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**Project Title:** Biologically Important Radiation Damage in DNA

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1. Subject.

General base damage assay and the contribution of base damage to LMDS.

2.1. General remarks.

The importance of the hydroxyl radical in DNA damage by ionizing radiation is well established. We have argued that clustering of the sites (referred to as locally multiply damaged sites, or LMDS) of radical attack is a feature of DNA damage by ionizing radiation (in contrast to chemical agents), and proposed an approach to characterize this clustering in terms of 1, numbers of individual lesions per cluster; and 2, inter lesion distances.

A minority (ca. 20%) of hydroxyl radical reactions with DNA lead to cleavage of the sugar phosphate backbone (i.e. strand breaks). These breaks are easily quantified using a plasmid nicking assay which has found extensive use in this laboratory. Plasmids containing strand breaks adopt less compact tertiary structures. These may be conveniently separated by agarose gel electrophoresis, and the strand break yield quantified by determining the relative proportions of the damaged and intact plasmid.

The remaining 80% of DNA damage by the hydroxyl radical is confined to the bases, and this base damage represents an important component of LMDS. The yields of the major damaged bases have been determined by gas chromatography mass spectrometry. For our proposed approach, it was necessary to convert a known fraction of these damaged bases to strand breaks and then assay these labile sites as the increase in strand break yield over the normally observed level. Three potential agents by which this strategy of conversion of base damage to strand break could be implemented were identified in the original application: 1, S1 nuclease; 2, piperidine; and 3, base damage specific enzymes.

2.2. S1 nuclease.

To emphasize the fraction of damaged sites in the form of LMDS, the plasmid DNA was irradiated in the presence of a high concentrations of DMSO and compared to DNA irradiated at low DMSO concentration. However, we found that the use of DMSO as a scavenger at very high irradiation doses suffers adversely from the problem of radiation induced anoxia. We found that under these conditions, the methyl radical (caused by OH radical attack on DMSO) can cause strand break formation in much greater yield than can the hydroxyl radicals available for reaction with DNA (a manuscript on the subject has been accepted by Radiation Research and is presently in the press). It is probable that the methyl radical also damages bases - we will check for this. Because of the relatively long lifetime of the methyl radical, strand breaks induced

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by it will not exhibit the clustering phenomenon that characterizes damage associated with the hydroxyl radical, i.e. it does not cause the types of damage in which we are interested. Therefore S1 nuclease treatment of such irradiated DNA gave results that were difficult to interpret.

We therefore decided to use a different scavenger, dimethyluracil since we had established that the secondary radical derived from this compound does not lead to strand break formation in DNA. However, after the S1 nuclease measurements were completed, subsequent observations revealed that in aqueous DNA solutions, dimethyluracil exhibits anomalous OH radical scavenging properties. The details remain unclear at present, but it appears that the explanation involves the self association of dimethyluracil molecules even at low (millimolar) concentrations.

Thus in our future investigations of this area we will repeat the examination of S1 nuclease as a reagent to detect base damage, again using DMSO as the scavenger. These experiments will require care to ensure that the problems of methyl radicals caused by anoxic conditions do not arise, i.e. we will use more frequent or continuous oxygenation.

### 2.3. Piperidine.

The lability of a number of damaged bases to piperidine treatment is well established in the literature. The reagent is used as part of the Maxam and Gilbert sequencing protocol to introduce strand breaks at various sites including alkylated purines, formamido pyrimidines (ring opened purines) and pyrimidine glycols. For this reason we had identified the use of piperidine as potentially useful in the measurement of the yields of damaged bases. When we attempted to use the standard piperidine treatment of our plasmids we found that the system is much more sensitive than the end labeling assays for which the piperidine hydrolysis is traditionally used: The background hydrolysis of undamaged plasmid DNA by piperidine is unacceptably high for our purposes, and the treatment leads to the essentially complete loss of unirradiated, supercoiled plasmid DNA after a 3 - 4 hr incubation with  $0.4 \text{ mol dm}^{-3}$  piperidine at  $60^{\circ}\text{C}$ . We are currently investigating the use of milder conditions, reducing: piperidine concentration, time of treatment and/or temperature of treatment.

### 2.4. Damage specific enzymes.

While the use of base damage specific enzymes might seem to be the most direct approach to the problem of cutting at base damaged sites we had identified two potential problems for their usage which led us to investigate the other approaches first. These problems were their possible lack of reactivity with damaged bases in the neighborhood of other damaged sites or within single stranded regions of DNA, either of which would compromise their suitability for our purpose, since LMDS would be expected to contain clustered damage sites and/or single stranded regions. However, given the difficulties we have experienced with the other approaches, we presently plan to investigate the use of such enzymes for this purpose. They have the advantage of requiring relatively mild conditions (i.e. pH 7 - 8,  $37^{\circ}\text{C}$ , moderate ionic strength).

### 3. Plan for the forthcoming year.

The S1 nuclease assay first requires the identification of reoxygenation conditions that avoid the problem of radiation induced anoxia. Since we have characterized the reactivity of the methyl radical, we can now easily determine when oxygen depletion is a significant problem in DNA solutions with DMSO as the scavenger. A recent report described the use of S1 nuclease under neutral conditions in the presence of magnesium ions (it is normally used at a

pH of 4 - 5 in the presence of zinc ions), and we will also examine the reactivity of the enzyme under these conditions.

For the piperidine induced breaks we will investigate the use of milder conditions of hydrolysis.

Damage specific enzymes will be obtained from the laboratories of Susan Wallace (University of Vermont, Burlington) and Michael Weinfeld (Cross Cancer Institute, Edmonton), and incubated with irradiated DNA under conditions described in the literature.

**JUSTIFICATION FOR CARRYOVER FUNDS:** ~\$13,000

The carryover amount was a result of the late hiring of the person to the position funded by this grant. There were problems in finding the correct person. We hired Mr. Aguilera initially at 50% time since he was already funded 50% on another grant. He is now available 100% time to work on this grant. The carryover monies will be used to hire a student worker at 25% time to assist Mr. Aguilera and bring the project up to speed during the second year.

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