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HAEMOPHILUS INFLUENZAE DOES NOT FIT THEORIES OF POSTREPLICATION **

REPAIR

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Studies with Escherichia coli have provided all the initial insights into the molecular bases of repair processes. It is the thesis of this article that especially if we believe that there are some general mechanisms in nature, it is important to consider more than one microorganism in arriving at an understanding of the biology of repair of DNA.

Escherichia coli cells lacking the ability to excise pyrimidine dimers can nevertheless survive ultraviolet radiation considerably better than a strain containing two mutations, which is recombination defective as well as excision defective (1). The first clue to the mechanism of the repair system defective in recA strains was provided by the discovery that the DNA synthesized after UV in an excision-defective E. coli on an irradiated template was shorter than in unirradiated cells, but that upon incubation the single-strand size of the DNA increased towards that of the control cells (2). The ability to accomplish this size increase was later found to be dependent on expression of the recA gene (3). All of these phenomena are also found in Haemophilus influenzae (4), including a mutation in the rec1 gene, somewhat analogous to recA, that causes the DNA size increase to be eliminated.

A further advance in understanding postreplication repair came when it was found that much of the increase in size of the fragments resulted from transfer of stretches of DNA from the

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parental to the daughter strand in E. coli, filling in the gaps in the daughter strand (5). Again H. influenzae was also found to undergo this process, but whereas in E. coli the number of gaps was approximately equal to the number of UV-induced pyrimidine dimers (2), in H. influenzae there appeared to be considerably more sister-strand exchange than could be accounted for by the number of dimers (6).

When sister-strand exchange takes place in E. coli, not only are the gaps in the daughter strands filled in, but in the process some of the dimers become transferred to the daughter strands (7). In H. influenzae this process is much more rapid and complete, so that the dimers become equally distributed between parental and daughter strands in a few minutes (8). This takes place even in a mutant lacking a functional recombination system for phage or transforming DNA. Thus it is clear that the recombination that leads to dimer exchange and gap-filling in H. influenzae is a different type from the recombination of transforming DNA or phage DNA.

The phenomena of postreplication repair have aroused great interest in recent years because of the connection between this type of repair and induced mutation by UV. It has been shown that part of the postreplication repair involves an inducible system in E. coli, and that functional recA and lexA genes are required for it (9, 10). If this inducible system is eliminated by treatment of E. coli cells with chloramphenicol before and twenty minutes after UV irradiation, part of the gap-filling and all of the UV-induced mutation is lacking (11).

A hypothesis was developed from these and other related phenomena in the reaction of E. coli to UV, that when cells with intact recA and lexA genes are irradiated, there is coordinate induction of a number of functions (lysogen induction, cell filamentation, Weigle (W) reactivation, gap-filling and induction of mutations) (9, 10). The discovery of a mutant (C600 T44, tif-1) that is thermally inducible without UV treatment, and mimics all these effects, strengthened the hypothesis (12, 13). Some of the difficulties with the hypothesis have been that various functions supposed to be coordinately controlled are clearly separable under certain circumstances. For example, E. coli carrying a recF mutation is normally mutable by UV, but W reactivation is reduced (14) and gap-filling is defective in excision-defective mutants (15, 16). Revertants of strains carrying the tif-1 mutation (zab) are lacking in W reactivation, but when lysogenized are UV-inducible (17).

H. influenzae shows many of the same responses to UV which have been considered to be coordinately controlled in E. coli.

Thus there is UV-induced lysogen induction (18), cell filamentation (19), W reactivation (18) and gap-filling (4, 6). Most of these responses have been shown to be recI-dependent (20). However, in a most important respect, H. influenzae is different, namely in being immutable by UV (21, and J. K. Setlow, unpublished experiments).

A recent study of W reactivation in H. influenzae has been made in an attempt to understand more about this phenomenon (22). We found that W reactivation could be carried out with UV-irradiated HPlcl phage when wild type but not recI mutant cells were treated with either UV or mitomycin C (MMC), resulting in an increase of viable progeny phage by more than a factor of ten. Similar treatment of cells infected with unirradiated phage only caused a decrease in the number of progeny. If the cells were incubated in growth medium following UV or MMC treatment, but before infection with UV-irradiated phage, there was a further increase in the number of progeny (up to a factor of eight), although the progeny from unirradiated phage were almost unaffected by the incubation. Chloramphenicol during this incubation eliminated the reactivation. Reversions of two temperature-sensitive phage mutations were measured as a function of conditions giving maximal W reactivation. There was no dose-dependent increase in mutations, and all the measured frequencies were well within previously measured spontaneous reversions at these loci (23).

Conclusions from these data were: (a) H. influenzae has an inducible repair system dependent on recI function that operates on UV-irradiated phage, although no reactivation of cells was observed when they were treated so as to produce maximal induced reactivation of phage. (In E. coli W reactivation conditions also do not contribute significantly to chromosome repair (24).) (b) This repair system, unlike the one in E. coli (25), does not result in phage mutations.

There are several responses of H. influenzae to UV radiation that differ from those of E. coli and that could have relevance to the problem of why H. influenzae is immutable by UV radiation. Whereas UV-induced stimulation of recombination in E. coli is readily observed (26), UV does not increase the genetic recombination of H. influenzae phage or transforming DNA (27). While part of the gap-filling is changed by chloramphenicol in E. coli, no chloramphenicol-induced change in the kinetics of this process could be observed in H. influenzae (21). We have already noted the much more rapid and widespread sister-strand exchange that takes place in H. influenzae after UV (6, 8).

Several hypotheses on the reasons for the UV immutability

of H. influenzae may be considered. Kimball, Boling and Perdue (21), taking into account the failure of certain agents including UV to induce mutations in H. influenzae cells, have proposed that H. influenzae lacks the inducible repair system. However, it is now clear that this bacterium does have such a system, dependent on the recI gene. Since the recI gene is known to affect postreplication repair (4, 6), it is reasonable to assume that postreplication repair is somehow involved with the inducible repair system in H. influenzae. The question remains how such an inducible system should be error free, unlike that of E. coli.

A second hypothesis is that UV causes induction of an inhibitor of proofreading function of a polymerase (28) in E. coli but not in H. influenzae. The evidence for such a function in E. coli is rather indirect. The hypothesis also explains the mutations resulting from UV irradiation of E. coli host cells subsequently infected with unirradiated phage (29, 30), and the lack of such mutation in H. influenzae phage. However, there are indications that the mechanism of such indirect mutagenesis in E. coli may be entirely different from that observed in the direct mutagenesis of W reactivation (30), providing some evidence against the hypothesis.

A third hypothesis is that in E. coli the inducible part of gap-filling forces synthesis past a pyrimidine dimer (31), particularly where there are overlapping daughter strand gaps, whereas the sister-strand exchange in H. influenzae results in an inserted piece of parental DNA that not only fills in the gap but also extends past the dimer responsible for the gap. Hence there is no synthesis possible on the dimer-containing template, and thus no mutation.

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