*THE RADIATION SENSITIVITY OF ESCHERICHIA COLI B: A HYPOTHESIS RELATING FILAMENT FORMATION AND PROPHAGE INDUCTION**

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Escherichia coli B is unusually sensitive to ultraviolet light (UV), compared to other wild-type strains and to its own radiation-resistant derivatives such as B/r.[1] Despite the large difference in survival after exposure to the same dose of UV, strains B and B/r differ little, or not at all, in: (1) number of thymine-containing dimers produced in the DNA,[2] (2) rate at which thymine-containing dimers are excised from the DNA,[2] (3) ability to promote "host-cell reactivation" of irradiated bacteriophages,[3] and (4) recovery time and rate of synthesis of DNA after irradiation.[4] Sensitivity to UV in strain B, unlike that in certain other UV-sensitive strains isolated from both B and B/r, is not associated with reduced ability to repair pyrimidine dimers or other kinds of UV damage that block DNA replication.

The most striking difference between strains B and B/r is the failure of cell division in the sensitive strain after exposure to very low doses of UV, resulting in the formation of greatly elongated filamentous cells upon subsequent incubation.[1] Survivors and nonsurvivors alike form filaments, the survivors ultimately regaining the ability to divide. Agents other than UV (e.g., crystal violet[5] and nitrosoguanidine[6]) also cause filamentous growth in B but not in B/r, and similar "snakes" are frequently observed in untreated cultures of the sensitive strain. Strains B and B/r differ mainly in the vulnerability of the cell division mechanism, which in strain B is readily deranged by a variety of initiating stimuli. In B/r given the same treatments, inhibition of the cell division mechanism either is not initiated or is promptly and invariably reversed.

Impressive similarities have been noted[7, 8] between filament formation in strain B and prophage induction in lysogenic strains such as K12 (λ), some of which are summarized below: (1) Both filament formation and prophage induction are mass effects, occurring in virtually every member of a population exposed to low doses of UV. (2) Both effects can be initiated by a variety of agents other than UV, all having in common the ability to produce damaging changes in DNA. Although no systematic comparison has been made to determine whether the same agents invariably produce both effects, strain B is more sensitive than strain B/r to many of the agents known to cause prophage induction (X rays,[1] mitomycin C,[9] and nitrogen mustard[10]). (3) Both filament formation in B and prophage induction in K12(λ) occur occasionally in untreated cultures, and especially in "old" cultures.[6] (4) UV-initiated filament formation is photoreversible,[11] as is prophage induction;[12] that is, both are greatly reduced by post-UV exposure to visible light. In strain B, efficient photoreactivation is obtained when wavelengths of light responsible for indirect effects are excluded, and most of this photoreactivation can be ascribed to the enzymatic splitting of pyrimidine dimers.[13] The same appears to be true of prophage induction in K12(λ).[6] This indicates that both effects when produced by UV depend upon the presence in the DNA of pyrimidine dimers. The doses of

UV that are effective in provoking filament formation[11] or prophage induction[14] are far too low, however, to permit the assumption that either effect requires the presence of one or more pyrimidine dimers in a particular gene, or in a particular operon. Doses producing only about 10–20 dimers per bacterium can cause filament formation or prophage induction in most of the exposed cells. This suggests that both effects are provoked by the presence anywhere in the DNA (or, at least, anywhere within a relatively large segment of it) of a small number of pyrimidine dimers. (5) Both filament formation and prophage induction are partially or wholly prevented by a number of pre-UV or post-UV treatments (e.g., chloramphenicol posttreatment[15,16] or pre-UV exposure to "photoprotecting" light[17,18]) having in common the ability to inhibit or reduce the rate of protein synthesis. (6) Certain posttreatments (caffeine and acriflavine), having in common the ability to inhibit the repair of UV-irradiated DNA, markedly increase both the induction of prophage in K12(λ)[19] and the UV sensitivity of strain B.[6]

Consideration of these parallels has led to the proposal that UV sensitivity in B may be due to the detachment of an integrated episome[7] or to the induction of a defective prophage.[8] Although there is every reason to seek a common mechanism underlying the UV sensitivity of strain B and prophage induction, it should be borne in mind that the primary event in prophage induction is the derepression of an operon.[20] Subsequent events (vegetative growth of phage, and lysis) are dictated by the potentialities of the particular operon that is derepressed, and it may be quite irrelevant to the comparison with UV sensitivity in B that this operon happens to be part of the prophage genome rather than of the bacterial genome proper.

The extraordinary feature of prophage induction is the sensitivity of the repressor responsible for the maintenance of the lysogenic state in K12(λ) to relatively slight impairment of the integrity of the DNA. The lambda repressor is not directly inactivated by UV, but loses its activity only after the occurrence of a complex biochemical process that includes postirradiation protein synthesis.[14] Although the nature of this process is still obscure, the direct photoreversibility of prophage induction indicates that the repressor is inactivated after UV irradiation only if pyrimidine dimers remain in the DNA for some time. This suggests that blockage of DNA replication plays an essential role in the inactivation of lambda repressor, perhaps by permitting the accumulation of a DNA precursor that participates in the inactivation process.[21] Although repressors exhibiting this sensitivity to the state of the DNA are probably rare (as indicated by the failure of prophage-inducing agents to induce the synthesis of certain inducible enzymes[22, 23]) they may not be associated uniquely with prophages. Colicin production, which is mediated by another kind of episome, can also be induced by UV.[24] It would be premature, however, to assume that such repressors are found only in association with episomes and never with normal bacterial operons. They are more likely to be detected if the consequences of induction are lethal or otherwise dramatically evident, and such drastic effects are probably unusual after induction of native bacterial genes. Repressors that are inactivated when DNA replication stops could, theoretically, play an important part in regulation of cellular activities normally geared to the cycle of cell division, especially in organisms characterized by a distinct pause between rounds of DNA synthesis.

The following hypothesis is offered to explain UV sensitivity and filament forma-

tion in strain B: (1) Strain B contains a repressor which, like the repressor of bacteriophage lambda, is inactivated by a complex process that starts with the presence of replication-blocking lesions, such as pyrimidine dimers, in the DNA. (2) This repressor (repressor B) is inactivated after UV only if protein synthesis occurs before the repair of pyrimidine dimers in the DNA is accomplished. (3) The inactivation of repressor B induces an operon (operon B), which may be part of an integrated episome or part of the bacterial genome proper. A product of operon B (presumably a protein) is an inhibitor of cell division or can lead indirectly to the production of such an inhibitor. (4) After UV induction, repression of operon B is restored when the repair of pyrimidine dimers in the DNA is completed. (5) Filamentous growth results from (*a*) induction of operon B and (*b*) synthesis of a sufficient quantity of the inhibitory product of operon B to cause persistent inhibition of cell division, even after DNA repair is accomplished and repression of operon B is restored. (6) Whether or not a filament will recover the ability to divide, and will thereby survive, depends upon the amount of inhibitor that accumulates in the cell while operon B is in the derepressed state (i.e., between the time the repressor is inactivated and the time DNA repair is completed). A filament survives only if the amount of inhibitor synthesized during this time is small enough to be diluted below a threshold concentration, as the filament elongates, before it reaches a "critical length"[25] beyond which recovery is no longer possible.

According to this hypothesis, the fate of irradiated B depends upon the relation between two postirradiation processes: (1) the rate of protein synthesis, and (2) the rate of DNA repair (in strain B, this coincides with the rate of excision of thymine-containing dimers from the DNA).[4] The effects of posttreatments known to increase or decrease survival in B (as compared to its survival when plated immediately after UV on nutrient agar and incubated at 37° C) are readily explained on this basis. Posttreatment with chloramphenicol, which inhibits protein synthesis but allows normal excision of thymine-containing dimers,[4] completely suppresses filament formation in B[6] and raises B survival to the same level as that of B/r.[15] Under these conditions, DNA repair is probably completed before any protein synthesis occurs, in which case repressor B should not be inactivated and strain B should exhibit the same UV sensitivity as B/r. Another posttreatment that converts strain B into a phenocopy of B/r is incubation at 45°C.[26] At this temperature protein is synthesized at about the same rate as at 37°C, but indirect evidence indicates that the rate of dimer excision is greatly accelerated.[6] Faster repair of DNA should reduce the period during which operon B remains derepressed, resulting in restoration of repression in every cell before a lethal quantity of inhibitor can accumulate.

Another kind of posttreatment that increases the survival of irradiated B, although never all the way to the level of B/r, is incubation in nonnutrient liquid medium before plating.[27] This "liquid holding" does not permit protein synthesis, but does allow dimer excision, albeit at a considerably reduced rate.[4] Since DNA repair is probably not completed during liquid holding, induction of operon B should occur upon subsequent plating. The head start in DNA repair provided by the incubation in liquid should hasten the restoration of repression, thereby reducing the amount of inhibitor produced. Thus, a larger fraction of the population should recover the ability to divide before reaching the point of no return. Neither liquid-

holding recovery[28] nor heat reactivation[6] occurs to any great extent in strains lacking the ability to excise pyrimidine dimers.

Acriflavine, which inhibits dimer excision,[29] reduces B survival after UV as would be expected if its effect on protein synthesis were less drastic.[30, 31] Caffeine, which delays DNA synthesis after UV with relatively little effect on protein synthesis,[32] also lowers the survival of irradiated B.[6] Any posttreatment having the *net* effect of reducing the amount of protein synthesized before DNA repair is completed should increase B survival; any posttreatment permitting more protein synthesis before DNA is repaired should decrease B survival. The relation between DNA repair and protein synthesis should similarly determine the fate of K12(λ) after UV induction. In both systems, protein synthesis before DNA repair would promote a lethal outcome in two distinct ways: (1) by permitting inactivation of a repressor, and (2) by permitting translation of a potentially lethal message coded by the operon thereby induced. Protein synthesis *after* DNA repair would be necessary for the production of active repressor, and thus for the restoration of repression, unless the inactivation of repressor after UV is a reversible process.

This hypothesis does not bear directly on the mechanism of inhibition of cell division that is promoted by the induction of operon B. Conceivably, such inhibition could be quite indirect; for example, operon B might contain a regulator gene coding a repressor (repressor X) for another operon (operon X). If operon X codes a product essential for septum formation, the induction of operon B would inhibit cell division by causing repression of the synthesis of this essential substance. A division-promoting agent present in *E. coli* extracts[33] could be such a product. According to this model, a filament could recover its ability to divide by producing a large enough number of DNA replicas to bind all units of repressor X synthesized while operon B was in the active state. Only after all accumulated units of repressor X are bound to DNA can the filament produce a DNA replica containing an active operon X and cell division resume (unless so much repressor X has accumulated that the "critical length" is reached before this point). This could account for the observation[34] that "multinucleate" B filaments appear to contribute genetic information from only one DNA molecule to the clones descended from them.

In this laboratory, filament formation in *E. coli* B and prophage induction in K12(λ) are being compared under a variety of conditions designed to test this hypothesis. In addition, an effort is under way to demonstrate transfer of the postulated inhibitor of cell division during conjugation between irradiated and unirradiated bacteria.

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