EFFECTS OF RADIATIONS ON DNA AND REPAIR OF THE DAMAGE

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ABSTRACT

Repair of DNA double-strand breaks produced by gamma rays takes place in E. coli. Such repair requires recA function and the presence of another DNA molecule of the same base sequence, so it may involve a recombination-like event. Ultraviolet light acting on DNA containing bromouracil produces double-strand breaks by single photochemical events, and a simple model can explain this, as well as other results.

Bromouracil mutagenesis of either E. coli or lambda phage does not involve the recA or red functions. Bromouracil mutagenesis is greatly increased in E. coli mutants such as uvrE, mutL, mutR and mutS, which are defective in mismatch repair. This, and other results, suggest that bromouracil mutagenesis occurs when cell enzymes fail to remove mismatched bases.

Ultraviolet mutagenesis of lambda phage may be a useful model for the study of mutagenesis in cells, because the effects of lesions in the gene mutated (i. e. in the phage) and changes in enzyme systems (by treating the host cells) can be examined separately. Quantitative data support this approach.

Most of the material in this progress report has been either published in the literature, or is fully described in a preprint submitted with this report, and will soon be sent to a journal. The references are to the list of publications at the end of the report. The quantitative information on lambda phage mutagenesis has not yet been written up for publication, and is described in somewhat more detail.

The results are summarized under three major headings.

Repair of DNA Double-Strand Breaks

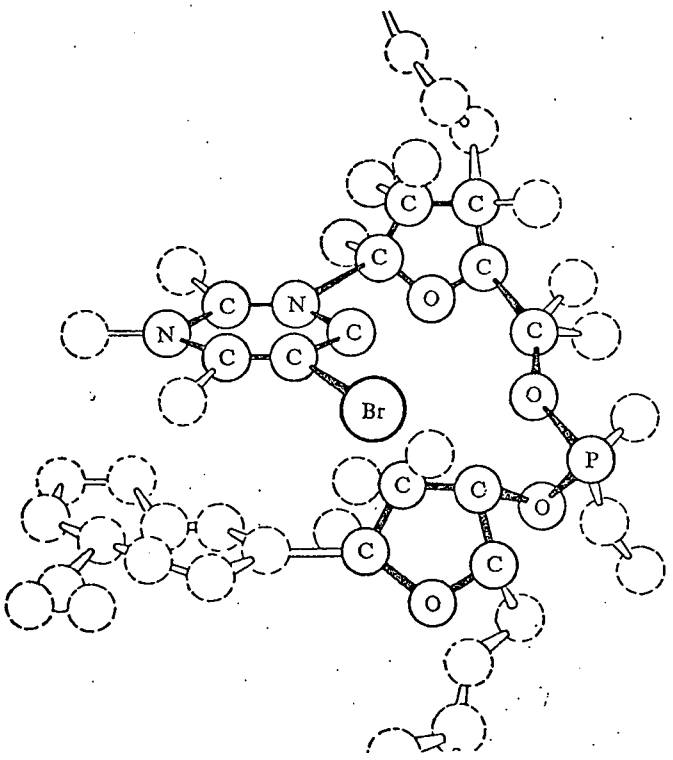
Despite conflicting reports from various laboratories (Hutchinson, 1975a), this process is shown to occur in E. coli cells. No such repair occurs in recA$^-$ cells, or in cells with only a little more than one genome per cell. It is hypothesized that repair may involve recombination-like events between the broken ends and another homologous DNA molecule, having the same sequence of bases as at the break (Krasin and Hutchinson, 1977a).

The method used for demonstrating repair in practically all studies is that of DNA sedimentation under neutral (i. e. nondenaturing) conditions. The distortion of very large DNA molecules caused by sedimentation as calculated by Zimm, presents serious difficulties for such studies (Hutchinson, 1975b). Some aspects of Zimm's theory have now been quantitatively verified (Hutchinson and Krasin, 1977), and suggest that current sedimentation techniques cannot measure the size of DNA larger than $2 - 3 \times 10^9$ daltons.

Radiation-Induced Lesions in DNA

Bromouracil incorporated in DNA, which is then irradiated with ultraviolet light, is shown to result in double-strand breaks as the result of single photochemical events. The yield is about one double-strand break per 100 single-strand breaks, as measured in alkali. This double-strand break may well be the lethal event in the sensitization of cells by bromouracil (Krasin and Hutchinson, 1977b).

Integrating this result, as well as other new observations, with results in the literature, has produced the following hypothesis concerning the ultraviolet lesion in DNA containing bromouracil. The primary photochemical event is dissociation of the bromine atom from bromouracil. The uracil free radical formed in this process extracts, with high efficiency, a hydrogen atom from the C'2 of the sugar on the 5' side of the affected bromouracil (see diagram on following page), forming uracil in the DNA.



Eighty of 100 Br· radicals combine with radicals in the sugar, forming a sugar with Br on C'2. This sugar is stable at neutral pH, but hydrolyzes in alkali to form a single-strand break with the release of a base. The remaining sugar free radicals, 20 out of 100, hydrolyze in some unknown manner to form a frank single-strand break. About one out of 100 Br· radicals diffuse to the complementary DNA strand and produces a frank single-strand break, thereby making a double-strand break in the DNA (Krasin and Hutchinson, 1977b).

The radioactive decay of ^{3}H on the C2 position of adenine incorporated in DNA forms a crosslink between the complementary strands with an efficiency of 0.5. This crosslink greatly reduces the ability of T4 phage to produce plaques (Krasin, Person, Ley and Hutchinson, 1976). (This project was a collaborative one, involving work at Penn State, Argonne National Laboratory and Yale.)

A four-strand DNA , isolated from B. subtilis, was shown to have many of the physical properties expected of a form postulated by Robin Holliday to be an intermediate in genetic recombination. The strain from which this form can be isolated was shown to be deficient in recombination. Therefore, it is hypothesized that this DNA may be an intermediate in genetic recombination which accumulates in this particular strain because of one or more mutations in the enzymatic pathways involved (Köhnlein and Hutchinson, 1976). (This work was a collaboration with Professor Köhnlein's group at the University of Münster, Germany.)

Mutagenesis

Bromouracil mutagenesis in E. coli seems to arise primarily by overloading a system in the cells which corrects mismatched bases in DNA. It does not involve the recA character of the cells. Mutagenesis is highly nonlinear in bromouracil incorporation, with little mutagenesis up to 10% replacement of thymine by bromouracil, and a rapidly increasing level for higher incorporation levels. Holding the cells under various conditions, such as starving without needed amino acids, decreases the observed mutagenesis (Rydberg, 1977a). Some strains which are highly mutable by bromouracil - uvrE$^-$, mutL, mutS, mutR - are deficient in mismatch repair as measured by transfection with heterologous lambda DNA. Since these strains also have a high rate of spontaneous mutability, the processes may be in part those insuring the faithfulness of DNA replication (Rydberg, 1977c).

When bromouracil is used as a mutagen, it is essential to measure incorporation in cellular DNA, because E. coli cells with different genotypes can have levels of incorporation differing by an order of magnitude (Rydberg, 1977b).

Forward mutagenesis by bromouracil of lambda phage does not depend on the recA character of the host cell or the red character of the phage, despite Pietrzykowska's report of such dependencies for back mutations. Bromouracil mutagenesis is not affected by treatments to the host cells which should give rise to Weigle mutagenesis (i. e. enhancement of λ phage mutagenesis by ultraviolet light, when the host cells are also irradiated) (Hutchinson and Stein, 1977).

Ultraviolet mutagenesis of λ phage as a model system for ultraviolet mutagenesis of cells is not unreasonable. The polymerases and the repair systems in both cases are those of the cell. Viewing the phage as a model system has the following advantage. The nonlinear (usually square-law) dependence of a number of mutations in many cells (particularly E. coli) on exposure to a mutagen has suggested to many investigators that mutagenesis involves:

(1) a lesion in the gene to be mutated;
(2) induction of some generalized cellular process.

In the phage model system, treatment of the phage creates the lesion in the gene whose mutation is being measured, and treatment of the host cell (Weigle mutagenesis, or W-mutagenesis) elicits the generalized cell response. A considerable amount of data has been obtained on this system, and the firm results to date may be summarized as follows.

(1) The mutagenesis of λ phage irradiated after adsorption to host cells is quadratic in UV fluence, as is mutagenesis of E. coli cells in this range of fluences.

(2) Little mutagenesis is found for forward mutations (clear plaque or gam$^-$) if the phage only, or the cells only, are irradiated.

(3) For phage assayed in host cells given some fixed fluence between 10 and 100 J/m^2 of 254 nm radiation, the number of mutants/plaque-forming-unit is about linear with UV fluence to the phage at lower exposures, then levels off to a plateau at high fluences.

(4) For phage given some fixed fluence between 10 - 200 J/m^2 of 254 nm radiation, mutants/plaque-forming-unit increase as roughly a linear function at low fluences, and the slope varies by a factor of 15, depending on the metabolic state of the cell. At higher fluences to the cell, there is a broad maximum in the number of mutants/plaque-forming-unit, followed by a precipitous decline for fluences to the cells above 100 J/m^2.

(5) Lesions formed by incorporating bromouracil in the host cell genome and irradiating with either 254 nm, or 313 nm, light are very efficient in eliciting W-mutagenesis. Approximately the same response is found for bromouracil in one strand only of the host cell genome, or for the same amount of bromouracil distributed evenly between the two strands (no increase in lambda phage mutation by incorporation of the bromouracil only is seen).

These results, and some others for which more data are still being obtained, will be written up and published soon.

Research Personnel Trained

Dr. Björn RYDBERG was a Postdoctoral Associate on this project for two years, and has returned to Sweden to a permanent position in the Swedish National Defense Research Establishment.

Dr. Frank KRASIN is completing two and one-half years as a Postdoctoral Fellow with this contract, and will continue work as a Research Associate on the repair of DNA double-strand breaks.

Three Graduate Students: Joseph FORAND, Daniel RABIN and Chi-Kuang HUANG, have each worked on projects associated with this contract for periods of a few months each.

In addition, two undergraduates have developed research experience on this contract: Oliver JONES (entering Graduate School at University of California) for one academic year; Kenneth CROEN (now in Medical School) full-time for one summer.

Publications on this contract, 1974-1977

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Rydberg, Björn (1977). Discrimination between bromouracil and thymine for uptake into DNA in drm$^-$ and dra$^-$ mutants of Escherichia coli K12. Biochim. Biophys. Acta, 476: 32-37.

Rydberg, B. (1977). Bromouracil mutagenesis in Escherichia coli evidence for involvement of mismatch repair. Molec. gen. Genet. 152: 19-28.

Rydberg, B. (1977). Bromouracil mutagenesis and mismatch repair in mutator strains of Escherichia coli. Ms. submitted.