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Cell Division in *Escherichia coli* B_{s-12} Is Hypersensitive to Deoxyribonucleic Acid Damage by Ultraviolet Light

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Escherichia coli B_{s-12} *uvrA lon* is hypersensitive to ultraviolet light. On minimal agar plates at densities in excess of about 10^7 bacteria per plate, as few as one or two photoreversible pyrimidine dimers in the entire genome are sufficient to cause inhibition of cell division. Most of the resulting filaments are unable to divide or form a viable colony. Inhibition of cell division appears to be a rapid consequence of replication of deoxyribonucleic acid containing a pyrimidine dimer. Photoreversibility of the inhibition of cell division persists indefinitely, indicating that the continued presence of the pyrimidine dimers (or the continued generation of daughter strand gaps) is necessary to maintain the division-inhibited state. In view of the kinetics for the production of filamentation by ultraviolet light and the extremely low average inducing fluence (0.03 J/m^2), it is concluded that the initiating signal is not the same as that causing other inducible phenomena such as prophage induction or Weigle reactivation.

Strains of *Escherichia coli* carrying a *lon* mutation (e.g., strain B) are prone to give rise to filaments, both spontaneously and in response to deoxyribonucleic acid (DNA) damage (1, 2, 14, 18), and in such strains the inhibition of cell division is involved in conferring sensitivity to many mutagens. Witkin (19) was the first to recognize that *lon* filamentation had many characteristics in common with prophage induction. Both belong to a group of phenomena that are "induced" in response to agents that damage DNA or interfere with DNA replication. Other phenomena in this group are Weigle reactivation and mutagenesis of phage λ, inhibition of respiration, and inhibition of DNA degradation (6, 11, 17). All are dependent upon the *recA*$^+$ and *lexA*$^+$ functions, and it has been suggested that they are coordinately induced by damage to DNA or inhibition of DNA synthesis (12, 13, 20). One suggestion for the inducing signal in ultraviolet light (UV)-irradiated DNA is an overlapping daughter strand gap formed by the replication of DNA containing photoproducts close together on opposite strands of the duplex (16). Certainly in strains deficient in the excision of photoproducts, the dose-response curves for Weigle reactivation and prophage induction are reasonably consistent with the overlapping daughter strand gap hypothesis, being quantitatively similar and usually proportional to the square of the dose.

Most experiments on filamentation have been carried out in excision-proficient backgrounds, including the classic paper in this area by Deering (5). Deering found that division delay in UV-irradiated *E. coli* B was a highly specific effect, with total mass, ribonucleic acid, and DNA continuing to increase at very nearly the same rate as for unirradiated dividing cells. Filamentation response was "single hit" with respect to UV fluence, and the fluence resulting in 37% nonfilamenting cells was 0.3 J/m^2 at 265.2 nm. Many of the filaments recovered the ability to divide at some later time and form normal macroscopic colonies, and filamentation was partly reversed by visible light if it was given within the first 20 min after UV. We might now interpret these data as indicating that pyrimidine dimers induce filamentation and that subsequent excision of dimers may, in a proportion of cases, allow cell division to be reinitiated. It must not be forgotten, however, that photoreversing light may exert indirect effects in excision-proficient strains that are not specific to pyrimidine dimers.

Such data as exist (9) suggest that filamentation in excision-deficient strains (*lon uvr*) might be rather more sensitive to UV than are prophage induction and Weigle reactivation. Our interest in the hypothesis of coordinate induction (12, 13, 20) has led us to examine inhibition of cell division in a *lon uvr* strain of *E. coli* as a case in which the inducing signal might be distinct from that for other *rec-lex* phenomena.

MATERIALS AND METHODS

Bacterial strains. We used *E. coli* B_{s-12}, a radiation-sensitive derivative of strain B isolated by Hill

and Feiner (9) and subsequently shown to carry a *uvrA* mutation (7). Stocks were maintained on nutrient agar plates.

Culture media. A colony was picked off the stock plate into Oxoid nutrient broth no. 2 and grown overnight at 37°C with shaking. A further subculture was made into the same medium and, when the density reached 2×10^8 to 4×10^8/ml, 0.2 ml of the culture was plated onto Davis and Mingioli (4) minimal agar with 0.4% (wt/vol) glucose but no further supplementation.

UV. Bacteria were exposed on the plate to UV at 209 cm from a Hanovia lamp (type 12555) at a fluence rate of 0.0096 J/m² per s. The fluence was measured at closer distances with a Latarjet meter. The output of our lamp is now given in units comparable to those of other laboratories and differs from those quoted in publications earlier than 1976. Earlier dosimetry had overestimated the output by a factor of around 1.6.

Photoreactivating light. The light source was two parallel Osman 24-inch (ca. 60.96-cm) fluorescent 20-W warm-light tubes placed 11 cm apart. Plates were exposed 8 cm from the lamps with an intervening Kodak Wratten filter no. 85 to remove wavelengths below about 380 nm.

Total counts. At various times after UV, bacteria were washed off the plate with 10 ml of phage buffer (3) and counted with a Hawksley Thoma counting chamber. A bacterium was classified as a filament if it exceeded 10 μm in length.

Viable counts. Bacteria at an appropriate dilution (100 to 1,000 per plate) were plated on minimal agar, and colonies were counted after incubation at 37°C for 2 days.

RESULTS

Dose-response for filamentation. Preliminary experiments revealed a number of interesting facts that have not been pursued, but that placed constraints upon the conduct of the subsequent experiments. It was, for example, not possible to show filamentation of UV-irradiated B_{s-12} in liquid medium, even in nutrient broth. In contrast, a strong tendency for spontaneous filamentation was almost universal on nutrient agar plates at all cell densities and on minimal agar plates at plating densities below about 10^7 per 9-cm-diameter plate. Only on minimal agar with an initial plating density in excess of 2×10^7 per plate was the spontaneous rate of filamentation sufficiently low (usually around 5%) to enable UV-induced filamentation to be observed after exposure to very low fluences.

Filamentation in response to UV has been expressed in two ways. In Fig. 1 the proportion of filaments is shown among the population washed off plates at 3 h after UV. This proportion cannot be simply related to the proportion of UV-irradiated cells in which division is inhibited since the number of nonfilamentous cells at that time depends on the number of cell cycles that have occurred. It serves, however, to show a roughly linear response to increasing UV fluence. Figure 2 shows the total number of nonfilamentous cells per irradiated plate after 3 h as a proportion of the number of cells per unirradiated plate. If we assume, as Deering found, that filamenting cells do not divide during this number of cell cycles and that nonfilamenting cells divide normally, then this figure should represent the fraction of UV-irradiated cells in which division is not inhibited. It can be seen that this fraction decreases approximately exponentially at a rate close to the rate of loss of colony-forming ability under comparable conditions (Fig. 2). It seems reasonable to conclude that under these conditions filamentation is in general not later reversed and is responsible for most of the ultimate inviability. This result is in contrast with that obtained with *E. coli* B, but is not unexpected since B_{s-12} contains no excision repair activity. DNA lesions (if these are the initiating events) are therefore likely to persist indefinitely in B_{s-12}, whereas they will be removed in strain B.

The slight shoulder usually observed on the dose-response curve for loss of colony-forming ability but absent from the curve for inhibition of cell division suggests that at times beyond 3 h some of the filaments with a very low number of DNA photoproducts, although ini-

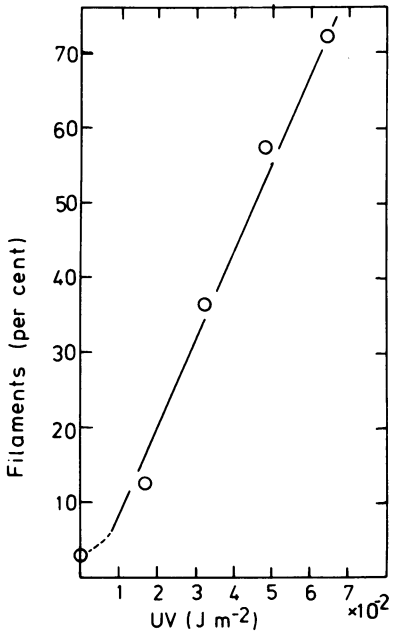

FIG. 1. *Fraction of filamentous E. coli* B_{s-12} *among bacteria washed off minimal agar plates at 3 h after exposure to various UV fluences.*

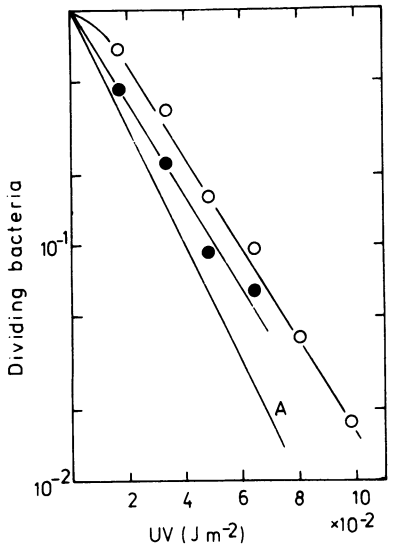

FIG. 2. *Fraction of dividing bacteria among a population of E. coli B_{s-12} on minimal agar after exposure to UV. Symbols: ●, total number of bacteria washed off irradiated plates after 3 h as a fraction of the number washed off unirradiated plates; ○, colony-forming ability of B_{s-12} at a lower plating density. Curve A shows the colony-forming ability of E. coli WP100 uvrA recA.*

tially division inhibited, eventually divide and segregate a daughter with no DNA damage that may then divide normally. Thus, at least two or more dimers per initial genome may be required to maintain the division-inhibited state as the cell becomes very long.

Photoreversibility of filamentation. The use of photoreversing light with B_{s-12} is made more difficult by the fact that the strain is so sensitive as to tend to filament after exposure to the light alone. This was minimized by filtering out wavelengths below 380 nm, and filamentation by light alone was absent or trivial in our experiments. It can be seen from Fig. 3 and 4 that, after UV, increasing exposure to visible light up to 20 min causes a marked reduction in division inhibition, although there was some variation between experiments in the kinetics of the effect. We conclude that photoreversible pyrimidine dimers are involved to some considerable extent in the inhibition of cell division in *E. coli* B_{s-12} after UV.

Loss of photoreversibility. UV-irradiated bacteria were incubated on minimal agar plates for varying periods of time, exposed to 20 min of visible light, and then incubated further for a total of 3 h. The inhibition of cell division was reversed by photoreactivation up to at least 80 min after UV, whether measured by filamentation or by the total count (Fig. 5a and b). The inhibition of cell division is therefore not fixed irreversibly, at least within the first 80 min. It needs the continuous presence of pyrimidine dimers or the continued generation of daughter strand gaps. If dimers are removed, cell division promptly resumes, even in a bacterium that has already filamented noticeably.

Kinetics of division inhibition. By counting the total number of cells washed off plates at various times after 0.06 J of UV per m^2, it was possible to show that cell division inhibition occurred very rapidly, probably within the first 10 to 15 min, on average (Fig. 6). A dose of 0.06 J/m^2 should form about four pyrimidine dimers per genome. In an average cell a replication fork should encounter a dimer within about 5 min, and 2 dimers should be encountered within about 12.5 min. If one of the resulting daughter strand gaps is the signal that inhibits cell division, one must conclude that the response to the signal is very rapid, probably occurring within 0 to 7 min.

DISCUSSION

The UV fluence at which 37% of the *E. coli* B_{s-12} population retained their ability to divide was about 0.03 J/m^2 in contrast to 0.3 J/m^2 for *E. coli B*. Although it could be argued that the measurements were taken in one case after growth on plates and in the other case in liquid medium, we think it is more likely that the significant difference between B and B_{s-12} is due

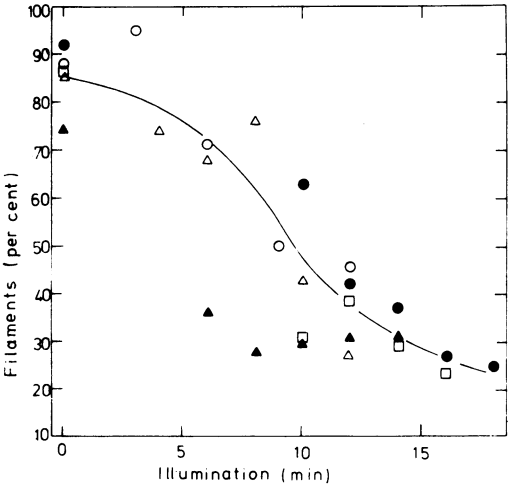

FIG. 3. *Decrease in the proportion of UV-induced filaments of B_{s-12} measured after 3-h incubation on minimal agar as a function of time of exposure to visible light. Different symbols represent different experiments. UV fluence was 0.096 J/m^2.*

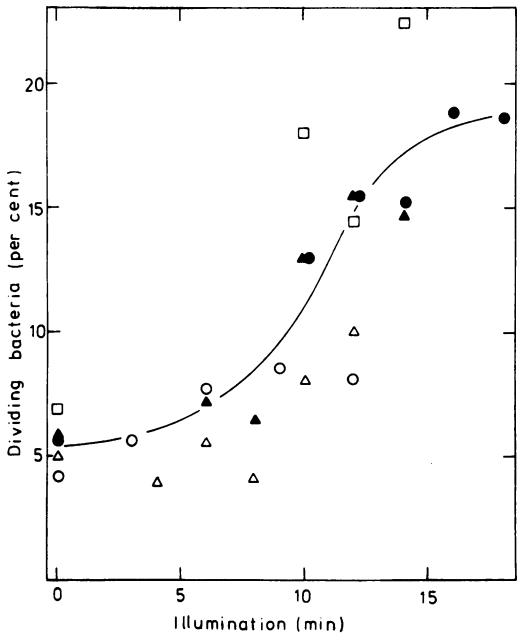

FIG. 4. *Increase in the number of dividing bacteria after exposure to 0.096 J of UV per m² (measured as the total number washed off irradiated plates) as a function of time of exposure to visible light. Different symbols represent different experiments.*

to the lack of excision repair ability in the latter. The difference in UV sensitivity therefore indicates that filamentation in B_{s-12} is triggered by DNA damage, most of which would normally be excised in B. One may calculate (by the method of Rupp and Howard-Flanders [15]) that after exposure to 0.03 J/m² approximately two pyrimidine dimers would be formed per *E. coli* genome. It is therefore possible to conclude that, if pyrimidine dimers are responsible, a very small number is enough to trigger the response, little more than the number required to kill a *uvrA recA* double mutant (Fig. 2 curve A).

Although it is clear that a very small number of pyrimidine dimers is enough to initiate filamentation and that the continued presence of dimers is necessary to maintain the inhibition of division, there seems to be no direct way to distinguish whether the filamentation signal is the dimer itself or the daughter strand gap that it gives rise to on replication. The latter is, however, strongly suggested by a comparison of the relative sensitivity of filamentation and of the relative number of daughter strand gaps in Uvr⁺ and Uvr⁻ strains.

As described above, the mean filamenting dose is 0.03 J/m² in B_{s-12} and 0.3 J/m² in B (5), giving a 10-fold difference in sensitivity. This correlates with the estimate from a closely related pair of strains that there are between 6 and 18 times as many daughter strand gaps in the Uvr⁻ strain as in the Uvr⁺ strain after a low dose of UV (8). If dimers themselves were the inducing signal, filamentation should occur almost as readily in a Uvr⁺ as in a Uvr⁻ strain. Moreover, the *recA*⁺ and *lexA*⁺ dependence of filamentation in *lon* strains supports the notion that daughter strand gaps are involved since these gene products appear to interact with gaps in DNA rather than photoproducts themselves. Finally the linear kinetics of filament induction and the dose range rule out the possibility of overlapping daughter strand gaps (16) being involved.

It has been suggested that the "inducible"

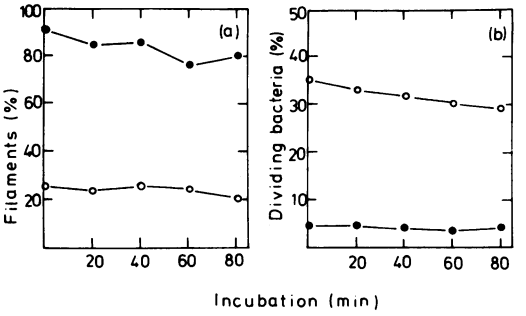

FIG. 5. *Persistence of photoreversibility of UV-induced division inhibition with time of incubation on minimal agar. Filamenting bacteria (a) and dividing bacteria (b), estimated as for Fig. 2 and 3, at 3 h after completion of photoreactivation. UV fluence was 0.096 J/m². Non-photoreactivated plates* (●) *were treated similarly to photoreactivated plates* (○), *but were not exposed to light.*

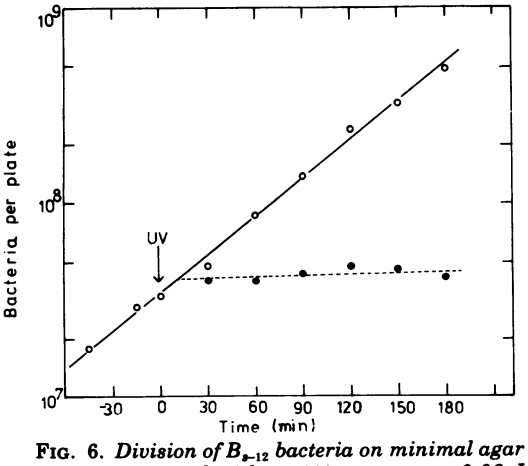

FIG. 6. *Division of B_{s-12} bacteria on minimal agar plates with* (●) *and without* (○) *exposure to 0.06 J of UV per m².*

recA$^+$- and *lexA*$^+$-dependent functions such as filamentation, prophage induction, and Weigle reactivation are coordinately induced (12, 13, 20). The extreme hypersensitivity of B_{s-12} filamentation argues against its inclusion in a scheme of coordinate induction. Whereas the mean fluence causing filamentation is about 0.03 J/m^2, the fluence causing induction of prophage or Weigle reactivation in Uvr$^-$ strains is of the order of 10 J/m^2 (6, 10, 20).

We emphasize that the control of cell division in *E. coli* B_{s-12} is a complex process. The fact that filamentation (presumably temporary) occurs spontaneously on agar plates and that it is supressed at high cell densities shows that pyrimidine dimers do not constitute the only signal whereby septation and cell division may be uncoupled from cell growth.

We conclude that inhibition of cell division (filamentation) in UV-irradiated *E. coli* B_{s-12} may be initiated when a few (probably about two) pyrimidine dimers are replicated and give rise to daughter strand gaps. When more than about two dimers are present per genome, continual generation of daughter strand gaps in each replication cycle of the excision-deficient B_{s-12} maintains the division-inhibited state indefinitely, and a visible colony is never produced. This contrasts with *E. coli* B, in which excision eventually removes the dimer and most filaments at low doses do not lead to loss of viability.

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