Interaction of the *exr* and *lon* Genes in *Escherichia coli*

JOHN DONCH, MICHAEL H. L. GREEN, AND JOSEPH GREENBERG

Palo Alto Medical Research Foundation, Palo Alto, California 94301

Strains of *Escherichia coli* carrying the gene *lon* typically produced excess capsular polysaccharide, and were sensitive to ultraviolet light (UV) irradiation, thymine starvation, and nalidixic acid, forming long filaments after these treatments. Sensitivity was reduced by a number of posttreatments. In the presence of a second UV sensitivity gene, *exr*, some of these properties were suppressed: long filaments were not formed, the effect of *lon* on UV and nalidixic acid sensitivity was greatly reduced, and irradiation posttreatments gave an enhancement of survival characteristic of *exr* rather than *lon* strains. Production of capsular polysaccharide was not affected by the *exr* gene.

A *lon* mutant of *Escherichia coli* is more sensitive to ultraviolet light (UV) and thymine deprivation than the wild-type, becomes filamentous after UV irradiation, and produces excessive amounts of mucoid polysaccharide (14). [As used in this report, mucoid strains are defined as those producing large spreading watery colonies at 37 C on minimal medium (7).] These properties appear to be pleiotropic expressions of a single mutation because they have been thus far inseparable genetically (7). Furthermore, mutations to UV resistance, B to B/r for instance, restore all the properties to wild-type. [The genotype of strain B/r is, in fact, *lon rad*, the *rad* mutation acting as a suppressor of *lon* (J. Donch, Y. Chung, and J. Greenberg, *submitted for publication*).] Some of the phenotypic expressions of the *lon* gene can also be turned off or modified in the direction of wild-type by the kind of treatment used after irradiation. These include photoreactivation (17), liquid holding (20), plating on minimal rather than complex media (20), incubating at elevated temperatures (2), and plating on medium containing pantoyl lactone (11, 23).

An *exr* mutant [mutants at a cistron linked to *malB* (8, 12, 19) and probably the same as the *lex* mutation (13)] produces an increase in UV sensitivity, estimated by Witkin (26) to be two- to threefold, and an increase in X-ray sensitivity. Furthermore, it reduces UV-induced mutations below detectable limits, but it does not affect spontaneous or chemically induced mutations (26). When it occurs in a *lon* strain such as B (12), it turns off filamentation following UV, and reduces sensitivity to death from thymine deprivation (5).

These investigations were designed to examine the effect of *exr* mutations on the mucoidy associated with *lon* mutations, as well as on posttreatment reduction of UV sensitivity. The results show that *exr* mutations do not turn off the excess mucoid polysaccharide production of *lon* mutants, nor do they eliminate the posttreatment reduction of UV sensitivity.

MATERIALS AND METHODS

Bacterial strains. The strains used in the investigation are given in Table 1.

Phage. P1 *vir* and the methods used in transductions were as described by Donch and Greenberg (7).

Media. Complete broth contained tryptone, 5 g; NaCl, 5 g; yeast extract, 5 g; and glucose 1 g per liter of deionized water. Viable counts, survival curves, and filament formation studies were performed on this medium, without glucose, solidified with 2.5% Agar (Difco). Minimal medium was as described by Donch and Greenberg (7). Dilutions and liquid holding were performed in phosphate-buffered saline: KH_2PO_4, 1.09 g; Na_2HPO_4, 1.69 g; NaCl, 10 g per liter of water (pH 6.8).

Filament formation. This formation was as described by Donch and Greenberg (7).

UV survival and recovery. Preliminary determinations of UV sensitivity were performed by the streak method of Greenberg (10). To determine UV survival curves and rescue, log-phase broth cultures were membrane-filtered, washed, and resuspended in phosphate-buffered saline at a concentration of 10^6 to 10^7 cells/ml and irradiated. They were diluted and plated on complete medium, complete medium with pantoyl lactone (0.75%, w/v), and minimal medium. Photoreactivation was determined by maintaining the diluted cell suspensions in phosphate-buffered saline for 30 min in a water bath at approximately 20 C, at an average distance of 12 cm from the filament of a

TABLE 1. *Characteristics of bacterial strains used[a]*

Strain	Relevant genetic markers						Phenotype			Sources
	exr	*lon*	*rad*	*arg*	*metA*	*malB*	Fil	Mu	UV	
B	+	−	+	+	+	−	+	−	−	R. Hill
B251	+	−	+	+	+	+	+	−	−	W. Arber
B/r	+	−	−	+	+	−	−	−	+	R. Hill
Bs2	−	−	+	+	+	−	−	−	−	R. Hill
Bs7	−	−	+	+	+	−	−	−	−	R. Hill
PAM 20	−	−	−	+	+	+	−	−	−	Transduction
PAM 44	−	−	+	+	+	+	−	−	−	Transduction
AB1157	+	+	+	−	+	+	−	−	+	E. Adelberg
AB1911	+	+	+	+	−	+	−	−	+	E. Adelberg
AB1899	+	−	+	−	+	+	+	+	−	P. Howard-Flanders
PAM 9951	+	−	+	+	−	+	+	+	−	Transduction
PAM 9942	−	−	+	+	+	−	−	+	−	Transduction
PAM 5717	−	+	+	+	+	−	−	−	−	Transduction

[a] Abbreviations are as recommended by Demerec *et al.* (6). Fil[+], forming long filaments after UV irradiation; Mu[+], forming excess capsular polysaccharide at 37 C on minimal agar medium; UV[+], resistant to UV irradiation.

300-w incandescent bulb. The suspensions were then plated on complete medium. With liquid holding, the diluted suspension was maintained in the dark in phosphate-buffered saline at room temperature for 6 hr before plating on complete medium. All operations, except photoreactivation, were carried out in subdued light.

Nalidixic acid (9) was the gift of W. A. Goss, Sterling Winthrop Research Institute, Rensselaer, N.Y. It was added at a concentration of 50 μg/ml to logarithmically growing broth cultures shaken at 37 C; viability was determined at 20- or 30-min intervals.

Optical density was determined at λ 610 nm with a Bausch & Lomb Spectronic-20 colorimeter. Nalidixic acid was removed by membrane filtration in this experiment.

All figures are based on the mean of at least three experiments.

RESULTS

Effect of the exr gene on mucoid production and and filament formation. Mucoidy is a phenotypic expression of the *lon* gene, which is, however, unexpressed in strain B. The *lon* derivatives of strain K-12, AB1899 for example, or K-12 strains into which the B *lon* gene has been transduced, are mucoid (7). To determine the effect of an *exr* mutation on the mucoid phenotype of the *lon* gene, the *exr* genes of strain Bs2 and Bs7 were transduced into AB1899 as follows: the *exr* gene is cotransducible by phage P1 with *metA; metA* is cotransducible with the *arg* locus of AB1899. The *metA* strain AB1911 was used as donor for P1 phage to transduce *arg+* to AB1899; *metA* clones were identified and isolated. One of these, PAM 9951 (*lon metA*), was transduced to *metA+* by P1

grown in strain Bs2 or Bs7. All of the *metA+* transductants examined were mucoid on minimal medium at 37 C. They were screened by the rapid streak method for UV sensitivity, and 4% were found to be more sensitive to UV than PAM 9951. These were considered to have acquired the *exr* gene of the donor. PAM 9942 is a typical transductant of this kind. Filaments were not induced by UV in the transductants with increased UV sensitivity. Therefore, the *exr* gene turned off filaments but did not suppress mucoidy.

In control experiments, B251 was used to transduce *metA+* to PAM 9951. All transductants were mucoid, and of 370 none was more UV-sensitive than PAM 9951. Furthermore, when PAM 9942 was used as a donor and B as a recipient in transductions selecting for *malB+*, UV-sensitive transductants were isolated, which were as sensitive as Bs2 itself. These latter two experiments confirmed that it is the actual *exr* gene of Bs2 and Bs7 which was transduced into a K-12 recipient.

Effect of exr on UV sensitivity of a lon+ strain (AB1157) and a rad strain (B/r). In analogous transductions, the *exr* gene of strain Bs2 was transduced into strain AB1157, the *lon+* parent strain of AB1899, yielding strain PAM 5717. It was also cotransduced with *mal+* from a *mal+* derivative of strain Bs2 into strain B/r (yielding strain PAM 20) and into strain B (yielding strain PAM 44). The latter was an extra control, confirming that the properties of strain Bs2 were the result of a single mutation to *exr*. The *exr lon* strains, Bs2, PAM 44, and PAM 9942 did not form filaments after UV irradiation, whereas the

exr^+ *lon* strains B and AB1899 did. Neither strain B/r nor strain AB1157, nor their *exr* derivatives PAM 20 and PAM 5717, formed filaments after UV irradiation. The UV survival curves of the derivatives of strain B are shown in Fig. 1A, and those of the derivatives of strain AB1157 in Fig. 1B. The derivatives of strain AB1157 were more resistant than the analogous derivatives of strain B, but within each genetic background, the pattern of sensitivities was similar.

Figure 1A shows that strain Bs2 (*exr lon*) was only marginally more sensitive to UV irradiation than strain PAM 20 (*exr* transduced into B/r). In Fig. 1B, strain PAM 9942 (*exr lon*) was only slightly more sensitive than strain PAM 5717 (*exr lon*$^+$). This is in contrast to *hcr lon* double mutants that are much more sensitive to UV irradiation than *hcr* or *lon* alone (15). [Strains carrying the *hcr* gene are UV-sensitive and do not reactivate UV-irradiated phage. They are unable to excise pyrimidine dimers from their deoxyribonucleic acid (DNA), as shown by Boyce and Howard-Flanders (3).] Since the increased sensitivity of *lon* strains to UV corresponds closely to the fraction of cells induced by UV to form filaments (1) and since filaments are not formed in the presence of the *exr* gene, the relatively small increase in UV sensitivity of *exr lon* double mutants was not surprising.

The *exr* gene might prevent filament formation by directly affecting the *lon* gene, or it might act by killing potential filament formers. Evidence that the *lon* gene is actualy suppressed is shown in Fig. 1A; strain Bs2 was actually slightly more resistant than strain B to low doses of UV irradiation. Furthermore, it is possible to find treatments to which *lon* strains are sensitive but to which *exr* strains are relatively resistant. This phenomenon was first shown by Cummings and Mondale (5), who found that *exr* derivatives of *E. coli* B (*exr lon*) show almost wild-type resistance to thymine starvation, although strain B (*exr*$^+$ *lon*) itself is highly sensitive. In Fig. 2, analogous results are presented for treatment of derivatives of strain B and strain AB1157 with nalidixic acid, a specific inhibitor of DNA synthesis (9).

It would appear that in strains (strains B, AB1899) which become filamentous after UV irradiation and nalidixic acid treatment (16), the addition of an *exr* gene reduced sensitivity to nalidixic acid (strains Bs2, PAM 44, PAM 9942), but that in strains not induced to form filaments by UV irradiation (strains B/r, AB1157), the *exr* gene increased sensitivity to nalidixic acid (strains PAM 20, PAM 5717).

Finally, *E. coli* B, even unirradiated, tended to filament on transfer of a log-phase culture to complete agar medium, and only 50 to 75% of the
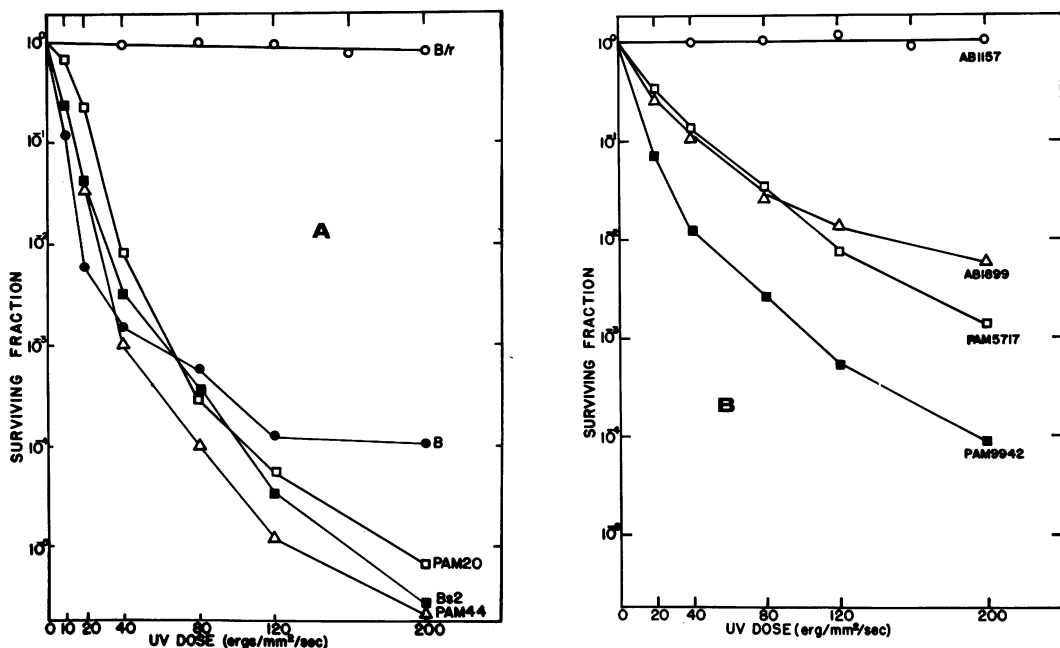

FIG. 1. *Survival after UV irradiation of exr and lon derivatives of E. coli. (A) Derivatives of strain B: B/r (○), B (●), Bs2 (■), PAM 20 (□), PAM 44 (△). (B) Derivatives of strain AB1157: AB1157 (○), AB1899 (△), PAM 5717 (□), PAM 9942 (■).*
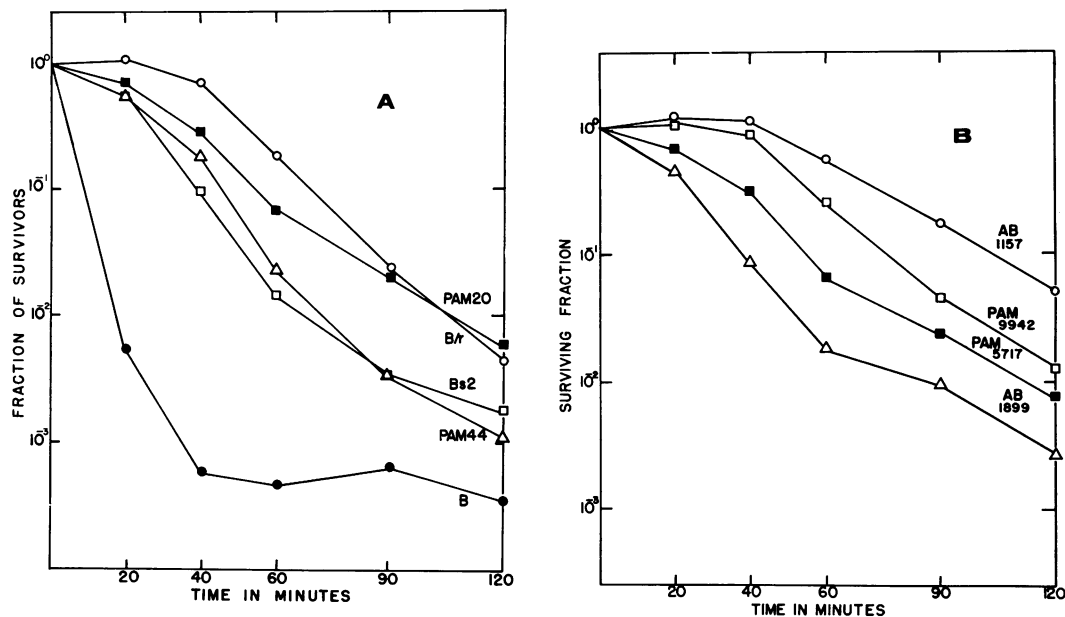
FIG. 2. *Survival of exr and lon derivatives of E. coli after growth in the presence of 50 μg of nalidixic acid per ml. (A) Derivatives of strain B: B/r (O), B (●), Bs2 (□), PAM 20 (■), PAM 44 (△). (B) Derivatives of strain AB1157: AB1157 (O), AB1899 (△), PAM 5717 (■), PAM 9942 (□).*

population might survive under these conditions. The *exr lon* strain Bs2 did not filament under these conditions, and no reduction in survival could be detected.

Effect of exr on treatments rescuing lon strains. In Fig. 3, the effects of pantoyl lactone, minimal agar, liquid holding, and photoreactivation on the survival after irradiation of strain B and its derivatives are shown. The increase in survival over control survival is plotted against control survival. Similar results were obtained within the AB1157 genetic background, with the exception that photoreactivation was not as effective in increasing survival.

The results with pantoyl lactone (Fig. 3A), plating medium response (Fig. 3B), and liquid holding (Fig. 3C) were similar. Each gave a large enhancement of survival of strain B, an intermediate enhancement of survival of Bs2 and PAM 20, and a small, sometimes negative or nonsignificant effect on survival of B/r. Thus, although the *exr* gene reduced rescue in strain Bs2, it did not completely eliminate it. The fact that strain PAM 20 underwent at least as much rescue as strain Bs2 shows that the residual rescue of strain Bs2 is related to its *exr* gene and is not a residual function of the *lon* gene. Likewise, the effect of pantoyl lactone on survival of strain B/r is not a residual property of the suppressed *lon* gene, since a similar result was obtained with the true wild-type strain AB1157.

Although results with liquid holding were broadly similar to those with plating medium response and pantoyl lactone, the results with this treatment were much more variable. Following doses of UV irradiation giving low kill, both minimal medium and liquid holding appeared to reduce survival of strain B/r.

The pattern of rescue with photoreactivation (Fig. 3D) was somewhat different from that with the other treatments. More substantial rescue of strain B/r occurred. At doses giving low kill, highly efficient rescue of strain B, Bs2, and PAM 20 occurred, whereas at doses giving higher kill, rescue of strain Bs2 and PAM 20 was greater than that of B.

Effect of exr on growth. Filaments result when growth of cells continues, but cell division is inhibited. A possible explanation for the effect of *exr* on filamentation associated with *lon* is that the *exr* gene prevented filaments by inhibiting cellular growth in the treatments described. The *exr* strains may have been growth inhibited after UV irradiation, but this was not true for nalidixic acid treatment, although the *exr* gene prevented nalidixic acid-induced filamentation. We determined growth of strain Bs2 *exr lon* by optical density during and after nalidixic acid treatment. Strain Bs2 grew as rapidly as strains B and B/r during treatment with nalidixic acid
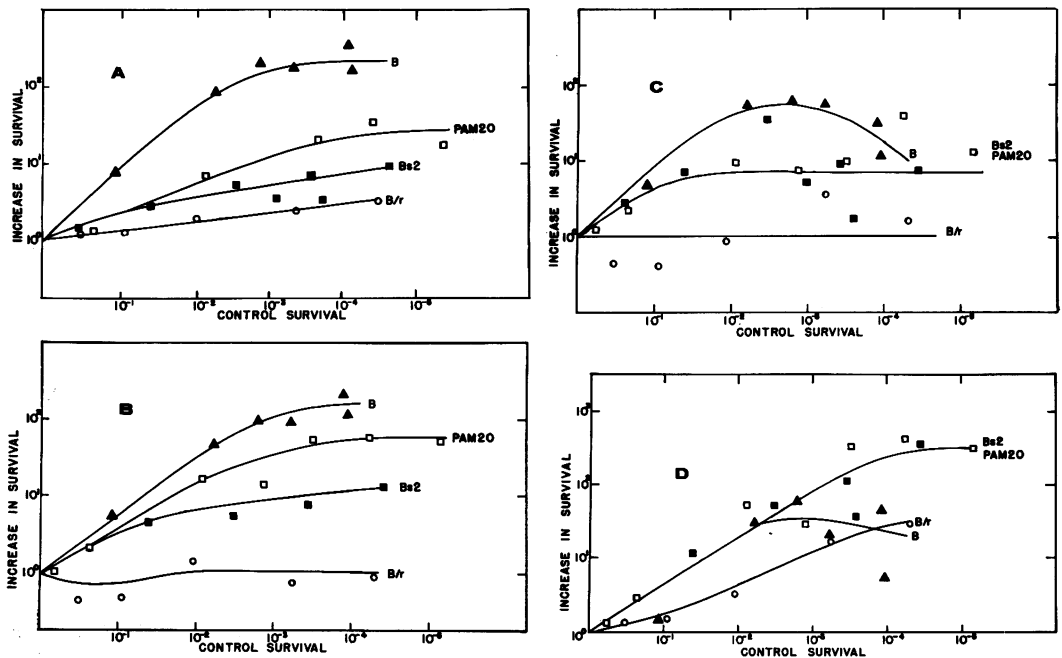
FIG. 3. *Treatments increasing survival of E. coli after irradiation. Increase in survival is plotted against the survival of the control. (A) Pantoyl lactone. (B) Minimal medium. (C) Liquid holding. (D) Photoreactivation. B/r (○), B (▲), Bs2 (■), PAM 20 (□).*

and almost as rapidly as strain B after treatment (Fig. 4). Hence, growth inhibition is not likely to account for the general effect of *exr* on *lon*.

DISCUSSION

If the *exr* gene does not prevent *lon* filament formation by simply inhibiting growth, the question of how it does exert its effect arises. Since it does not affect mucoid production, it would seem likely that it does not act directly on the *lon* lesion.

Two hypotheses to account for the properties of *lon* strains have recently been proposed by Witkin (25) and Walker and Pardee (24). Walker and Pardee have suggested that cell envelope metabolites inhibit recovery from division inhibition in *lon* and *lon*⁺ bacteria, that these metabolites are overproduced in *lon* strains, and that these metabolites may be converted to capsular polysaccharide under suitable growth conditions. Witkin has suggested that initial inhibition of cell division is a process analogous to prophage induction. Treatments such as UV irradiation may inactivate a repressor of vegetative prophage production, and, in the same way, such treatments may inactivate a repressor of an inhibitor of cell division. The *lon* strains might repress this inhibitor ineffectively. These hypotheses may prove, in part, complemen-
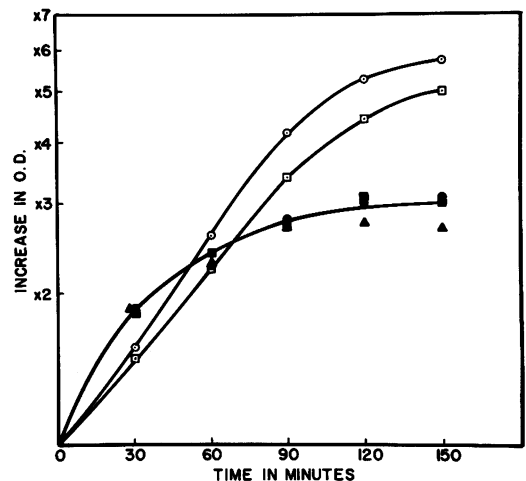

FIG. 4. *Increase in optical density of E. coli strains B (●), B/r (▲), and Bs2 (■) during growth in the presence of 50 µg of nalidixic acid per ml. Optical density of B (○), and Bs2 (□), after 20-min treatment with nalidixic acid.*

tary, since one concerns inhibition of cell division and the other reinitiation.

The salient feature of Walker's and Pardee's hypothesis is that it suggests a direct relationship

between capsular polysaccharide production and filament production. Although we have shown another instance in which these two properties can be modified independently, this would not contradict their model if the *exr* gene affected initial inhibition rather than reinitiation of cell division.

Since the *exr* gene appears to be concerned with DNA repair (26), and since the primary target of UV on *lon* sensitivity appears to be DNA (24), it would certainly seem plausible that the *exr* gene should affect initial inhibition of cell division. This would extend Witkin's analogy between prophage induction and filament formation; Brooks and Clark (4) have shown that in one class of recombinationless UV-sensitive mutants, the repressor of bacteriophage is not inactivated after UV irradiation. Moreover, there is some indication that this *rec* mutation also suppresses filament formation (18), and experiments in this laboratory indicate that *exr* strains are not inducible for prophage (J. Donch, M. H. L. Green, and J. Greenberg, *in preparation*).

If the *exr* gene does prevent inhibition of cell division, a likely corollary would be that a strain such as Bs2 would continue to divide, even under conditions where a wild-type strain was inhibited. However, we have been unable to demonstrate this.

The processes of UV sensitivity of the *lon* type, prophage induction (21), and UV-induced mutation seem closely related. In particular, all three appear to be prevented by *exr* (26) and reversed by appropriate posttreatments. It is clear from the data that the inhibition of colony formation by UV of *exr* as well as *lon* mutants is reversible by a variety of posttreatments. Witkin (26) suggests that to survive UV irradiation, *E. coli* plated on complete medium must repair and replicate its chromosome and undergo cell division within about 5 hr. It is not surprising that photoreactivation reverses the UV sensitivity of *exr* mutants, since this merely indicates that the lethal lesions are pyrimidine dimers that are split by photoreactivating enzymes in the presence of visible light (21).

Dark repair, in which pyrimidine dimers are excised together with adjacent nucleotides and are replaced by using the adjacent strand as a template, is an alternative method for removing these lesions from DNA (3, 22). Liquid holding and plating on minimal medium may act by increasing the time available for dark repair. Pantoyl lactone appears to be a specific promoter of cell division, and may increase the ability of *lon*⁺ as well as *lon* cells to reinitiate cell division within the 5-hr period. Microscopic observations have not revealed a fraction of filaments in the populations of *exr* and B/r corresponding to the fraction

rescued by pantoyl lactone and other treatments, so that it is possible that nonfilamentous cells can also be rescued.

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