gene show significantly increased chromosome instability when exposed to the carcinogen diepoxybutane (DEB) (Auerbach and Wolman, Nature 261:494-496, 1976; and Nature, in press). Untreated homozygous cells have 0.20 to 0.36 breaks per cell. Untreated heterozygous cells have the same amount of intrinsic chromosome instability as normal cells. After treatment with DEB (at a concentration which has no clastogenic effect on normal cells) homozygous and heterozygous cells have a 3- to 5-fold increase in the amount of aberration. The ability to distinguish readily the three genotypes makes available a test for the prenatal detection of the FA gene. We have determined the genotype of a 13-week-old abortus from a female who is an obligate heterozygote. Fibroblasts from the fetus and chorion were cultured and treated with DEB. Untreated cells have low intrinsic instability (0.02 and 0.04 breaks per cell in the fibroblast and chorion derived cells). Treated cells have a significantly increased ($p<0.001$) amount of instability (0.14 and 0.20 breaks per cell in the fibroblast and chorion derived cells). The pattern of instability is similar to that reported in heterozygous cells and suggests that the fetal cells are heterozygous.

207 SISTER CHROMATID EXCHANGES IN DYSKERATOSIS CONGENITA AFTER EXPOSURE TO TRIMETHYL PSORALEN AND UV LIGHT, D. Martin Carter, Alan Gaynor and Joseph McGuire, Yale University School of Medicine, New Haven, CT 06510

Dyskeratosis congenita (DKC), a rare, X-linked recessive genodermatosis, is characterized by mucosal leukokeratosis, nail dystrophy, patterned telangiectatic hyperpigmentation, pancytopenia, and heightened susceptibility to infection and malignancy. Peripheral blood leukocytes from 2 unrelated persons with DKC, their mothers, an unaffected brother and normal controls were cultivated in McCoy's medium with phytohemagglutinin. After 24 hours, some cells were exposed to trimethylpsoralen (TMP), 6.46×10^{-8}M and/or UV light (UV-A, 365nm, 0.3-0.9 J/cm^2). All flasks received BrdU (20ug/ml) for 48 hours and colchicine, 3×10^{-6}M for 90 min. Spreads of mitotic chromosomes were prepared, stained with Hoechst 33258 and Giemsa, and the number of sister chromatid exchanges (SCEs) was determined per metaphase. All cells treated with TMP and UV-A had more SCEs than untreated cells but the rate of increase was about 1.5 times greater in DKC cells than in normals; in cells from the mothers, an intermediate value was obtained. Conditions used were sufficient to produce psoralen-DNA, crosslinking photoadducts in cutaneous fibroblasts as shown by zone sedimentation in alkaline sucrose of DNA from cells labeled with tritiated thymidine. We have previously shown that the number of SCEs promoted by psoralens plus light increases with the crosslinking potential of the drug. Our data suggest that chromosomes of patients with DKC are more susceptible to DNA crosslinks than normals; possibly because they are defective in removal of the photoadducts.

208 EFFECTS OF DNA DAMAGING AGENTS ON CULTURED FIBROBLASTS DERIVED FROM PATIENTS WITH COCKAYNE SYNDROME, M.H. Wade and E.H.Y. Chu, Univ. of Michigan, Ann Arbor, MI. 48109.

The cytotoxic action of physical and chemical agents on ten skin fibroblast strains in culture derived from individuals with Cockayne's syndrome was measured in terms of colony forming ability. As compared to fibroblasts from normal donors, all Cockayne cell strains tested exhibited a significantly increased sensitivity to UV light but a comparable sensitivity to X-rays. Cells from two sets of parents of unrelated Cockayne children showed an intermediate level of UV sensitivity. There was no effect of .5mM caffeine on UV survival in normal and two Cockayne strains tested, indicating that postreplicational repair in Cockayne cells as measured by caffeine sensitivity was operational.

Sensitivity of normal and Cockayne cells to the chemical carcinogens and mutagens 4NQO, N-AcO-AAF, ICR-170 and EMS was also compared. An increased sensitivity of Cockayne cells to 4NQO or N-AcO-AAF was observed. However, unlike the intermediate UV sensitivity, the cell strain from one parent of a Cockayne patient showed the same sensitivity to N-AcO-AAF or 4NQO as fibroblasts from normal individuals.

Quantitation of damage to the DNA after 20 $J \cdot M^{-2}$ UV irradiation indicates normal repair synthesis in the Cockayne cells, in contrast to UV-irradiated xeroderma pigmentosum cells (XP 12BE) in which there was a very low level of repair synthesis. Moreover, we have shown previously normal excision of UV-induced pyrimidine dimers in two of the ten Cockayne cell strains. Thus, although there is no clear answer to the molecular defect present in Cockayne cells, these results are consistent with decreased fidelity of a DNA polymerase, or a defective ligase specific for long patch repair.

Biological Effects of DNA Damage

209 ULTRAVIOLET IRRADIATION AND THE MAMMALIAN CELL CYCLE, Andrew R.S. Collins, Robert T. Johnson, C. Stephen Downes, Foch F. Yew and Kornel Burg, University of Cambridge, Department of Zoology, Downing Street, Cambridge, CB2 3EJ, U.K.

We have attempted to answer the question whether the probability of survival of UV-irradiated mammalian cells is determined solely by the amount of damage inflicted on the DNA, by a comparison at different points in the cell cycle of the efficiency of thymine-thymine dimer production and of colony forming ability over a range of UV doses. A computer model designed to estimate the proportion of incident radiation absorbed by the DNA target, from changes during the cell cycle in DNA concentration and packing and in shielding by other regions of the cell, has predicted dimer yields consistent with our experimental findings.

The fluctuation in UV sensitivity through the cell cycle bears some relation to changes in DNA damage, but cannot entirely be accounted for in this way. It is likely that survival is also a function of the cell's ability to repair DNA damage. We have evidence that this ability may be limited by the size of the DNA precursor pools; the addition of exogenous deoxyribonucleosides is found to enhance repair and reduce the chromosome decondensation which normally follows UV, to different extents at different points in the cell cycle and in different cell types in accordance with known variations in pool size. A further factor to be considered is the role played in survival by possible variations in intrinsic activities of the repair enzymes.

210 CELL CYCLE PERTURBATIONS CAUSED BY CHEMICAL CARCINOGENS, James C. Bartholomew, Andrew L. Pearlman, Joseph R. Landolph, and Kenneth M. Straub, University of California, Berkeley, California 94720

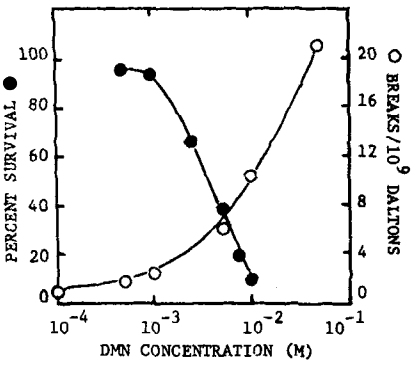
The mechanism of action of chemical carcinogens is not known, but evidence is accumulating that they act by causing mutations. These mutations presumably arise through a direct binding of the carcinogen to DNA, causing errors in the replication of the genone. This binding to the DNA may also cause alterations in the DNA replication rate which can be detected by analysis of the kinetic parameters of the cell cycle. We have used flow cytometry (FCM) to monitor the alterations in cell cycle distributions caused by chemical carcinogens. The *closely derived mouse liver cell strains growing in culture have been studied with regard to* the effect of benzo[a]pyrene (BaP) and derivatives of BaP on DNA synthesis. The derivatives tested were 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene(7,8-diol); 7,8-dihydro-7,8-dihydroxybenzo-[a]pyrene-9,10-oxide (Diol-Epoxide), and 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[a]-pyrene (tetrol). One cell strain used in this study, NMuLi cl 7, is not highly inducible for the enzyme system (Arly Hydrocarbon Hydroxylase, AHH) that converts the parent compound into the derivatives listed above. The second strain, NMuLi cl 8, is highly inducible for AHH. Correlated with the high level of metabolic activity is an increased sensitivity to the cytotoxicity of the parent compound. However, both strains were equally sensitive to the Diol-Epoxide. FCM analysis showed that the Diol-Epoxide increased the number of cells involved in DNA synthesis, but that the rate of DNA synthesis was greatly reduced. BaP and 7,8-Diol had this same effect on NMuLi cl 8, but not in NMuLi cl 7.

211 CORRELATION OF CELL LETHALITY AND DIVISION DELAY WITH THE INDUCTION AND REPAIR OF THREE TYPES OF DNA DAMAGE, Arthur Cole, Peter Corry, and Ruth Chen, The University of Texas System Cancer Center, Houston, Texas 77030

Repair of DNA single strand breaks (SSB), double strand breaks (DSB), and reassembly of large DNA arrays were assayed using sedimentation techniques. Both high linear energy transfer (LET) alpha particles and low LET gamma rays were used to induce the initial cell damage. Alpha particle irradiation induced DNA SSB at low efficiency but induced DNA DSB and the disassembly of the large DNA arrays at high efficiency. Kinetics for post-irradiation repair of both SSB and DSB were similar with 10 minute half-repair times, whereas half-times for reassembly of large DNA arrays were in the order of 45 minutes. Essentially complete repair of all three forms of damage was observed following low LET irradiation, but only 60% of the DSB were rejoinable following high LET irradiation. DNA repair kinetics were comparable for cells plated either 24 hours or immediately before repair incubation, or for cells suspended in media during repair incubation. Repair did not occur at 4°C or in the presence of EDTA. Pre-irradiation hyperthermic treatment at 43°C for 45 minutes inhibited repair by increasing the repair half-times by a factor of four or more. Various pre- and post-irradiation temperature treatments are being investigated. Although limited repair occurred during post-irradiation incubations in low-nutrient balanced salt solutions, pre-treatment hyperthermia in such solutions caused an appreciable increase in cell killing and inhibition of post-treatment repair. Our studies to date provide correlations of cell lethality, division delay, and the repair of sub-lethal damage with the induction and repair of DNA DSB and the breakdown and reassembly of the large DNA arrays, but not with the induction of DNA SSB.

212 DNA DAMAGE AND CELL SURVIVAL IN CHO CELLS AFTER IN VITRO METABOLIC ACTIVATION OF CARCINOGENS, George R. Douglas and Caroline E. Grant, Environmental Health Directorate, Department of National Health and Welfare, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2.

The induction of single strand breaks and/or alkali-labile bonds was studied in relation to cell survival in CHO cells. Metabolic activation was accomplished using a crude rat liver microsome preparation (S9) plus appropriate co-factors (S9mix). The optimum treatment mixture contained 24.3% S9mix, 61.4% MEM medium, with 2.5% newborn calf serum, and 14.3% diluted carcinogen. Cells are treated for 1 hour. ^{14}C-TdR labelled control and ^{3}H-TdR labelled treated DNA were co-sedimented in 5-20% alkaline sucrose gradients (4.8 ml) after 10 hour lysis, and the number of breaks calculated from the weight average molecular weights. Most DNA damage induced by dimethylnitrosamine was observed at toxic concentrations (see figure). The effective dose range for DMN was wide (10^{-4}-10^{-1}M) compared to benzo(a)pyrene (10^{-5}-10^{-4}M). The ability to quantitatively measure DNA damage and cell survival with metabolic activation makes this technique particularly useful for the detection of environmental mutagens and carcinogens.



213 REPAIR OF DNA DAMAGE IN MAMMALIAN CELLS TREATED BY X-RAYS OR MMS : ENHANCEMENT BY UNCOUPLERS. Françoise LAVAL, Radiobiologie Clinique, Institut Gustave Roussy, 94800, VILLEJUIF, France.

Incubation of mammalian cells with uncouplers before or immediately after irradiation or MMS treatment increases cell survival (LAVAL F.LITTLE J.B. Rad. Res. 71,1977,571). We report here the relationship between survival, DNA repair capacity and the induction of mutations in cells treated in the above conditions.

- utilizing the 5-BrUdr density labeling method, we have shown that uncouplers stimulate both DNA repair and DNA synthesis in cells treated by X-rays or MMS.
- zone sedimentation of cellular DNA under alkaline conditiions shows an increase in the lenghts of the newly-replicated pulse-labeled DNA when cells are treated by uncouplers.
- the frequency of 8-azaguanine mutation per unit of survival is higher in irradiated cells treated by uncouplers than in untreated cells.

This work was suported by grants from INSERM and CNRS.

214 UNSCHEDULED DNA SYNTHESIS IN MIXED-CELL PRIMARY CULTURES OF ELEVEN-WEEK AND TERM HUMAN PLACENTAE, David Lefkowitz, Robert C. Johnson, W. Page Faulk, and Russell A. Vincent, Jr., Medical University of South Carolina, Charleston, South Carolina 29403.

The human placenta may provide a model to study the relationship of DNA repair with aging. The placenta is of fetal origin, and as the organ matures, histological evidence of aging is observed. Placental cells cultured in vitro attain a lower cell population doubling level than cells from fetus and mother. In this study, primary cultures were established by continuous flow trypsin-DNAse digestion from eleven-week and term placentae. These cultures consisted of a mixture of cell types as measured by periodic acid-Schiff (PAS) and immunofluorescent (antitransferrin) staining. PAS-positive cells, judged to be trophoblasts, exhibited low levels of ultraviolet-light-induced unscheduled DNA synthesis (UDS), whereas PAS-negative cells were found to have high levels of UDS. The latter cells were judged to be stromal cells, largely fibroblasts. These observations are evidence that trophoblasts are capable of UDS, but not to the extent of the stromal cells of the chorionic villi. However, no differences in UDS were observed in identically studied cells derived from eleven-week and term placentae. The failure to detect age-dependent differences in UDS in human placentae is evidence that the DNA repair capacity of cells isolated from an organ in primary culture may not necessarily be an index of the age of the organ. This is particularly striking in placentae in which morphological criteria of aging are so obvious.

215 AGING AND SISTER CHROMATID EXCHANGES, Edward L. Schneider, David Kram, Yoshitumi Nakanishi, Robert Monticone, Brian Gilman, Michael Nieder and Robert Dein, Laboratory of Cell. & Molec. Biol., Gerontology Res. Ctr., NIA, NIH, Baltimore, MD 21224

To examine the effect of aging on cellular response to DNA damage, we have investigated SCE induction by a variety of mutagens. In vitro, human fetal lung fibroblasts (IMR-90) at different levels of passage were incubated with bromodeoxyuridine (BrdU, 10 μg/ml) and either mitomycin C (MMC, 10 ng/ml), N-acetoxy-acetyl-aminofluorene (AAF, 1μg/ml) or ethyl methanesulfonate (EMS, 50 μg/ml). With all three mutagens, induced SCE frequencies were significantly ($p < 0.05$) reduced in late passage cells (O) when compared to cells at early passage (Y): MMC,O = 18.9 ± 1.7 vs. Y = 29.6 ± 1.2; AAAF,O = 18.1 ± 1.5 vs. Y = 28.0 ± 1.5; EMS,O = 16.1 ± 0.8 vs Y = 20.6 ± 1.3. A similar decline in mutagen-induced SCE frequencies was observed in old (O) donor skin fibroblast cultures when compared to young (Y) donor cell cultures at equal levels of early passage: MMC,O = 67.9 ± 1.4 vs. Y = 56.1 ± 1.4; AAAF,O = 19.1 ± 1.2 vs. Y = 26.5 ± 1.3. In vivo C57B1/6 mice were intravenously infused with 50 mg/kg wt/hr BrdU and either MMC 5 mg/kg or cyclophosphamide (CP, 45 mg/kg). As with the in vitro studies, a significant ($p < 0.05$) decrease in mutagen-induced SCE was observed in the old (O, 24 mo) mouse bone marrow cells when compared with cells derived from young (Y, 6 mo) animals: MMC,O = 46.3 ± 2.4 vs. Y = 76.7 ± 2.7; CP,O = 87.6 ± 3.3 vs. Y = 123.0 ± 3.9. Both in vitro and in vivo, diminished SCE levels were accompanied by increased numbers of chromosomal aberrations. If SCE are a form or result of DNA repair as has been suggested by several investigators, the above results would indicate impairment of DNA repair in old cell populations.

216 **ARE AGING AND REJUVENATION IN UNICELLULAR EUKARYOTES EVOLUTIONARY BY-PRODUCTS OF A RECOMBINATION PATHWAY INVOLVING DAMAGE AND REPAIR OF DNA?** **Rolf Martin, Dept of Chemistry, Brooklyn College, Brooklyn, NY 11210** **In most eukaryotes, genetic recombination takes place primarily during meiosis, between homologous non-sister chromatids. We may wonder how mechanisms evolved to restrict the nature and timing of recombination in eukaryotes. To begin to answer this question we can speculate that the expression of genes which control recombinational (rec) repair in bacteria became coordinated with those that result in conjugation. This would tend to limit rec repair to periods of mating, and may have had these additional evolutionary consequences: (1) Cells could accumulate certain kinds of DNA damage in the absence of rec repair, and, perhaps partly as a result of this, would age. (2) Rejuvenation could take place when rec repair enzymes are synthesized during mating. (3) Relatively frequent mating would be ensured if only those cells that mate, and are rejuvenated, avoid aging and death[1]. (4) Chromatid exchange would occur preferentially between homologous rather than sister chromatids, as mentioned previously[2]. (5) Genetic recombination during repair at mating could be more efficient because of an accumulation of recombinogenic DNA damage during the preceding cell divisions[2]. Evidence of repair during conjugation and meiosis will be discussed to emphasize similarities between the two processes.**

(1) Sonneborn, T.M. (1977) In "A New Look at Biological Aging" Behnke, J.A., Finch, C.E. and Moment, G.B. (eds.) Plenum Press, New York, in press.
(2) Martin, R. (1977) In "Molecular Human Cytogenetics, ICN-UCLA Symposium Proceedings" vol. Comings, D.E., Sparkes, R.S. and Fox, C.F. (eds.) Academic Press, New York, in press.

217 Patterns of DNA repair in mouse embryo cultures during aging and spontaneous transformation in vitro. R. L. Meek, T. Rebeiro and C. W. Daniel, Div. Natural Sciences, Univ. of Cal., Santa Cruz, CA

Serially passaged cultures of Balb/c mouse embryo cells exhibit a decline in proliferative capacity with increasing in vitro age. We have described changes in these early passage cultures which are comparable to changes that occur in aging human fibroblasts subcultured in vitro. However, instead of phasing out,the mouse cells spontaneously transform at the 9th or 10th passage into permanent cell lines.

It has been reported that the capacity to perform DNA repair measured by unscheduled DNA synthesis (UDS) is reduced in old cultures of human cells. In the present study the efficiency of U.V. induced UDS was determined for mouse embryo cells during serial passage. We found that the capacity to perform UDS declined in aging cultures. The ability of cultures to repair damaged DNA was correlated with the fraction of cells which retained the ability to perform scheduled DNA synthesis. The decline of UDS in older cultures was associated with a decrease in the fraction of cells which could enter the S period and traverse the cell cycle. When the cultures transformed spontaneously, at about the 10th passage, UDS capacity increased as did the fraction of cells in the proliferating pool.

These experiments indicate that early passage mouse cells capable of proliferating, and cells recently transformed to a continuously dividing cell type,display high levels of DNA repair. This suggests that a relationship between scheduled and unscheduled DNA synthesis may exist.

218 MORE EVIDENCE SUGGESTING A REPAIR FUNCTION FOR DNA POLYMERASE-β, Christopher Chetsanga and Lynne Pentler, Scientific Research Labs., Ford Motor Co. and University of Michigan, Dearborn, Michigan 48121

We are studying DNA polymerase-β from livers of old and young mice. The purpose of the study is to determine if the specific activity and other properties of this enzyme change with cell senescence. DNA polymerase α activity is assumed to function in DNA replication because its level increases in cells undergoing rapid proliferation. The low rate of proliferation of senescent cells makes them a suitable system in which to determine whether β-polymerase is a DNA repair enzyme as other investigators have proposed.

Our results show that the specific activity and apparent K_m of DNA polymerase-β from young and old mice are comparable. The activity of the α enzyme has been shown to change with cell age. While different pH conditions did not show age associated changes in β enzyme activity, the enzyme from old mice was sensitive to higher temperatures. We have studied the template copying fidelity of the β enzyme from the two age groups of mice using several types of synthetic deoxypolynucleotides as primer-templates. The β enzyme from old mice did not show a proneness to catalyzing the incorporation of wrong nucleotides as the α enzymes has been shown to do. The fact that β-polymerase specific activity is not affected by the slowed metabolic rate of old cells suggests, as does other evidence, that it is a DNA repair enzyme.

219 U.V. MAPPING OF THE HISTONE GENES IN HELA CELLS, Perry B. Hackett, Peter Traub* and Dieter Gallwitz**, Univ. of Calif. San Francisco; *Max-Planck-Institut, Wilhelmshaven, Germany; **University of Marburg, Germany.

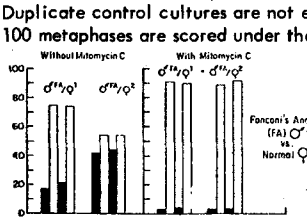
We have investigated whether in human cells the five major histone genes are transcribed as a single polycistronic transcriptional unit. We employed the method of u.v.-mapping. The fundamental premise of this procedure is that the sensitivity of transcription of a gene can be expressed as $R_{x,d}=e^{-k_x d}$ where $R_{x,d}$ is the level of transcription of gene x after irradiation with a u.v. dose d, and k_x is the inactivation coefficient of gene x; k_x is directly proportional to the distance between the promoter and the 5' end of gene x. The dose-response kinetics for histone genes H1 and H3 are first order, indicating a single type of transcriptional unit for each gene. The inactivation kinetics for genes H2A, H2B and H4 are first order with two distinct rates: 10-15% of the genes appear to be much more u.v. sensitive than are the majority. As determined by the inhibition of histone protein synthesis in vivo, the k_x's for the major component of each histone are (in units of mm^2/ergs): H1, 907; H2A, 878; H2B, 871; H3, 965; H4, 792. The sensitivities of histone mRNA synthesis to u.v. were similar. Assuming an inactivation rate of 1 transcription-terminating lesion per 1300 base pairs for each 1000 ergs/mm^2 of u.v., the target sizes of the histone genes (in base pairs) are: H1, 1190; H2A, 1240; H2B, 1250; H3, 1130; H4, 1380. The similarity in target sizes indicates that these genes are transcribed from individual transcriptional units.. In addition, during the first hour after irradiation, there is no measurable repair of the u.v.-induced lesions which cause premature termination of transcription of both the histone genes and those for 18S and 28S rRNA.

220 CYTOMETRIC AND CORRENDONUCLEASE ANALYSES OF BSA-PROTECTED MOUSE MYELOMA CELLS, Tatsuo Matsushita, Anita Shotola, Avrom Brendzel, and Aaron Simms, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439
Electronic growth curve analyses of mouse myeloma suspension cultures exhibit a bimodal distribution of electrical resistance. Electronically "smaller" (less resistant) cells generally correlate with nonviable cells, with nonviability defined by both trypan blue uptake and light scattering (flow cytometry) techniques. Centrifugation and suspension of myeloma cells in phosphate-buffered saline (PBS) result in a greatly increased peak of electronically "smaller" cells (nonviable) and a corresponding decrease of trypan blue-excluded (viable) cells. When cells are suspended or centrifuged in PBS containing bovine serum albumin (BSA), the appearance of electronically "smaller" cells and the disappearance of trypan blue-excluded, viable cells are minimized. UV-survival curves of myeloma cells in PBS plus BSA show an increased survival compared to PBS-suspended cells. The increased survival could be due to the screening of UV light by BSA, or an increased cloning efficiency in the PBS-BSA cells. To examine these two possibilities, the number of pyrimidine dimers in myeloma DNA is determined for various UV doses in the presence and absence of BSA. Dimers are quantitated by gentle extraction of DNA, treatment of the DNA with Fraction D of M. luteus correndonuclease preparation [S. Riazuddin and L. Grossman, J. Biol. Chem. (1977) 252 6280], and alkaline sucrose gradient centrifugation. The use of BSA for UV-survival and DNA repair studies will be discussed. (This work was supported by the U. S. Department of Energy.)

Mutagenesis II

221 A GENERAL METHOD FOR ISOLATION OF REPAIR DEFICIENT MUTANTS. Aziz Sancar and Claud S. Rupert. The University of Texas at Dallas, Richardson, Tx 75080.
We have developed a rapid method for isolation of UV-sensitive mutants of Escherichia coli. The selection method involves production of growth and/or division delay in repair-deficient cells with low UV fluences and killing of repair-proficient cells by penicillin treatment. Using this technique we have achieved about a 3000-fold enrichment for photoreactivationless (phr⁻) mutants of E. coli K-12. We have also shown that the method can be used to isolate mutants deficient in dark-repair functions.
A modification of this technique using incorporation of BUdR followed by white light treatment to kill repair proficient cells is being evaluated for the isolation of repair deficient mutants of eukaryotes since there is no good method currently available for this selection. This work was supported by Research Grant GM-16547 from the National Institute for General Medical Sciences, U.S. Public Health Service.

222 A SIMPLE CYTOGENETIC TEST FOR INCREASED MUTAGEN-SENSITIVITY, J. German, S. Caskie, and S. Schonberg, New York Blood Center, New York, NY 10021
Variable clinical phenotypes often make difficult the diagnosis of human disorders in which defective DNA repair has been implicated or suspected, particularly the Louis-Bar syndrome (LBS) and Fanconi's anemia (FA). To facilitate diagnosis, and eventually for use in screening, we have developed a simple cytogenetic test based on the known sensitivity to specific mutagens of cultured cells from persons with certain of these diseases. Blood from the suspected individual is co-cultivated in PHA-containing medium along with an equal volume of blood from a normal person of opposite sex. The environmental insult is administered shortly after the culture is set up. Thus, mitomycin C is used as the noxious agent in the case of FA, γ-rays in LBS. Duplicate control cultures are not exposed to the agent. After 5 days, metaphase preparations are made and 100 metaphases are scored under the fluorescence microscope to determine the proportion with a Y chromosome.



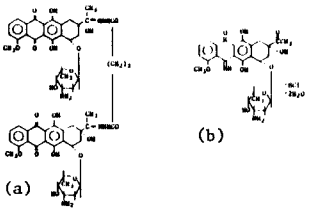
Comparison of the percent Y-bearing metaphases in the control (untreated) cultures with that in the treated cultures provides a clear indication of whether either cell type (♂or♀) is particularly sensitive to the mutagen used, as judged by their relative ability to proliferate and divide. (Results from 1 experiment with FA (1♂) and normal (2♀'s) lymphocytes are shown in the fig.) This test system could prove useful in determining sensitivity to mutagens of cells from other genetic diseases or less clearly defined entities (e.g. cancer-prone families).

223 FIXATION AND EXPRESSION OF RECESSIVE MUTATIONS IN MAMMALIAN CELLS AS A MODEL FOR THE STUDY OF CARCINOGENESIS, Anne Kinsella, Suzanne Mousset, Claude Szpirer and Miroslav Radman, Department of Molecular Biology, Université Libre de Bruxelles, B 1640 Rhode-St-Genèse, Belgium.

Epidemiological studies, which implicate a multi-stage process in the development of human cancer, lead to hypotheses that several distinct heritable changes are necessary to alter a normal cell into a malignant cell, the proliferation of which results in tumour formation. On the other hand, experimentally induced carcinogenesis is considered to occur in two steps, called initiation and promotion. Initiators, which are radiation or chemical carcinogens, cause rapid, irreversible changes, probably mutational events, which result in a "pre-malignant" state. Promoters are neither significantly carcinogenic nor mutagenic but, when added after an initiator, they greatly increase tumour frequency and shorten the lag-time for tumour appearance. Thus, tumour promoters complete a process begun by initiators and not vice versa. Consideration of these facts prompted us to consider the possibility that initiated cells contain specific, recessive, autosomal, somatic mutations, and that promotion causes the expression of these mutations by some segregational event leading to homozygosity or hemizygosity.
The effects of tumour promoters, antipromoters and X-ray irradiation on three possible segregational events have been studied. (i) Chromosome loss by non-disjunction was followed by analysis of thioguanine-resistant cells (X-linked, $hprt^-$, recessive character) from a heterozygous ($hprt^+/hprt^-$) cell hybrid. (ii) Chromosomal rearrangement or deletion was analysed cytologically, by assessment of chromosomal aberrations. (iii) The analysis of quadriradial chromosomes and of sister chromatid exchanges was performed under assumption that they are correlated with mitotic recombination, which has not yet been demonstrated by genetic experiments. The preliminary evidence favours mitotic recombination as the mechanism of tumour promotion and as an essential step in carcinogenesis.

224 MECHANISM OF MUTAGENICITY OF ANTHRACYCLINE ANTICANCER AGENTS, Dennis G. Kleid and David V. Goeddel, Stanford Research Institute, Menlo Park, CA 94025.

Although the anthracycline antibiotics adriamycin and daunomycin demonstrate high clinical anticancer activity, the cardiotoxic side effects shown by these drugs seriously limits their usefulness. The mode of action of these drugs has been postulated to involve DNA intercalation. These compounds have also been shown to bind to DNA covalently in vitro. This reaction is dependent on reduction of the chromophore and results in nicking of superhelical plasmid DNA in vitro. These compounds also induce error prone repair, as shown by induction of rec A protein [J. W. Little & D. G. Kleid, J. Biol. Chem. 252, 6251 (1977)] in rfa^- E. coli. We have attempted to understand the mechanism of action of these drugs by investigating the DNA-drug interactions of a double intercalating analog, bis-daunomycin succinhydrazone (a), and an analog incapable of chromophore reduction, 5-imino daunomycin (b). The mutagenicity of these drugs in the Ames tester strain TA98, indicates two possible modes of mutagenicity, one may involve bio-reductive alkylation, the second may be dependent on oxidation.



225 ENU INDUCED MUTAGENESIS IN CHO CELLS, Regine Goth-Goldstein and H. John Burki, Donner Laboratory, University of California, Berkeley, CA 94720

N-ethyl-N-nitrosourea (ENU), which is known as a potent carcinogen, was tested on its mutagenic effect in asynchronous and synchronous Chinese hamster ovary cells. Although these cells are quite resistant to induced reproductive death by ENU ($D_0 \sim 1$ mM ml^{-1}), they were very sensitive to the induction of mutations to drug resistance. For 6-thioguanine resistance the mutagenic potential of ENU was 270×10^{-6} mutants (cell mM $ml^{-1})^{-1}$ and for ouabain resistance, 220×10^{-7} mutants (cell mM $ml^{-1})^{-1}$. As expected, many oubain mutations were expressed immediately after ENU-treatment; however, the numbers of mutants increased with longer expression times. 6-thioguanine resistant clones first arose about 4 days after treatment, and reached a maximal number after 8 days.

When synchronous cell cohorts from an automatic mitotic selector were treated with high doses of ENU, there was a strong age response for induction of reproductive death, cells in early S phase being more sensitive by a factor of 10. Cell cohorts treated with low doses of ENU did not show any systematic "hot" periods for mutagenesis in the cell cycle.

226 CELL-MEDIATED MUTAGENESIS OF HUMAN CELLS, Rodger D. Curren, Charles J. Homer, Paul J. Price and Aaron E. Freeman, Microbiological Associates, Torrey Pines Research Center, La Jolla, California 92037

Mutations to ouabain resistance (Oua^r) were induced in human cells by the procarcinogen 3-methylcholanthrene (MCA). Primary explants of human skin epithelial cells were co-cultivated with excision-repair defective xeroderma pigmentosum (XP) cells before application of MCA. XP cells alone were also treated with MCA. Oua^r mutants were only found in cultures derived from the mixed cell population receiving MCA. MCA did not induce mutations in XP fibroblasts cultivated without a feeder layer. Presumed mutants recovered after growth in ouabain had a human karyotype and retained their Oua^r phenotype in the absence of selection.

227 ULTRAVIOLET LIGHT INDUCED MUTAGENESIS IN SACCHAROMYCES CEREVISIAE, Christopher W. Lawrence and Roshan Christensen, Dept Rad Biol & Biophys, Univ of Rochester, Rochester, NY 14642.

Genetic analysis of UV-induced mutagenesis in Bakers' yeast, using a variety of radiation sensitive mutations and reversion of a number of well defined cyc1 alleles, has led to the following observations. First, genetic control of radiation mutagenesis is site specific; certain gene products concerned in this process act at some but not other sites in the cyc1 gene. A similar result is found with both UV and gamma rays, suggesting that it does not depend on the type of premutational lesion. Second, variation in the types of base pair change and in their frequency induced by UV at different sites in repair proficient strains is largely independent of nucleotide sequence at these sites. Such variation more probably depends on the type and amount of mutagenic repair. Third, UV-induced mutations, at least in some instances, can arise at some distance from the site of the premutational lesion. Finally, the work of Sherman, Stewart and others has shown that the great majority of UV-induced mutations are due to single base pair changes. These observations seem to be incompatible with the Radman/Witkin model for UV-mutagenesis in bacteria, and suggest that it be inappropriate for this single eukaryote.

228 MUTAGENIC ACTIVITY OF PHOTOSENSITIZING DRUGS - A COMPARATIVE STUDY, Emanuel Riklis, Michal Green, Ehud Ben-Hur and Ariela Prager, Nuclear Res. Center Negev, BeerSheva, Isr.

Drugs used clinically sometimes cause photoallergic and phototoxic reactions in patients, and may be potential mutagens and carcinogens. While working on the photochemistry, photobiology and radiobiology of the interaction and synergism existing between radiation and sensitizers, we have tested and compared the mutagenic activity of some of these compounds, using a modified Ames test system, with S.typhimurium mutant strains exposed to UV light in the presence or absence of the photosensitizung drugs. Far UV, 254 nm, or Near UV, 360 nm light were used. Furocoumarins (psoralens), used in photochemotherapy of skin disorders such as psoriasis and vitiligo, form interstrand DNA crosslinks. 4,5',8-trimethylpsoralen indeed induces very high histidine independence in S.typhimurium AT-100 cells, and is thus shown to be highly mutagenic, when exposed to NUV light. Hematoporphyrin, which increases the yield of production of thymine dimers in UV-irradiated thymine, was suggested in combination with red light as a treatment modality for superficial tumors. There is no mutagenic activity of this combination, and even with far UV light only very slight mutagenic activity was seen. Chlorpromazine, commonly used in psychotherapy causes photoallergia, and is shown to be mutagenic in conjunction with NUV light, although a 1000 fold less than psoralen.
The relative mutagenicity of these compounds over a wide range of doses and concentrations seems to be of the order of 10000:15:1 for Psoralen:CPZ:HP respectively. UV light, 360 nm, greatly enhances the mutagenicity of psoralen and chlorpromazine.

229 THE CONSEQUENCES OF DNA INTERACTION WITH ALKYLNITROSOUREAS ON ITS CODED ENZYME SYNTHESIS IN A CELL FREE SYSTEM Shu-jing C. Wei, Beatrice P. Chen and Jerry M. Rice National Cancer Institute, NIH, Bethesda, MD 20014

Both N-methyl-N-nitrosourea (MNU) and its ethyl homolog (ENU) are potent mutagens and carcinogens, but differ in toxicity and in efficiency of transplacental carcinogenesis. The interactions of MNU and ENU with lac gene DNA were studied to determine the relative effects of these agents on the fidelity of gene function in a cell-free enzyme synthesis system. DNA from bacteriophage λh80dlacp^S containing the lac operon is incubated with the 30,000 xg supernant (S-30) of an extract from a lac deleted strain of E. Coli., plus salts and cofactors. The activity of the coded enzyme β-galactosidase (βG) is assayed from the extent of hydrolysis of its substrate o-nitrophenyl β-galactoside during 1 hour. (Chen, Kung & Bates, Chem. Biol. Interact. 14, 101 (1976). In a typical experiment, DNA was first allowed to recat for 10 min. at 37° with 2.5, 5 and 16.7 mM MNU or ENU, then was dialyzed and added to S-30. Using treated DNA as template, the activity of the newly synthesized βG decreased with the increasing concentrations of the alkylnitrosourea. For MNU treated DNA, the βG activity became 26, 11 and 2.5% respectively of the control. However, in the case of ENU treated DNA, the βG activity was 100, 100 and 84% respectively. The results show that under comparable conditions MNU inhibits DNA coded enzyme synthesis to a greater extent than ENU. Both MNU and ENU treated DNA sedimented more slowly than the control DNA in alkaline sucrose gradients, whenever there was inhibition of βG activity. The inhibitory effects of MNU and ENU on DNA templates is currently under investigation.

230 O6-ALKYLGUANINE, A DNA-ADDUCT RELEVANT FOR CHEMICAL CARCINOGENESIS ? E.Scherer, A.P.Steward and P.Emmelot, The Netherlands Cancer Institute Amsterdam, The Netherlands

O6-alkylation of guanine has been considered as a DNA alteration relevant for cancer formation by alkylnitrosourea in the nervous system of rats. This is based partially on the low enzymatic removal rate of this lesion from DNA of the target organ (brain) as compared to very efficient removal from DNA of a non-target organ (liver). In spite of the effective removal of O6-alkylguanine from liver DNA, liver is highly susceptible to cancer formation by dialkylnitrosamine. The question therefore arose whether or not the liver is a target for the carcinogenic action of alkylnitrosourea. To test this,experiments concerning (a) the formation of precancerous foci in liver (Scherer and Emmelot, Cancer Res.36,2544;1976) by, and (b) the contribution to liver carcinogenesis (completed by repeated doses of diethylnitrosamine,Scherer and Emmelot,Europ.J.Cancer 11,145;1975) of a single dose of N-ethyl-N-nitrosourea(ENU) were performed. The results indicate that a single dose of ENU effectively contributes to liver carcinogenesis, 50 mg ENU/kg being at least as effective as 10 mg/kg diethylnitrosamine. High carcinogenic action of ENU in the rat is therefore not correlated with a low removal rate of O6-ethylguanine from DNA. The significance of O6-alkylguanine for carcinogenesis remains to be established.

231 PERSISTENT DAMAGE IN RAT LIVER DNA AFTER DIETHYLNITROSAMINE (DEN), Leo Den Engelse, Ben Floot and Erik Philippus, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

At equimolar doses DEN is more potent than DMN(dimethylnitrosamine) in inducing (pre)cancerous liver lesions in the rat. This difference might be related to the alkylation pattern or the repair of nitrosamine-induced DNA lesions. Effects of DEN and DMN on the sedimentation pattern of H3-TdR-labelled SD female rat liver DNA in alkaline sucrose gradients were studied with regard to time and dose dependency. In experiments at 1, 6, 14 and 56 days after injection it was observed that damage induced by DEN (134 mg/kg, ± 45% of LD50; or 13.4 mg/kg) was repaired at a low rate. At 56 days the sedimentation pattern was still grossly abnormal. Identical experiments after DMN (10 mg/kg, ± 35% of LD50) showed (almost) completely control sedimentation patterns at 8 weeks after injection. Experiments at 6 or 56 days after the last of a series of 5 or 10 weekly injections of DEN (13.4 mg/kg) showed that a major part of DEN-induced DNA damage (measured as potentially s.s. breaks) is of a persistent and accumulating character. It is concluded that DNA fragmentation and lack of DNA repair is not a consequence of aspecific hepatotoxicity. As far as we are aware this is the first time that persistent damage after nitrosamine administration has been observed in liver DNA. The relevance of above lesions (probably ethylphosphotriesters) in the process of liver tumour formation will be discussed.

Replication/Inhibition

232 INHIBITION OF DNA-REPAIR SYNTHESIS BY ADRIAMYCIN AND DAUNOMYCIN, Ulrik Ringborg and Bo Lambert, Radiumhemmet and Dept of Clin Gen, Karolinska Hospital, S-104 01 Stockholm, Sweden.

The anthracyclines adriamycin and daunomycin are widely used in the treatment of tumor patients. They bind to the DNA and are potent inhibitors of DNA- and RNA-synthesis. With the purpose to investigate whether adriamycin and daunomycin induce DNA-repair synthesis, human peripheral leukocytes were incubated with different concentrations of the drugs in presence of 10^{-3} M hydroxyurea and ^{3}H-thymidine. No measurable amount of DNA-repair synthesis was detected.

Since both adriamycin and daunomycin are strong inhibitors of replicative DNA-synthesis, their effect on DNA-repair synthesis was tested. Peripheral human leukocytes were stimulated for DNA-repair synthesis with nitrogen mustard, methylmethanesulphonate and UV-light. Both drugs were potent inhibitors of the stimulated DNA-repair synthesis, and the nitrogen mustard induced DNA-repair synthesis was most sensitive.

The nitrogen mustard induced DNA-repair synthesis was significantly decreased by concentrations of adriamycin used for treatment of patients. From studies on both animal tumors and tumor patients there is evidence for synergistic effects between alkylating agents and adriamycin. Our data are in the line with the hypothesis that this synergism may be caused by inhibition of the repair of alkylation lesions by adriamycin.

233 DNA REPAIR CAPACITY IN THE GENERAL POPULATION AND IN OPIATE ADDICTS, John J. Madden, Arthur Falek, and David Shafer, Emory University School of Medicine and Georgia Mental Health Institute, Atlanta, Georgia 30306.

Previous studies on genetic damage in opiate addicts have been limited to identification of unstable chromosome abberations. We have evaluated DNA repair mechanisms in heroin addicts, methadone maintainence patients, and control subjects to see if opiates might induce stable mutations by acting at this biochemical level. DNA repair capacity was quantitated in peripheral lymphocyte cultures by stressing the cells with known mutagens including far UV, 8-methoxypsoralen + near UV, mitomycin C, and ethyl methanesulfonate, and by measuring the increase in the sister chromatid exchange frequency cytologically and in unscheduled DNA synthesis. Unscheduled DNA synthesis was assayed by DNA extraction and perchloric acid precipitation, and also by sodium iodide isopycnic centrifugation. While the average response of the 100+ control subjects to far UV stress agreed closely with the saturation value ($\sim 20 J/m^2$) reported by Ahmed and Setlow (Proc. Nat. Acad. Sci. 74 1548 (1977)) and others, the range of response was extraordinary, extending from practically zero to 10-fold greater capacity than average. A similar pattern was found in response to the chemical mutagens. This variation in DNA repair capacity in a "normal" population has important implications for humans at both the genetic and environmental levels. The 12 opiate addicts studied thus far have a drastically reduced level of unscheduled DNA synthesis, and experiments are in progress to confirm the significance of this result. This work is supported by National Institute of Drug Abuse Grant #DA01451.

234 INHIBITION BY CAFFEINE AND THEOPHYLLINE OF 3-METHYLCHOLANTHRENE (MCA) INDUCED MALIGNANT TRANSFORMATION OF CULTURED C3H-10T1/2 MOUSE FIBROBLASTS, R. L. Merriman and J. S. Bertram, Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14263.

One proposed mechanism of chemical carcinogenesis is that chemically-induced DNA damage is converted by an "error-prone" repair process to an altered genome which leads to malignant transformation. This investigation examined the effect of the known inhibitors of post-replication DNA repair, caffeine and theophylline, on malignant transformation. Exposure of cultured 10T1/2 cells to 2.5 μg/ml of MCA alone for 24 hr resulted in a 1% transformation frequency (TF). The TF was decreased by 40% when cells were exposed for 24 hr to 2.5 mM caffeine or theophylline 24 hr after removal of the MCA. This decrease was time dependent. Little or no effect was seen on TF when cells were treated with caffeine immediately after MCA exposure. TF decreased by 90% when the cells were treated with caffeine 48 hr after MCA treatment; however, when cells were treated with caffeine 120 hr after MCA exposure, no effect on TF was seen. Comparable results on the temporal aspects of the inhibition of TF were obtained with theophylline. Preliminary experiments using sedimentation in alkaline sucrose gradients to examine nascent DNA synthesis indicate that caffeine inhibits post-replication repair of DNA damage in MCA-treated 10T1/2 cells. The results of this study are consistent with the hypothesis that an error-prone DNA repair process is a mechanism for chemical carcinogenesis. (Supported by USPHS, NCI Grants CA18197 and CA05319).

235 THE INFLUENCE OF A DNA REPAIR INHIBITOR ON INDUCTION OF TYPE C RNA TUMOR VIRUS, Kiki B. Hellman and Pamela P. Brewer, Bureau of Radiological Health, Food and Drug Administration, DHEW, Rockville, MD 20857

Induction of endougenous mouse type C virus occurs following exposure to principally 254 nm far ultraviolet (UV) radiation. [1] Irradiation of A1-2 cells, a cloned line of murine sarcoma positive-helper negative (S+H) cells derived from the BALB/c mouse, induced endogenous xenotropic helper virus permitting rescue of the sarcoma virus genome as determined by focus formation in normal rat kidney (NRK) cells. The dose response for virus induction by UV radiation compared to that for the halogenated pyrimidine, 5-iododeoxyuridine (IdUrd). Optimum induction of virus from A1-2 cells by UV radiation occurred at exposures corresponding to the shoulder of the A1-2 cell UV survival curve, suggesting that repair of UV-induced DNA damage or maintenance of the cell's capacity to support replication of induced virus may be important for UV induction of endogenous type C virus. Studies with caffeine confirmed that DNA repair may be necessary for type C virus induction by UV radiation. Caffeine presumably inhibits postreplication repair in rodent cells, by inhibiting the gap-filling process in UV-irradiated or otherwise damaged DNA. This study shows that caffeine decreases endogenous mouse xenotropic virus induction by UV radiation. This decrease was caffeine concentration dependent. A higher caffeine concentration was required to decrease the quantity of virus induced by IdUrd, to a similar level as seen for virus induction by UV radiation. Caffeine most likely influences some aspect of the virus induction rather than viral replication. Presumably postreplication repair, appears to affect the induction efficiency of endogenous type C virus by UV radiation and IdUrd.

[1]Hellman, K.B., Brewer, P.P., and Hellman, A.: Proc. 25th Mtg. Rad. Res. Soc., May 8-12, 1977

236 THE EFFECT OF DNA DAMAGE ON THE INDUCTION OF SIMIAN VIRUS 40 (SV40) IN TRANSFORMED HAMSTER CELLS, G. B. ZAMARSKY, J. B. LITTLE, P. H. BLACK, and J. C. KAPLAN, Dept. Medicine, Massachusetts General Hospital and Depts. Microbiol. & Mol. Genetics, and Medicine, Harvard Medical School, Boston, Massachusetts 02114

The relationship between DNA repair processes and the induction of infectious virus from SV40-transformed cells has been investigated. Studies of the induction of SV40 from a group of cloned transformed hamster cells by various chemical and physical agents, which produce DNA strand breakage either directly or indirectly via DNA repair systems, had suggested that breaks or gaps in DNA play an important role in virus activation. Recent experiments demonstrated that caffeine enhances the uv light induction of SV40. In order to investigate further the mechanism of this enhancement, the effect of caffeine on postreplication repair of uv light damage was studied utilizing alkaline sucrose gradient sedimentation. Caffeine at concentrations of 0.5, 1.0 or 2.0 mM inhibited the filling of gaps during postreplication repair. In addition, caffeine enhanced the mitomycin C induction of SV40 suggesting that caffeine may act at a common step in the repair of uv light and mitomycin C damaged DNA. We believe that the persistence of gaps in DNA, caused by the presence of caffeine, results in the enhancement of SV40 virus induction, and that virus induction may provide a sensitive biological marker for examining mammalian DNA repair mechanisms.

237 MICE, MEN, CAFFEINE, AND CYCLOPHOSPHAMIDE. J.E. Byfield, M. Lynch, F. Kulhanian, and J. Murnane. Div. of Radiation Oncology, Univ. of Calif., San Diego, 92103.

Human and rodent cells were compared for the effect of clinically useful alkylating agents (AA) on clonogenicity and molecular repair of DNA, with an emphasis on UV excision repair (ER) systems. Unscheduled DNA synthesis by human lymphocytes (HL) significantly exceeded rat LC after exposure to either UV or alkylating agents; the UV repair pahtways therefore appear to process some alkylated damage in humans. Rat cells with no UV ER were only slightly more sensitive to AA, suggesting that ER contributes per se only slightly to AA survival. All useful AA, except nitrogen mustard, showed a small shoulder(D_q) on their AA survival curve. The D_q depended on exposure duration, indicating a pharmacokinetic contribution. In both UV ER competent and deficient cells, the D_q and D_0 were reduced by methylated xanthines (caffeine, theobromine, and theophylline), the degree of reduction being concentration-dependent. Split-dose expts. for AA gave variable kinetics, dependent on the drug and method used for the split-dose expt. Both DNA single and double strand breaks were found following exposure to AA. To date, the data suggests (a) the UV repair systems process some, probably mono-alkylated, sites; (b) sub-lethal damage repair is small following AA in contrast to x-ray damage; (c) the methylated xanthines may be useful during chemotherapy in man with theobromine probably the drug of choice. Supported by CM-43791.

238 PURINE SYNTHESIS, CAFFEINE AND REPAIR. C. Waldren and D. Patterson, The Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado 80262.

Caffeine increases killing of cultured rodent cells by UV light presumably by inhibiting genome repair processes which normally act to decrease reproductive death. The mechanism of caffeine action is not well understood.

In Chinese hamster ovary cells (CHO-K1) and purine-requiring mutants of CHO-K1, we find that concentrations of caffeine which enhance UV killing also inhibit de novo purine synthesis, as measured by inhibition of accumulation of α-N-formylglycineamide ribonucleotide (FGAR) intracellularly or 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) intracellularly or extracellularly. FGAR and AICAR are the third and eighth intermediates respectively of the de novo purine biosynthetic pathway. Inhibition is dose-dependent, with a caffeine concentration of 7.5 mM producing a 90% reduction in 15 minutes. High pressure liquid chromatographic analysis reveals that caffeine causes a marked decrease in intracellular nucleoside triphosphate concentrations, even in the presence of purines in the growth medium, indicating that caffeine may also interfere with utilization of pre-formed purines.

Purine deprivation either by starvation of purine-requiring mutants or by treatment of CHO-K1 cells with methylmercapto purine ribonucleoside (MMPR), a known inhibitor of purine synthesis, also results in sensitization to UV light.

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239 THE EFFECT OF CAFFEINE AND HYDROXYUREA ON VICIA FABA ROOT-TIP CHROMOSOMES PREVIOUSLY EXPOSED TO MALEIC HYDRAZIDE. Beryl Hartley-Asp, Research Lab., AB Leo, S-251 00 Helsingborg, Sweden.

Hydroxyurea, an inhibitor of desoxyribonucleotide reductase, was tested in conjunction with caffeine, a repair inhibitor, to acquire information concerning hydroxyurea's disputed repair inhibitory capacity.

Maleic hydrazide was used as the inducer of chromosomal breaks in Vicia faba root-tip chromosomes. Caffeine when given as a 5h post-treatment at various times during the recovery period caused a potentiation of the chromosomal aberration frequency. Hydroxyurea when given simultaneously with colchicine after maleic hydrazide also produced an enhancement of the aberration frequency but to a lesser degree. However, when the Hydroxyurea/colchicine mixture was given in conjunction with a 5h caffeine treatment after maleic hydrazide, a substantial increase in potentiation above that found for caffeine was obtained. This potentiation could be mimicked by a similar caffeine plus caffeine/colchicine treatment.

This indicates that the potentiation of the chromosome aberration yield found after treatment with hydroxyurea could be due to an inhibition of DNA repair. The results also suggest that lesions sensitized by caffeine are more susceptible to an inhibition of repair at a later stage in replication than unsensitized lesions. Hydroxyurea, however, appears to effect a somewhat different mechanism in the repair process than caffeine as the types of aberrations found after the two treatments differ noticeably.

240 EFFECTS OF CAFFEINE ON THE SURVIVAL OF CULTURED MAMMALIAN CELLS EXPOSED TO FAST NEUTRONS AND GAMMA RAYS, Carter B. Schroy, Paul S. Furcinitti, and Paul Todd, The Pennsylvania State University, University Park, Pennsylvania 16802

It is known that caffeine reduces the survival of cultured human and Chinese hamster cells exposed to x rays. Caffeine added to complete medium at a concentration of 2.0 mM immediately after irradiation for a 44 hr period reduced by approximately one-third the gamma ray dose required for 90% inhibition of colony forming ability of cultured human kidney (T-1) cells. The effects of caffeine treatment of these cells after exposure to fractionated gamma irradiation or single fast neutron irradiation were also evaluated. It appears that caffeine differs from other radiation modifiers (such as halogenated pyrimidines, radioprotective sulfhydryl compounds, and hypoxic sensitizers), because it is similarly effective when high and low LET (linear energy transfer) radiations are used. In terms of currently popular cell survival curve analysis (linear plus quadratic dose dependence of double-strand DNA break induction) caffeine appears to promote the effect of and/or to inhibit the repair of the linear, or "single-hit" component (blunt-end double-strand breaks?) of cell lethality.

241 ACTION OF 4NQO, ADRIAMYCIN AND ETHYLENIMINE ON DNA REPLICATION IN HELA CELLS, Robert B. Painter, University of California, San Francisco, CA 94143

Although the three drugs, 4NQO, adriamycin, and ethylenimine (aziridine) damage DNA quite differently and these damages are repaired differently, all of them inhibit initiation of HeLa replicons. 1) 4NQO is "UV-like" in many respects, but its first observable action at concentrations that inhibit DNA synthesis by 50-60% is on replicon initiation. There are no observable strand breaks at this concentration and there is no effect on chain elongation. 2) Adriamycin does not induce repair replication at concentrations that strongly inhibit DNA synthesis. Replicon initiation is inhibited but the largest effect of this drug seems to be to inhibit the rate of chain elongation. 3) At concentrations that inhibit DNA synthesis by 50-60%, ethylenimine causes breaks in parental DNA that are very slowly repaired. Accompanying this damage is a marked inhibition of replicon initiation. Chain elongation cannot proceed past the damage in the parental DNA so that nascent molecules of the size of the broken parental strands accumulate. These data suggest that most kinds of DNA damage alter the conformation of a large subchromosomal subunit of DNA whose structural integrity must be reestablished before normal DNA replication can resume.

242 EFFECTS OF IONIZING RADIATION AND METHYLMETHANE SULFONATE ON REPLICON INITIATION AND REPLICON ELONGATION.* T. D. Griffiths and D. B. Dahle
University of Rochester

Abundant data clearly indicates that exposure of cultured mammalian cells to ionizing radiation or to methylmethane sulfonate (MMS) results in a transient depression in the rate of DNA synthesis. We have compared the effects these two agents have on DNA synthesis in Chinese hamster V-79 cells by, 1) kinetic analysis of thymdiine incorporation into PCA precipitable material and 2) by the use of DNA fiberautoradiography. Results from fiber data strongly support models proposed by others, which require X-irradiation to inactivate whole banks of parasynchronously initiating replicons instead of randomly inactivating individual replicons within a bank, since X-irradiation decreases the ratio of post-pulse to pre-pulse replicons without affecting the inter-origin distances between replicating units within replicon banks. In fact, the biphasic nature of the X-ray dose response curve for thymidine incorporation can be entirely accounted for by the presence of replicon banks with different sensitivities to the inhibitory effects of ionizing radiation. On the basis of our work with MMS we conclude that there are effects on both replicon initiations and replicon elongation. We have proposed elsewhere that MMS-induced single strand breaks inhibit initiation events, while MMS-induced base alterations induce elongation events. Further data testing this proposal and its relation to DNA repair will be presented.

*This paper is based on work performed under contract with the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1281.

243 LONGWAVE ULTRAVIOLET (UVA) INHIBITION OF DNA SYNTHESIS IN HUMAN LYMPHOID CELLS Kenneth H. Kraemer and Haywood L. Waters, Chemistry Branch, NCI, Bethesda, MD. 20014

This study indicates that high intensity UVA in the absence of exogenous photosensitizers can inhibit tritiated thymidine (H3TdR) incorporation and cell proliferation in human lymphoid cells in vitro. Earlier studies (Kraemer and Weinstein, J. Invest. Dermatol., 69:211, 1977) have demonstrated decreased H3TdR incorporation in circulating leucocytes from some psoriatics immediately after UVA plus oral 8-methoxypsoralen in vivo. Fresh lymphocytes obtained from normal donors by leucophoresis were purified by Ficoll-Hypaque sedimentation and cultivated for 2-4 days in RPMI 1640 medium with 17% fetal calf serum (complete medium). Long term lymphoblastoid cell lines established by EB virus treatment of lymphocytes from normal individuals were also grown in suspension cultures in complete medium. For UVA exposure the cells were resuspended in a solution containing only the salts and glucose present in RPMI 1640 medium. Cell suspensions in plastic flasks at 37° were exposed to UVA from a bank of 4 Sylvania fluorescent lamps (FR40T12 PUVA) with incident flux 1.2-1.5mW/cm^2 through a 6mm plate glass filter. Peak wavelength was 350nm and minimum detectable wavelength passing filter was about 320nm. There were no significant differences in the amount of post-UVA decrease in H3TdR incorporation in cultured fresh lymphocytes (3 donors), or in 3 lymphoblastoid lines (GM 1953-9 experiments, GM 1652 -3 experiments, El -10 experiments). H3TdR incorporation for the first 2hr after UVA exposure was found to be 50-90% of that in identically treated unirradiated cells after 5min UVA exposure, 40-85% after 10min UVA, 30-75% after 20min UVA, 25-65% after 40min UVA, and 20-55% after 60min UVA. Increase of log phase viable lymphoblastoid cells (GM1953) after UVA exposure was inhibited for 1day after 20min UVA and 9davs after 60min UVA.

244 CHROMATIN REPLICATION IN VITRO. Ronald L. Seale. National Jewish Hospital and Research Center, Denver, Colorado 80206 and University of Colorado School of Medicine, Denver, Colorado 80262.

Isolated HeLa cell nuclei synthesize DNA for 60-90 min. Half of this DNA is in nucleosomes spaced with a similar neighbor-neighbor distance as in parental chromatin. By comparison of these structures with models for segregation, it is clear that nucleosomes pass from parent to daughter chromatid by a non-random process.

The structure of newly replicated chromatin is different from mature chromatin in three ways; 1) nucleosomes on newly replicated chromatin have an altered conformation, 2) nucleosome phasing is extraordinarily precise, i.e., spacer DNA heterogeneity is lost, and 3) the nucleosomal repeat size is reduced from 185 bp of mature chromatin to 170 bp in newly replicated chromatin.

During replication the nucleosome undergoes a conformational change. In both micrococcal nuclease limit digest and DNAase I digest patterns, the higher molecular weight oligonucleotides are either diminished in content, or lost. These patterns are strikingly similar to those derived from trypsinized chromatin; a model is proposed for the replicative state of the nucleosome wherein the histone N-terminal arms are neutralized by chemical substitution, thus preventing their interaction with DNA.

A soluble activity resides in cell extracts which catalyzes the conversion of the replicative conformation of the nucleosome to the "normal", non-replicating conformation.

245 PHOTOCHEMISTRY AND BIOLOGICAL EFFECTS OF BISBENZIMIDAZOLE DYE 33258HOECHST WITH BUdR AND BUdR-SUBSTITUTED DNA, Ehud Ben-Hur, Ariela Prager and Emanuel Riklis Nuclear Research Center-Negev, Beer-Sheva, Israel.

We have recently shown that the increased radiation sensitivity of bacteria with BUdR-substituted DNA is partly due to inhibition of the excision repair pathway of DNA damage by BUdR. Exposure of BUdR-substituted E.coli cells to 360 nm light in the presence of 33258 Hoechst further increases their sensitivity dramatically. Mutant cells deficient in excision repair of DNA damage (uvr B) are more sensitive than wild type cells, indicating the latter ability to repair partly this type of damage. They perform, however, only a limited amount of liquid holding recovery, an excision repair dependent process. Exposure of the dye with BUdR to Near UV light in solution results in appearance of two BUdR-derived photoproducts, deoxyuridine and an adduct of BUdR-dye. The adduct is acid-labile, and as a result only uracil is observed in acid hydrolyzates of DNA from exposed cells. The production of uracil is linearly dependent on light exposure. Cells in which 85% of thymidine was replaced by BUdR are unable to remove uracil from their DNA during postirradiation incubation. However, when only 4% of thymidine is replaced, about 50% of the uracil is removed during 30 min incubation after exposure. The results support our previous conclusion that BUdR interferes with repair via excision-resynthesis. It is suggested that the repair enzymes are more tightly bound to BUdR-substituted DNA and are thus less available for performance of their function.