Recovery of Division Ability in Ultraviolet-irradiated *Escherichia coli* Induced by Photoreactivation, Photoprotection, and Liquid Holding Treatment

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Small doses of ultraviolet light (UV, 265 m\textmu) cause *Escherichia coli* B to grow into long, multinucleate, nonseptate, filamentous cells. This UV-induced filament formation can be prevented by irradiating with photoprotecting light (335 m\textmu) prior to UV irradiation, and by irradiating with photoreactivating light (406 m\textmu), or by liquid holding treatment, after UV irradiation. It is concluded that UV-induced division inhibition in *E. coli* B is initially induced by repairable lesions in the deoxyribonucleic acid, probably pyrimidine dimers.

Cells of *Escherichia coli* B irradiated with relatively low doses of ultraviolet light (UV, 265 m\textmu) continue to increase in mass, but cell division is inhibited (3, 23). Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis continue following these low doses, and, after a period of growth, long multinucleate nonseptate filamentous cells are formed (3). This property of filament formation causes *E. coli* strain B to appear more sensitive to UV than strain B/r when the assay for survival is colony-forming ability (8, 11, 21). The detailed mechanisms by which UV delays division in filament formers such as *E. coli* B are not known. The experiments reported here were done in an effort to get a better understanding of this process. We report results of direct observations of filament formation which show that UV-induced division delay in *E. coli* B can be prevented by post-UV treatment with photoreactivating light (10, 12) or liquid holding (15), and by pre-UV treatment with photoprotecting light (10, 22). Although photoreactivation (PR) of UV-induced division delay in *E. coli* B has previously been reported (3, 5), it was desirable to reinvestigate these results in view of a recent report by Jagger and Stafford (10). They presented evidence for two types of PR, which occur through completely different mechanisms, thus making the interpretation of some earlier PR data questionable.

Three different wavelengths of radiation are used in the experiments described in this paper. "UV" light will refer to 265 m\textmu radiation, "PP light" will refer to 335 m\textmu radiation used for photoprotection (PP), and "PR light" will refer to 406 m\textmu radiation used for photoreactivation. These particular wavelengths were used for reasons that are explained later. Direct and indirect PR and PP were defined by Jagger and Stafford (10) and are used in this paper in accordance with their definitions.

MATERIALS AND METHODS

**UV-irradiation techniques.** The UV-irradiation techniques have been described in earlier papers (3, 11). Briefly, all UV-irradiations including irradiations for PR and PP were done in 1-cm quartz cuvettes in a water-prism monochromator similar to that described by Fluke and Setlow (6), with a Philips SP500W UV source. Incident intensities were determined with a photocell by methods similar to those previously described (3). Samples were stirred during irradiations with a small glass-enclosed iron flea and magnetic stirrer. The temperature of the cultures was maintained at 37 C during the irradiations unless otherwise specified. Irradiated bacterial suspensions were dense enough so that the corrections calculated by Morowitz (13) were used to obtain an average effective intensity throughout the sample.

**Induction of division delay.** Log-phase cells of *E. coli* B (Yale subculture) at a concentration of approximately 10^8 cells/ml grown in aerated C-1 minimal medium (14) at 37 C were used in all experiments. These cells were irradiated at 37 C with approximately 20 ergs/mm^2 of 265-m\textmu UV light to induce division delay, and they were incubated in aerated liquid C-1 medium at 37 C. Details of this procedure were described previously (3). After 100 min of growth (approximately two mass doubling times), a drop of the culture was placed between a cover slip and microscope slide and was examined under a microscope. The number of normal and filamentous (four to six times normal length) cells in several random fields
was counted. All irradiations and incubations after irradiations were done either in the dark or under subdued yellow lights.

**Photoreactivation and photoprotection.** Immediately after cells were irradiated with 265-mu UV light, they were exposed to 2.5 \( \times 10^6 \) ergs/mm\(^2\) of 406-mu light for PR. The time required for this exposure was 6 min. These PR conditions were used in order to employ an exposure time of less than 10 min (3), even though the not give maximum PR of colony-forming ability using the conditions of Jagger and Stafford (10). Since PR is temperature-dependent (12, 16), attempts to photoreactivate UV-induced division delay were made at 4 and 37 C. After irradiation with 406-mu light, the cultures were incubated with aeration at 37 C in the dark. A drop of the culture was examined under a microscope after 100 min of growth, and the relative number of normal and filamentous cells was determined.

For PP, log-phase cells from 10 ml of a culture were collected on a membrane filter (0.45 \( \mu \) pore size; Millipore Corp., Bedford, Mass.), washed once with 10 ml of warm C-0 (C-1 without glucose), and re-suspended in 10 ml of C-0 medium. This culture was immediately exposed to 4.2 \( \times 10^6 \) ergs/mm\(^2\) of 335-mu light for PP. The irradiation temperature was maintained at 37 C, and the irradiation time was 3 min. The dose and dose-rate used here were similar to those used by Jagger and Stafford (10) to obtain maximum PP as measured by colony-forming ability. A glass slide was placed between the beam exit slit and the sample to filter out wavelengths shorter than 300 mu. Immediately after the 335-mu irradiation, cells were irradiated with 265-mu UV light. Cells were again collected on a membrane filter, re-suspended in fresh C-1 medium, and incubated at 37 C with aeration. Rates of mass increase of these cultures were determined by measuring turbidity with a Bausch & Lomb Spectronic-20 colorimeter at 625 mu. After the turbidity had doubled twice, a drop of the culture was examined under the microscope, and the relative number of normal and filamentous cells was determined.

**Liquid holding recovery.** To observe the effects of liquid holding on UV-induced division inhibition, cells were collected on a membrane filter, re-suspended in C-0 medium, and held in the dark at various temperatures for various lengths of time. After a specified time, cells were collected on a membrane filter, re-suspended in fresh C-1 medium and incubated in a 37 C water bath. After two mass doubling times, samples were examined under the microscope for filaments.

**RESULTS**

**Photoreactivation.** A comparison of action spectra determined for PR and PP of *E. coli* (10) shows that light of wavelength 406 mu causes direct PR but does not cause PP or the growth delay associated with PP. This wavelength was therefore used to see if UV-induced division delay was photoreactivable by the direct mechanism. The data from a typical experiment are shown in Table 1. If a culture was exposed to 24 ergs/mm\(^2\) of 265-mu UV light and allowed to grow, examination after 100 min of mass increase (two mass doubling times) showed that the culture contained 92% filamentous cells four to six times normal length. Treatment with 406-mu light at 37 C immediately after UV irradiation decreased the amount of filament formation observed after the same growth period by about two-thirds but was without effect at 4 C. PR treatment alone had no effect on division ability. Figure 1 shows that irradiation with PR light had no effect on growth as determined by turbidity increase, and that 100 min of growth was in all cases equivalent to two mass doubling times.

**Photoprotection.** Exposure of *E. coli* cells to 335-mu light induces a growth delay (10) that is thought to be responsible for the phenomena of PP (22) and indirect PR (10) of far UV damage (265 mu). To see what effect PP had on UV-induced division delay, cells were irradiated with 335-mu light prior to irradiation with 265-mu UV light. The results of a typical experiment are shown in Table 2. Treatment with PP light at either 37 or 4 C prior to irradiation with UV light decreased the amount of filament formation observed after two mass doubling times by about two-thirds. Similar results were obtained when exposure to PP light followed UV-irradiation. Irradiation of cells with PP light alone did not cause filaments to be formed. The dose of PP light used here caused a growth delay, as measured by increase in turbidity, of approximately 70 min. This is shown in Fig. 2. The time required for the irradiation with PP light was 34 min. Holding cells in buffer for this time did not affect growth, division, or UV-induced filament formation when subsequently grown in C-1 as is shown in Fig. 2 and Table 2.

**Liquid holding.** If UV-irradiated cells of *E. coli* B are held in non-nutrient medium for a period before being plated on agar, an increased survival is observed when the assay for survival is colony-forming ability (15). Here we have examined the effect of holding in non-nutrient medium on UV-induced division delay. The results are shown in Fig. 3. A culture exposed to 22 ergs/mm\(^2\) of 265-mu UV light contained 92% filamentous cells after 100 min of growth in C-1 medium. If cultures were held at 37 C for various times in C-0 medium after exposure to UV light, fewer filamentous cells were observed after 100 min of subsequent growth in C-1 medium. The number of filamentous cells observed depended on the holding time and the holding temperature. In general, the longer the holding time, the fewer the fila-
Immediately after 100 min of growth, cultures of medium were exposed to 265-nm UV light at the temperature indicated and then incubated at 37°C in C-1 medium. After 100 min, cultures were examined for filaments.

*Cells exposed to 265-nm UV light were immediately exposed to 406-nm light at the temperature indicated and then incubated at 37°C in C-1 medium. After 100 min, cultures were examined for filaments.

<table>
<thead>
<tr>
<th>Filament-forming dose, 265 mp</th>
<th>PR dose 406 mp</th>
<th>Temp</th>
<th>Filaments after 100 min of growth</th>
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<tr>
<td>0</td>
<td>2.5 × 10⁴</td>
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<td>4</td>
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* Cells exposed to 335 mp light at the temperature indicated were immediately exposed to 265-nm light and then incubated in C-1 at 37°C. After two mass doubling times, cultures were examined for filaments.

* Held in buffer for the interval required for the above PP dose (34 min).

**TABLE 1. Photoreactivation (PR) of division inhibition**

**TABLE 2. Photoprotection (PP) from division inhibition**

**FIG. 1. Turbidity of UV-irradiated Escherichia coli**

B cultures exposed to photoreactivating light. All irradiations were finished at t = 0. ○, 24 ergs/mm² UV (265 mp); △, 24 ergs/mm² of UV plus 2.5 × 10⁴ ergs/mm² of PR (406 mp) at 37°C; ●, 2.5 × 10⁴ ergs/mm² of PR; ▲, 24 ergs/mm² of UV plus 2.5 × 10⁴ ergs/mm² of PR at 4°C.

**FIG. 2. Turbidity of UV-irradiated Escherichia coli**

B cultures exposed to photoprotecting light. All irradiations were finished at t = 0. ○, 22 ergs/mm² of UV (265 mp); △, 4.2 × 10⁴ ergs/mm² of PP (335 mp) at either 4°C or 37°C plus 22 ergs/mm² of UV; ●, 4.2 × 10⁴ ergs/mm² of PP at either 4°C or 37°C; ▲, held for 35 min in C-0 medium with no UV, and no PP.

**DISCUSSION**

Photoprotection is a pre-UV treatment, and photoreactivation and liquid holding are post-UV treatments; all treatments increase the colony-forming ability of UV-irradiated E. coli B [see
Rupert and Harm (17) and J. Setlow (18). It was desirable to observe the effects of these treatments directly on division inhibition in UV-irradiated E. coli B since division occurs at an earlier time and is a necessary event in colony formation. It is an immediately assayable effect in contrast to colony formation which is rather far removed from the primary photochemical changes. The effect of these treatments can also be used to help understand the molecular mechanisms leading to UV-induced division inhibition.

Much evidence exists which indicates that direct PR of UV damage in E. coli B involves photoenzymatic splitting of pyrimidine dimers in DNA (see 17, 18), thus removing blocks to DNA synthesis. Studies by Jagger and Stafford (10) show that a second type of PR, called indirect PR, which does not involve photoreactivating enzyme, can also be observed in E. coli B and operates by the same mechanism as PP. The action spectra for direct PR and indirect PR (or PP) determined by Jagger and Stafford (10) differ from one another so that the two types of PR can be distinguished by use of the proper wavelength for photoreactivating light. Light of 406 µm causes direct, but not indirect, PR. Our results (Table 1) show that UV-induced division inhibition in E. coli B can be prevented by post-UV irradiation with 406-µm light. This recovery of division ability caused by 406-µm light is also temperature-dependent (occurring at 37 C but not at 4 C), as is PR of colony-forming ability (12) and in vitro photoreactivation of Haemophilus influenzae. The transforming principle using E. coli photoreactivating enzyme (16). The recovery of division ability observed here is not due to a liquid holding effect since the time required for the PR light exposure was only 6 min, a time which causes very little liquid holding recovery. The UV lesions causing division inhibition are, therefore, subject to direct PR. This direct PR of UV damage suggests that the primary event causing division inhibition is the induction of pyrimidine dimers in DNA. The splitting of these dimers restores the ability of the cell to divide. That the initial UV damage-causing filaments is in a nucleic acid was suggested earlier by the action spectrum for filament formation in E. coli B (3). The action spectrum parallels the absorption spectra of nucleic acids. It had previously been shown that UV-induced filament formation is photoreactivable (3, 5), but no distinction could be made between direct and indirect PR in these earlier experiments.

UV-induced division inhibition is also photoprotectable as the data in Table 2 illustrate. PP is presumed to operate by means of induction of a growth delay allowing more time for dark repair (9). The growth delay caused by PP light may allow repair of molecular damage other than DNA lesions. Therefore, we cannot argue on the basis of this that the initial UV-damage is in the DNA, and that repair of DNA reduces division inhibition. But we point out that a treatment (PP) that causes higher colony-survival, presumably by allowing more time for repair of DNA, also causes higher survival of division ability.

Liquid holding in non-nutrient medium is also presumed to operate by allowing more time for dark repair (7, 9). Our data (Fig. 3) show that UV-induced division inhibition is reduced when irradiated cells are held in C-0 medium at 37 C for a period before growth in C-1 medium. The results of Fig. 3 further show that recovery of division ability occurs to a greater extent in cells held in non-nutrient medium at 37 C than at 3 C. It had previously been shown that recovery of colony-forming ability of UV-irradiated E. coli B during liquid holding is also temperature-dependent, occurring to a greater extent at 37 C than at lower temperatures (2, 15). As with PP, the holding period could allow time for repair of systems other than DNA synthesis. However, since dark repair of UV-damage leading to higher colony survival occurs during liquid holding (7), and since recovery of both division ability and colony-forming ability in non-nutrient medium is temperature-dependent, we strongly suspect that the steps of dark repair are involved in recovery of division ability during liquid holding.
UV-induced filament formation in *E. coli* is a genetically determined function (1, 8, 21). The filament-forming strain *E. coli* B is capable of excising UV-induced pyrimidine dimers from its DNA (19); it can reconvert UV-irradiated T1 phage (4); and it can resume DNA synthesis after UV at least as well as the nonfilament forming strain *E. coli* B/r (20). Yet *E. coli* B is more sensitive to UV than *E. coli* B/r when colony formation is measured. This greater sensitivity is due to the property of filament formation (8, 21). Little is known about the molecular events caused by UV that lead to filament formation. It has been shown here that UV-induced filament formation in *E. coli* B can be reduced by PR, PP, and liquid holding. One of these, PR, strongly suggests that the initial UV lesions causing filament formation are pyrimidine dimers in the DNA. The possibility that filament formation is induced by UV damage to some other cellular component such as RNA could not be excluded by previous experiments. PP from UV-induced division inhibition and liquid holding recovery of UV-induced division inhibition show that division ability can be repaired if a growth delay following UV-irradiation is somehow induced. Possibly this growth delay allows more time for dark repair processes to occur before growth, as suggested by Jagger (9).

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**LITERATURE CITED**


