EXCISION-REPAIR OF DNA DAMAGE IN AN AUXOTROPHIC STRAIN OF ESCHERICHIA COLI

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Escherichia coli B/r WP2 is a tryptophan-requiring organism which has been used by many workers in studies on the mutagenic and lethal action of radiation. From it Dr. Ruth Hill has isolated a mutant, E. coli WP2 hcr^-, which cannot host-cell-reactivate phage, is extremely sensitive to the lethal and mutagenic actions of UV (Hill, 1965; Ashwood-Smith, Bridges and Munson, 1965; Ashwood-Smith and Bridges, to be published) and has an ability to excise thymine dimers from its DNA at one tenth or less of the rate of E. coli B/r WP2 (R.B. Setlow, quoted by Hill, 1965). In most of these respects it behaves like the very radiosensitive strain E. coli B_{s-1} (Hill and Simson, 1961). We have used it to study the possible involvement of the thymine dimer excision-repair (abbreviated as hcr) system in extending or repairing damage by ionizing radiation.

Susceptibility of ionizing radiation damage to excision repair

There is now abundant evidence that hcr^+ but not hcr^- strains are able to excise damage by alkylating agents as well as by UV and this has led to the suggestion that the hcr system is rather non-specific and capable of dealing with many types of DNA damage (Haynes, 1964b; Papirmeister and Davison, 1964; Kohn, Steigbigel and Spears, 1965; Lawley and Brookes, 1965). Since E. coli B_{s-1}, which is also unable to excise thymine dimers (Setlow, 1964), is some three or four times more sensitive to ionizing radiation than E. coli

B/r (Hill and Simson, 1961; Haynes, 1964a,b), it appeared likely that a considerable proportion of lethal damage by ionizing radiation may also be repaired by the hcr system. We have found, however, that E. coli WP2 hcr$^-$ is no more sensitive to gamma rays than E. coli B/r WP2. Survival curves for these two strains together with E. coli B_{s-1} are shown in Figure 1.

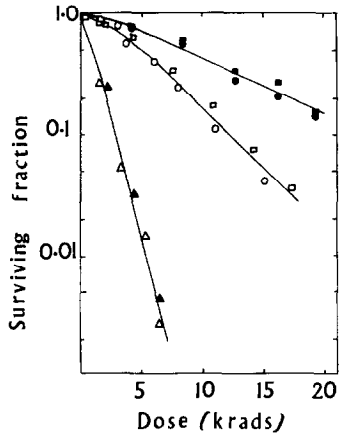

Figure 1. Gamma ray survival curves under oxygenated conditions for E. coli B/r WP2 (circles), E. coli WP2 hcr$^-$ (squares), and E. coli B_{s-1} (triangles). Solid symbols indicate bacteria grown before irradiation in 1% unbuffered peptone and plated on nutrient agar; open symbols indicate bacteria grown in and plated on glucose-salts medium (supplemented with tryptophan in the case of the auxotrophic strains).

Two simple explanations are possible: I. that there is a separate repair system for gamma ray damage present in B/r WP2 which is absent from B_{s-1} but not from WP2 hcr$^-$, II. that there is only one multi-enzyme repair system for both UV and ionizing radiation damage but that repair of gamma ray damage involves fewer steps than repair of UV damage. One could then postulate on hypothesis II that B_{s-1} lacks one or more enzymes common to both repair pathways, whilst WP2 hcr$^-$ lacks one or more enzymes in the UV pathway only.

There is evidence against the first hypothesis from the work of Haynes (1964a,b) who has shown fairly convincingly that a repair system present in B/r but not B_{s-1} operates on both UV and X-ray damage. One is therefore faced with the problem of which repair step functioning in WP2 hcr$^-$ might be lacking in B_{s-1} and vice versa.

The first steps in the repair process as postulated by Setlow and Carrier (1964) and Boyce and Howard-Flanders (1964) are the breakage and subsequent excision of a small fragment of the strand containing the lesion. (The gap in the DNA strand resulting from excision may be extended by exonucleases but is ultimately filled by a DNA polymerase using the intact single strand as a template.) Both B_{s-1} and WP2 hcr$^-$ are deficient in the initial steps since they cannot release thymine dimers from DNA, and yet they appear to be deficient in different enzymes. It is not unreasonable to suppose that strand breakage and excision might be performed by two distinct enzymes. Single strand breaks are known to be caused by ionizing radiation (Shooter, 1957; Hagen, 1964) but not by UV (Smith, 1965); thus the strand breakage enzyme might not be required for repair of damage due to ionizing radiation.

If WP2 hcr$^-$ could not make the initial incision but could perform excision and all subsequent steps, its sensitivity to UV, inability to excise dimers, and resistance to ionizing radiation would be explained. The sensitivity of B_{s-1} to both UV and ionizing radiation and inability to excise dimers would follow if it were unable to perform the actual excision step. A prediction of the sensitivity of these strains to nitrogen mustard and methylmethanesulphonate may be made on the basis of this hypothesis. Nitrogen mustard produces crosslinks which are apparently excised and repaired by the hcr system (Lawley and Brookes, 1965; Haynes, 1964a,b). Methylmethanesulphonate produces single breaks (Wahl, 1965). We might therefore expect strain B/r WP2 to be resistant to both chemicals, B_{s-1} to be sensitive to both, and WP2 hcr$^-$ to be resistant to methylmethanesulphonate and sensitive to nitrogen mustard. These predictions have been confirmed (Fig. 2). Searashi and Strauss (1965) have isolated a strain of B. subtilis resistant to methylmethanesulphonate but sensitive to UV; if excision-repair is carried out in a similar manner to E. coli we might expect their strain to behave similarly to WP2 hcr$^-$ and be resistant to ionizing radiation.

It may also be seen from Figure 1 that the well known effect of an en-

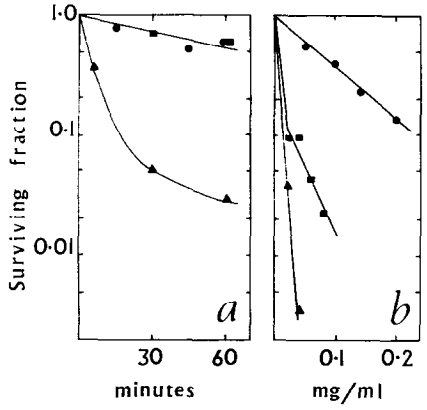

Figure 2. (a) Survival of three strains of E. coli with time of incubation at 22°C in 0.5% (v/v) methylmethanesulphonate.
(b) Survival of three strains of E. coli after incubation for 2 hours at 22°C in nitrogen mustard.
● , E. coli B/r WP2; ■ , E. coli WP2 hcr⁻; ▲ , E. coli B_{s-1}. Bacteria were grown before treatment to logarithmic phase in glucose-salts medium (with tryptophan supplement for WP2 strains) and plated on nutrient agar.

riched medium in increasing resistance to ionizing radiation is shown by both B/r WP2 and WP2 hcr⁻ but not by B_{s-1}. It therefore seems likely that medium enrichment is effective because in some way it increases the number of enzyme molecules (or in some way enhances enzyme action) in the repair process which the first two strains possess but which B_{s-1} lacks.

Susceptibility of ionizing radiation mutation damage to excision repair

It has been inferred that the lesions which result in mutation after UV treatment are susceptible to the excision repair system (Hill, 1965; Bridges, to be published). To test the susceptibility of the X-ray mutational lesion we examined the induced reversion rate for E. coli WP2 hcr⁻ under the precise conditions used previously for E. coli B/r WP2 (Munson and Bridges, 1964) where the induced reversion rate at 37° throughout for B/r WP2 was 6.55×10^{-11} ($\pm$ 0.45×10^{-11}) per nucleus per rad. The figure for WP2 hcr⁻ is 7.1×10^{-11} ($\pm$ 1.0×10^{-11}) per nucleus per rad. It must be concluded that B/r WP2 does not possess any means of removing X-ray mutational lesions which are not also

possessed by WP2 hcr$^-$, thus eliminating excision repair as a repair system for X-ray induced mutation lesions.

Possible involvement of excision repair in extending damage from one to both strands of DNA

In studies on the segregation pattern following induction of revertants in B/r WP2 by X-rays we came to the conclusion that when the gene is first replicated after irradiation the mutation is present in a stable form on both daughter genes (Munson and Bridges, 1964; Bridges and Munson, 1964). Since there were theoretical grounds for expecting that most of the initial X-ray lesions would involve damage to only one strand of the double helix (and thus be transmitted to only one daughter gene at replication) we therefore supposed either that the lesion was transferred to the other strand before replication or that in the process of replication only one of the strands was used to code both daughters. A similar explanation has been suggested to account for apparently double stranded segregation after base analogue-induced mutation (Witkin and Sicurella, 1964). The excision repair system might provide a feasible way of transferring damage from one to both strands if it were capable of recognizing a double stranded region of the DNA which has been damaged but not of recognizing the mutant strand. In half of the excisions the mutational lesion would then be excised and replaced by "correct" nucleotides. In the other half, the "correct" nucleotides would be excised and replaced by nucleotides complementary to the mutational lesion.

We have therefore examined the segregation pattern for newly-induced prototrophic revertants of E. coli WP2 hcr$^-$ (which is deficient in thymine-dimer excision enzymes) after a dose of 2,500 rads X-rays. The theory and technique were exactly as described previously (Munson and Bridges, 1964). We found an average of 1.44 $\pm$ 0.12 independently mutable units of the try gene per nucleus as compared with 1.5 $\pm$ 0.2 in the hcr$^+$ strain E. coli B/r WP2. If the lesions had remained confined to a single strand in the hcr$^-$ strain we would have found twice as many independently mutable units (i.e.

approx. 3). We may therefore conclude that if the mutational lesion is transferred from one to two strands before the first replication, then the excision repair system which can excise thymine dimers is not the responsible agent.

Conclusions

1. Although several enzymic steps would appear to be common to the excision repair of thymine dimers and nitrogen mustard damage and the repair of lethal damage due to methylmethanesulphonate and gamma radiation, at least one step exists in the former system which is not required for the latter. It is suggested that this step might be the production of single strand breaks in the sugar-phosphate backbone of DNA.

2. The gamma-radiation damage which results in mutation to prototrophy is not excisable by the thymine dimer excision system.

3. The excision-repair system is not responsible for the extension from one to both strands of mutational events induced by ionizing radiation.

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