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Overproduction of FtsZ Suppresses Sensitivity of *lon* Mutants to Division Inhibition

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Escherichia coli lon **mutants are sensitive to UV light and other DNA-damaging agents. This sensitivity is due to the loss of the *lon*-encoded ATP-dependent proteolytic activity which results in increased stability of the cell division inhibitor SulA. Introduction of the multicopy plasmid pZAQ containing the *ftsZ* gene, which is known to increase the level of FtsZ, suppressed the sensitivity of *lon* mutants to the DNA-damaging agents UV and nitrofurantoin. Alterations of pZAQ which reduced the expression of *ftsZ* reduced the ability of this plasmid to suppress the UV sensitivity. Examination of the kinetics of cell division revealed that pZAQ did not suppress the transient filamentation seen after exposure to UV, but did suppress the long-term inhibition that is normally observed. *lon* strains carrying pZAQ could stably maintain a multicopy plasmid carrying *sulA* (pBS2), which cannot otherwise be introduced into *lon* mutants. In addition, the increased temperature sensitivity of *lexA*(Ts) strains containing pBS2 was suppressed by pZAQ. These results suggest that SulA inhibits cell division by inhibiting FtsZ and that this interaction is stoichiometric.**

Escherichia coli lon mutants are pleiotropic and display a number of phenotypic properties, which include sensitivity to DNA-damaging agents (12, 33), mucoidy (22), decreased degradation of many abnormal (11) and some wild-type proteins (25, 27), and decreased frequency of lysogenization by some phage (28, 30). The *lon* gene product has been purified and has been shown to be an ATP-dependent protease (4, 5). The absence of this protease activity in *lon* mutants presumably results in the pleiotropic phenotype.

The increased sensitivity of *lon* mutants to DNA-damaging agents can be suppressed by mutations at two loci, *sulA* and *sulB*, which do not suppress the other *lon*-dependent phenotypes, with the possible exception of the defect in phage lysogenization (8, 9, 10, 14). Mutations at the *sulA* locus, including insertion mutations, inactivate the SulA protein, which has been shown to be an inhibitor of cell division (13, 15). The *sulA* gene is under control of the LexA-RecA regulatory circuit and is induced following DNA damage (15, 18, 29). Induction of the *sulA* gene results in an increase in the level of the SulA protein which inhibits cell division, presumably by inhibiting the FtsZ protein, an essential cell division protein (19, 20). Repair of the damaged DNA results in repression of SulA synthesis (24) and in a rapid loss of SulA in wild-type strains due to the Lon protease (25, 27). This removal of SulA allows cell division to resume, as the FtsZ-SulA inhibition is reversible (E. Maguin, J. Lutkenhaus, and R. D'Ari, submitted for publication). In *lon* mutants, SulA is more stable and its continued presence results in lethal filamentation, which is observed as an increased sensitivity to DNA damage.

sulB mutations, which map in the *ftsZ* gene (16, 19) make cells refractory to the induction of SulA. The *sulB* mutations result in an altered *ftsZ* gene product, which is slightly temperature sensitive and still active in cell division, but which no longer interacts with SulA (17, 19). Recently, we have shown that overproduction of the FtsZ protein leads to increased division potential, which is expressed as the minicell phenotype (32). In addition, we obtained preliminary evidence that increasing the level of FtsZ suppressed the sensitivity of a *lon* missense mutant to the mild SOS inducer nitrofurantoin (Maguin et al., submitted for publication). In this report we asked if increasing the level of FtsZ could suppress the sensitivity of *lon* mutants to DNA damage by overcoming the lethal filamentation caused by the increased stability of SulA.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used in this work are derivatives of *E. coli* K-12. JFL101 [*ftsZ*(Ts)], JFL110 [*ftsA*(Ts)], and TOE-1 [*ftsQ*(Ts)] were used in complementation tests to assess the genes carried by the various plasmids. These strains have been described previously (20). Strain DM511 [*lexA*(Ts)] is thermoinducible for the SOS response and has been described (26). RGC103 carries a missense mutation in the *lon* gene that has been labeled *capR9* (8). HB101 was the strain used for assessment of minicell induction by plasmids that overproduce FtsZ. JFL125, which carries the *lon-100* mutation, was constructed by P1 transduction of W3110 with P1 grown on SG20252 (F^- *lac lon-100 zba*::Tn*10 araD139 rpsL thi*, obtained from Sue Gottesman), selecting for Tet^r, and screening for mucoidy. One such mucoid isolate was cured of Tet^r to give JFL125. The plasmids used for cloning were pBR322 and pACYC184. The plasmids pZAQ carrying the *ftsQ*, *ftsA*, and *ftsZ* genes and conferring tetracycline resistance (32), pJW5 carrying the *ftsA* and *ftsZ* genes and conferring ampicillin resistance (35), and pTU302 carrying the *sulA* gene and conferring ampicillin resistance have been described (1). They are all derivatives of pBR322. Plasmid pZ4-2 contains the *ftsZ* gene and confers ampicillin resistance (34). Plasmid pBS2 was constructed by subcloning the 1.78-kilobase *Bam*HI fragment from pTU302 into the single *Bam*HI site of pACYC184. The isolation of plasmid DNA and recombinant DNA techniques were as described previously (21).

Cell growth and determination of sensitivity to DNA-damaging agents. The medium used throughout these experiments was LB broth (23). Sensitivity to UV irradiation was assessed as follows. Cells growing exponentially in LB broth were collected by centrifugation and suspended in buffer (10 mM Tris hydrochloride, pH 7.5, 1 mM $MgCl_2$). The cell

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suspension was placed in a petri dish and was exposed to UV radiation. At various intervals samples were removed and appropriate dilutions were spread on LB agar plates to determine the viable count. In experiments in which the kinetics of cell division was determined, the cells, after UV irradiation, were diluted into warmed LB broth at an optical density at 540 nm = 0.05. At various times, 100-μl samples were removed and were added to 100 μl of 20% formaldehyde. The cell number in these samples was determined with a model ZB Coulter Counter (Coulter Electronics, Inc.) with a 30-μm orifice. Sensitivity to nitrofurantoin was assessed on LB agar plates containing 2 μg of nitrofurantoin per ml.

Determination of the level of FtsZ. The level of FtsZ was determined by Western blot analysis as described previously (32). Samples (1 ml) from exponentially growing cultures (at the same optical density at 540 nm) were centrifuged in a Microfuge tube. The cell pellet was suspended and lysed by the addition of 100 μl of sodium dodecyl sulfate (SDS)-sample buffer (62.5 mM Tris-HCl, pH 6.8, 1% SDS, 10% glycerol, 5% β-mercaptoethanol) and heating at 100°C for 5 min. Samples electrophoresed on a 12.5% SDS-polyacrylamide gel were transferred to nitrocellulose overnight at 150 mA as described by Burnette (2). FtsZ was visualized by an indirect immunostaining procedure with a rabbit polyclonal antiserum against FtsZ and goat anti-rabbit immunoglobulin G antibodies coupled to horseradish peroxidase, and by the Immuno-Blot assay (Bio-Rad Laboratories).

Isolation of Tn*5* insertions in pZAQ. The Tn*5* mutagenesis of the plasmid pZAQ was carried out essentially by the procedure described by De Bruijn and Lupski (7). W3110 pZAQ was infected with λ467, and kanamycin-resistant colonies were selected. The colonies were pooled, and plasmid DNA was isolated. The plasmid DNA was transfected into strains carrying a mutation in *ftsZ*, *ftsA*, or *ftsQ*, and was screened for the loss of complementation. Plasmids which no longer complemented were further analyzed by restriction enzyme mapping for location of the Tn*5* element.

RESULTS

Suppression of *lon* by pZAQ. In a previous study we showed that introduction of the multicopy plasmid pZAQ into cells increased the level of FtsZ sevenfold (33). Subsequently, it was shown that this plasmid suppressed the sensitivity of RGC-103 (*capR9*) to nitrofurantoin (Maguin et al., submitted for publication). The presence of this plasmid also suppressed the sensitivity of JFL125 (*lon-100*) to nitrofurantoin (data not shown). The suppression of the sensitivity of *lon* mutants to UV light was also examined. JFL125 is much more sensitive to UV light than is its isogenic parent and, therefore, behaves as a typical *lon* mutant (Fig. 1). Introduction of the plasmid pZAQ into JFL125 restored UV resistance to that of the parental strain. Thus, the plasmid pZAQ completely suppressed the sensitivity of this *lon* mutant to DNA-damaging agents.

In the above experiments we assessed the ability of pZAQ to suppress the decreased viability of *lon* strains observed after exposure to DNA-damaging agents. We next wished to determine directly if pZAQ suppressed the filamentation induced by UV light in *lon* mutants. JFL125(pBR322) and JFL125(pZAQ) were grown to exponential phase and were treated with UV light. The cell number was monitored throughout the experiment, and the results are presented in Fig. 2. In both cases, cell division was inhibited by UV light, but cells containing pZAQ were able to resume division after a transient inhibition, whereas cells containing the control

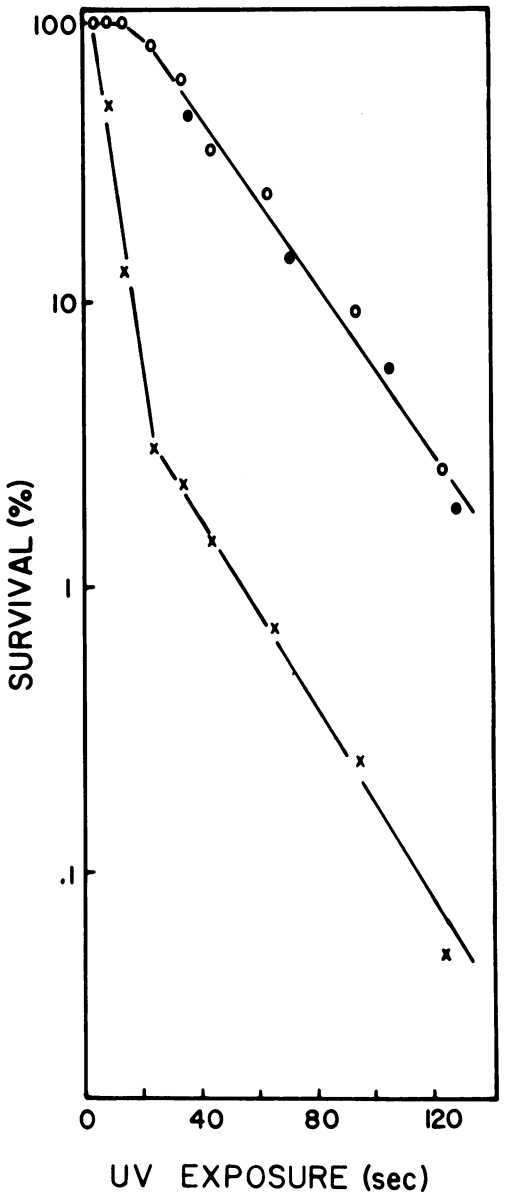

FIG. 1. Suppression of UV sensitivity of *lon*. Cultures growing exponentially in LB broth were centrifuged, and the cells were suspended in buffer. At various times after UV irradiation, samples were removed and plated on LB agar plates to determine the number of survivors. Symbols: ×, JFL125(pBR322); ○, JFL125(pZAQ); ●, W3110(pBR322).

plasmid were unable to do so. Another difference between the two strains was the extent of residual division after UV treatment. Cells containing pZAQ continued to divide for a longer time and to a greater extent than cells containing the control plasmid. Thus, the presence of pZAQ suppressed the lethal filamentation that occurs in *lon* mutants after DNA damage, but it did not suppress the transient filamentation seen after DNA damage. Transient filamentation is seen in *lon sulA* double mutants and therefore is not due to SulA (10).

Since pZAQ contains the *ftsQ*, *ftsA*, and *ftsZ* genes, and some flanking DNA sequences, we set out to determine the region of pZAQ that is essential for suppression of *lon*. Two approaches were used. In the first approach, various plas-

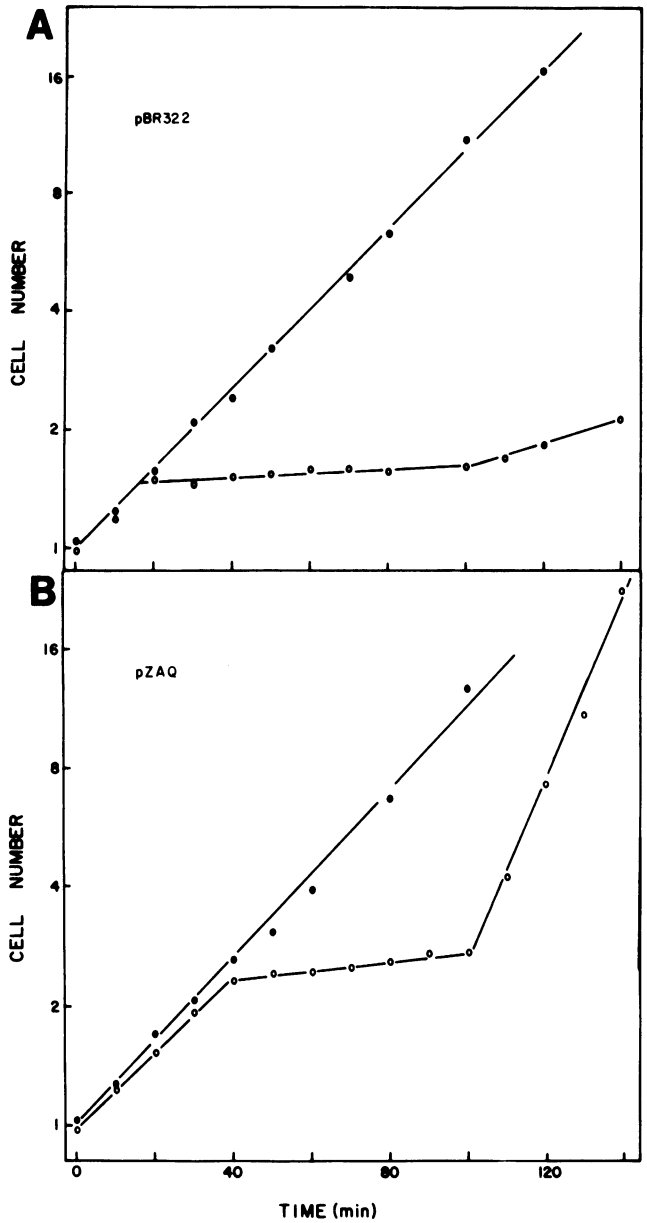

FIG. 2. Kinetics of cell division in a *lon* strain carrying pZAQ or pBR322 after UV irradiation. Exponential cultures of JFL125(pBR322) (A) and JFL125(pZAQ) (B) were centrifuged, suspended in buffer, and exposed to UV irradiation. At time zero, the cells were suspended in LB broth, and cell number was monitored with a Coulter Counter (Coulter Electronics, Inc.). Symbols: ●, no UV irradiation; ○, UV irradiation for 10 s.

mids containing inserts smaller than that present in pZAQ were examined for their ability to suppress *lon* (Fig. 3). None of these deletion derivatives of pZAQ was able to suppress nitrofurantoin sensitivity (data not shown). This suggested that the presence of all three *fts* genes was required for suppression. However, we previously showed that approximately 2 kilobases of DNA upstream of the *ftsZ* gene, including the *ftsQ* and *ftsA* genes, was required for maximal expression of *ftsZ* (35). This raised the possibility that only the *ftsZ* gene was required for suppressing *lon* and that the requirement for the region upstream of *ftsZ* was for increased expression of *ftsZ*.

In another approach, pZAQ was mutagenized with Tn*5* and the resultant plasmids screened for their ability to suppress *lon*. A map of pZAQ and the location of the insertions that were examined further are shown in Fig. 4. Plasmids carrying each of these insertions were examined for their effect on the ability of pZAQ to complement mutations in the *fts* genes. Each of these plasmids was then introduced into JFL125 to determine whether it could suppress *lon* by looking for growth in the presence of nitrofurantoin. The results obtained with these plasmids are presented in Table 1. Plasmids containing insertions within *ftsQ* (insertions 41, 14, 4, and 22) were able to completely suppress *lon*, whereas plasmids containing insertions within *ftsZ* (insertions 28 and 26) could not. The plasmids containing insertions within the *ftsA* gene had an intermediate effect. These plasmids could in fact be divided into two groups based on their effect on the plating efficiency of the *lon* strain on nitrofurantoin. The insertions that mapped distal to *ftsZ* within the *ftsA* gene (insertions 3, 12, 5, and 10) increased the EOP about 100-fold, whereas those that mapped proximal (insertions 45 and 1) had only a slight effect. On plates containing slightly less nitrofurantoin, the *lon* mutant containing any of the plasmids with an insertion within *ftsA* was able to grow, suggesting that the *ftsA* gene was not absolutely required.

The ability of the above plasmids to suppress *lon* was also assessed by examining sensitivity to UV light (Fig. 5). Again cells containing plasmids carrying insertions within the *ftsA* gene displayed a response intermediate between that of pZAQ and that of pJW9, a control plasmid lacking the *ftsZ* gene. Cells containing plasmid pJW5 showed poor suppression of UV sensitivity, about the same as those containing pZAQ45. Overall we observed that the further an insertion mapped upstream of the *ftsZ* gene, the more efficient the corresponding plasmid was at suppressing UV sensitivity. This was consistent with the hypothesis that it was the level of FtsZ that was important in suppressing *lon*, since we knew that the 2-kilobase region upstream of *ftsZ* contributes to *ftsZ* expression.

Determination of the level of FtsZ. Previously we showed that the plasmid pZAQ stimulated a sevenfold increase in the level of FtsZ (32). Additional plasmids that contained the *ftsZ* gene, but less DNA to the 5′ side of the gene, resulted in a smaller increase in the level of FtsZ. The Tn*5*-mutagenized derivatives of pZAQ should result in various levels of FtsZ, and this might correlate with their ability to suppress *lon*. The level of FtsZ induced by a plasmid can be assessed indirectly by determining the effect the plasmid has on minicell formation (32). The percentages of cells with minicell septa induced by each of the plasmids are shown in Table 1. The results show that the plasmids can be separated into groups based upon the extent of minicell formation that is induced. Those containing an insertion in *ftsZ* induced no minicell septa, those with insertions in *ftsQ* induced a high level of minicell septa, and those with insertions in *ftsA* induced an intermediate level. These results correlate very well with the ability of these plasmids to suppress nitrofurantoin sensitivity.

The relative amount of FtsZ present in JFL125 containing the various plasmids was determined by Western blot analysis (Fig. 6). Plasmids pJW2, pZAQ28, and pZAQ26 had no effect on the level of FtsZ. The latter two plasmids have insertions that inactivate the *ftsZ* gene, and pJW2 carries only the *ftsQ* and *ftsA* genes. The insertion in pZAQ26 occurs near the middle of the *ftsZ* gene, and a band of approximately 20 k was seen in the extract of cells carrying

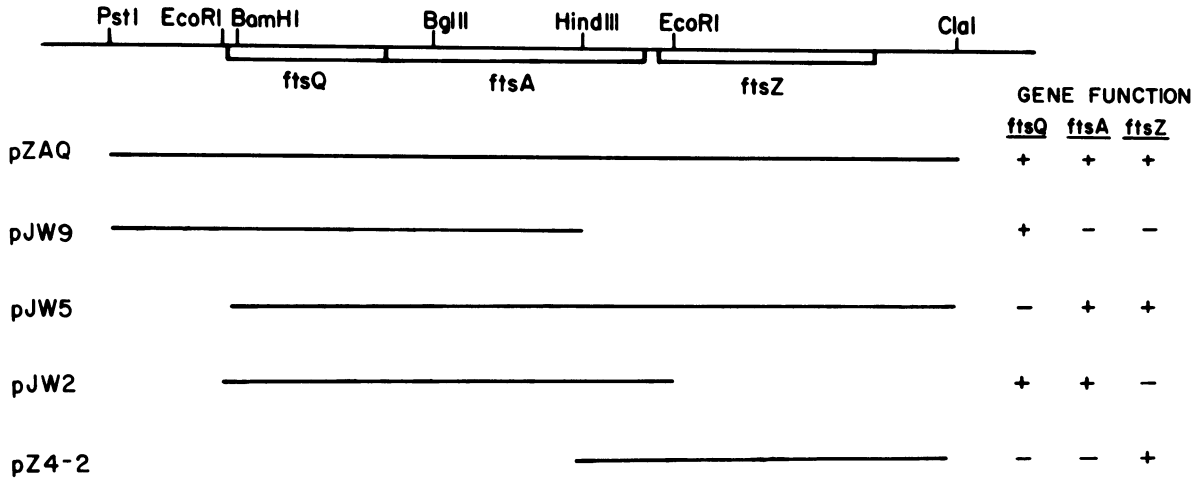

FIG. 3. Physical map of the plasmids used in this study. The insert present in each plasmid is indicated by the solid line. Gene function was determined by complementation of a strain carrying a temperature-sensitive mutation in the genes indicated.

this plasmid. Since this band is indirectly stained with the antibodies to FtsZ, it must be a truncated *ftsZ* gene product. The remaining plasmids result in higher levels of FtsZ. Plasmids pZAQ and pZAQ14 stimulate the highest levels of FtsZ, followed by pZAQ41, pZAQ45, pZAQ1, and pJW5. Plasmid pZAQ45, which has an insertion near the middle of the *ftsA* gene, is perhaps the most interesting. It appears to be on the threshold of suppressing *lon*. In the UV sensitivity test, it showed some protection (Fig. 5), and it also showed some protection in the nitrofurantoin tests, for example, if the tests are done on plates that contain reduced nitrofurantoin. Thus, suppression of *lon* depends on the test used, but increasing suppression occurs with increasing levels of FtsZ.

Multicopy *sulA* plasmid. The *sulA* gene has been cloned, and the DNA sequence has been determined (1). Multicopy plasmids that carry the *sulA* gene can be introduced into *lon*$^+$ strains but not into strains with a *lon* mutation (27). Presumably, the increased gene dosage combined with the increased stability of the SulA protein results in too high a level of SulA in strains that lack *lon*. This affords us an additional opportunity to look at the interaction between SulA and FtsZ. We first subcloned the *sulA* gene from pTu302 into a pBR322-compatible plasmid, pACYC184. The resultant plasmid, pBS2, could not be transfected into the *lon* strains RGC103 or JFL125(pBR322). However, pBS2 could be transfected into RGC103 or JFL125 containing pZAQ with the same frequency as a *lon*$^+$ strain.

We next analyzed the ability of the Tn*5*-containing derivatives of pZAQ to suppress the inability of *lon* strains to support multicopy plasmids carrying the *sulA* gene. The results of transfections in which we attempted to introduce pBS2 into JFL125 containing the various pZAQ::Tn*5* derivatives are shown in Table 1. The only derivatives that could not suppress the *lon* defect were those that contained a Tn*5* insertion within the *ftsZ* gene. JFL125 containing any of the other pZAQ::Tn*5* derivatives showed normal transfection frequencies with pBS2. Thus, increasing the level of FtsZ allowed the *lon* strain to maintain pBS2 by making the *lon* strain refractory to the increased gene dosage of *sulA*. In addition, pBS2 transfectants were selected on plates containing nitrofurantoin, which should cause a further increase in the level of SulA. Under this condition, only JFL125 containing plasmids with insertions within *ftsQ* and expressing a relatively high level of *ftsZ* could be transfected by pBS2.

Effect of a temperature-sensitive *lexA* allele. We next introduced multicopy plasmids carrying *sulA* (pBS2) or *ftsZ* (pZAQ) into DM511, which carries a temperature-sensitive allele of *lexA*. This allele codes for a repressor that has some activity at 30°C but little at 42°C (13). Cells from a culture of DM511 (pBS2) were filamentous at the permissive temperature due to poor repression of *sulA* by the *lexA*(Ts) allele. The repressor specified by this allele is impaired even at the low temperature (13). In contrast, cells from a culture of DM511 pZAQ displayed the expected minicell phenotype due to elevated FtsZ. Since the two plasmids are compatible,

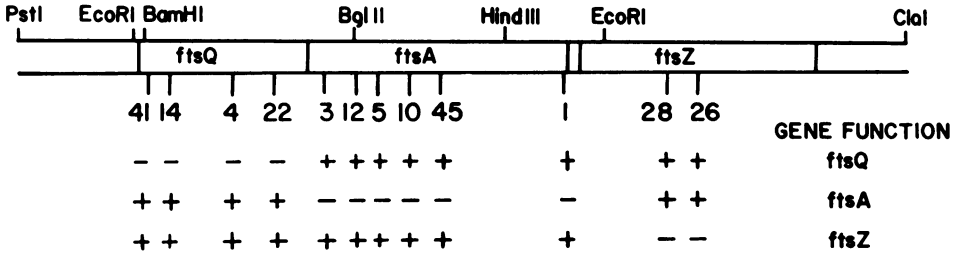

FIG. 4. Location of Tn*5* insertions in pZAQ. The bacterial DNA insert (a 4.5-kilobase *Pst*I-*Cla*I fragment) contained in pZAQ is depicted. The location of the Tn*5* in 10 different insertions was determined by restriction enzyme mapping with *Eco*RI, *Hin*dIII, and *Bgl*II. The position of the Tn*5* in each insertion is indicated by the number below the DNA fragment. Gene function was determined as described in the legend to Fig. 3.

TABLE 1. Suppression of *lon* by pZAQ and its Tn*5*-mutagenized derivatives.

Plasmid	Relative EOP on NF[a]	Minicell[b]	Transfection by pBS2[c]	
			L agar	L agar + NF
pBR322	1.4×10^{-5}	0	−	−
pZAQ	1.0	19	+	+
pZAQ28	0.7×10^{-5}	0	−	−
pZAQ26	0.2×10^{-5}	0	−	−
pZAQ1	2.0×10^{-5}	1	+	−
pZAQ45	6.7×10^{-5}	2	+	−
pZAQ10	5.9×10^{-3}	6	+	−
pZAQ5	1.5×10^{-3}	8	+	−
pZAQ12	0.8×10^{-3}	8	+	−
pZAQ3	1.6×10^{-3}	8	+	−
pZAQ22	0.8	12	+	+
pZAQ4	0.8	14	+	+
pZAQ14	1.0	10	+	+
pZAQ41	0.7	13	+	+

[a] The efficiency of plating (EOP) of JFL125 containing each of the plasmids listed was determined on plates containing 2 ug nitrofurantoin (NF) per ml.

[b] Each of the plasmids listed was transfected into HB101 by selection for Tet^r. Samples from an exponentially growing culture were then examined by phase-contrast microscopy to determine the percentage of cells that exhibited minicell septa (32).

[c] Strain JFL125 containing each of the plasmids listed was transfected with pBS2 and Cm^r selected on L agar plates with or without 2 μg of nitrofurantoin per ml. +, >200 colonies observed; −, No colonies observed.

they can be simultaneously introduced into the same strain. Cells from DM511 (pBS2) (pZAQ) displayed the minicell phenotype at the low temperature, indicating that the overproduction of FtsZ was suppressing the division-inhibitory effects of the increased level of SulA.

We next tested these strains at the nonpermissive temperature. The strain DM511 is temperature sensitive for colony formation due to SulA induction as a consequence of the thermolabile LexA repressor. A temperature of 42°C is required to demonstrate this thermosensitivity, as this strain will grow at lower temperatures. In contrast, DM511(pBS2) was much more temperature sensitive, as it was unable to form colonies at the intermediate temperature of 37°C. DM511(pZAQ) was temperature resistant and formed colonies at 42°C, demonstrating that overproduction of FtsZ could suppress increased SulA. DM511 carrying both plasmids was temperature sensitive at 42°C, indicating that the elevated level of FtsZ due to pZAQ was not sufficient to suppress the effects of this level of SulA. However, DM511(pBS2)(pZAQ) did form colonies at 37°C, indicating that the level of FtsZ supplied by pZAQ was sufficient to overcome this intermediate level of induction of SulA.

Effect on other *lon* phenotypes. Sensitivity to DNA-damaging agents is only one of the manifestations of the *lon* phenotype. We next asked if overproduction of FtsZ could suppress other aspects of the *lon* phenotype. Colonies of *lon* strains carrying pZAQ were as mucoid as colonies containing no plasmids or control plasmids. Therefore, overproduction of FtsZ suppressed the sensitivity of *lon* mutants to DNA-damaging agents but did not suppress the mucoid phenotype.

DISCUSSION

Damage to DNA in *E. coli* results in inhibition of cell division. This inhibition can result from the expression of any of three mechanisms. The best-characterized mechanism is the SulA-dependent mechanism, which involves an inducible inhibitor of cell division that is under the control of the *recA-lexA* regulatory circuit (13). *sfiC* is a second inducible inhibitor, which is present in some strains, and its expression is dependent upon *recA* but not upon *lexA* (6). Both of these inducible inhibitors appear to act on FtsZ since *sulB* mutations, which map in *ftsZ*, can suppress the inhibition of cell division brought about by these inhibitors. The third mechanism, termed the alternate pathway, is revealed when mutations are present that inactivate the two inducible systems (3, 10). This latter pathway probably results from the inhibition of DNA replication by DNA damage, which in turn would delay completion of chromosome replication and nucleoid segregation. Blocking these events may directly block cell division.

The *lon* mutants have increased sensitivity to DNA-

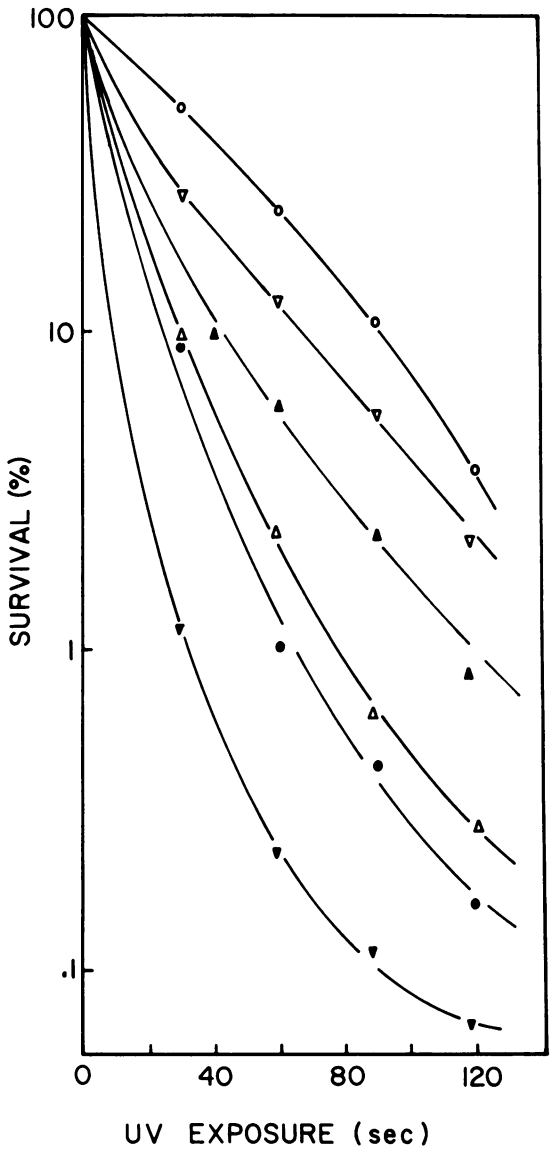

FIG. 5. Suppression of UV sensitivity of *lon*. Survival after UV irradiation was measured as described in the legend to Fig. 1. Strain JFL125 contained the following plasmids: pZAQ (○), pZAQ::14 (▽), pZAQ3 (▲), pZAQ::45 (△), pJW5 (●), and pJW9 (▼).

damaging agents such as UV irradiation and nitrofurantoin, since SulA, once induced, is fairly stable and results in a lethal filamentation (12). This irreversible filamentation (in *lon* mutants) is due to the *sulA*-dependent mechanism since *lon sulA* double mutants are UV resistant. The two other mechanisms are not affected to any great extent by *lon* mutations. Mutations in the *ftsZ* gene, termed *sulB*, also render *lon* cells UV resistant, presumably because they result in an alteration of the FtsZ protein such that it no longer interacts with SulA (17, 19). In this investigation we have shown that overproduction of the target molecule FtsZ also suppresses the sensitivity of *lon* mutants to DNA-damaging agents. Introduction of pZAQ into *lon* mutants restored UV resistance to a wild-type level (Fig. 1) and allowed *lon* mutants to grow on plates containing nitrofurantoin (Table 1). Thus, the lethal effect of increased stability of SulA caused by *lon* mutations can be suppressed by either alterations in the FtsZ protein or by overproduction of the wild-type FtsZ protein.

Various approaches were used to manipulate the levels of SulA and FtsZ in order to examine the effect on *lon* suppression and to gain further insight into the interplay between these two proteins. Advantage was taken of the observation that multicopy plasmids containing the *sulA* gene cannot be introduced into *lon* strains (27). Presumably the increased gene dosage, along with increased stability of SulA due to the *lon* mutation, results in a steady level of SulA that induces filamentation and cell death. Increasing FtsZ by introduction of pZAQ, however, allowed the introduction of a compatible multicopy plasmid containing the *sulA* gene. In addition, the *lon* strain carrying both plasmids exhibited the minicell phenotype, which is indicative of excess FtsZ (32). Since the *lon* mutant carrying both plasmids is expected to have an elevated level of SulA, and yet displays the phenotype of excess FtsZ, it is clear that a small number of SulA-FtsZ complexes does not block cell division.

The levels of FtsZ and SulA can also be manipulated in the *lexA*(Ts) strain. By introducing the *sulA* or *ftsZ* plasmid, or both, in addition to varying the temperature, the levels of the two gene products can be altered. Conditions that favor excess SulA result in inhibition of cell division, and conditions that favor excess FtsZ favor cell division. These results are consistent with SulA directly inhibiting FtsZ, and the level of free FtsZ determines if cell division is to take place.

The Tn*5*-mutagenized derivatives of pZAQ allowed us to set the amount of FtsZ within the cell at different levels and then to examine the effect this has on suppression of *lon*. The level of FtsZ caused by a given plasmid correlated with the ability of that plasmid to suppress the sensitivity of a *lon* mutant to DNA-damaging agents (Fig. 5 and 6; Table 1). In addition, the ability of the *lon* mutant to cope with a multicopy *sulA* plasmid depended upon the level of FtsZ. These results also suggest that by increasing FtsZ, the SulA inhibitor is titrated out.

Our earlier results concerning the expression of *ftsZ* led us to the conclusion that approximately 2 kilobases of DNA upstream of the *ftsZ* gene was required for maximal expression (36). Those studies were done with a single-copy phage vector. In this study we employed a multicopy plasmid vector, but we reached the same conclusion: maximal expression of *ftsZ* requires the presence in *cis* of upstream DNA that includes the *ftsQ* and *ftsA* genes. The most probable explanation is that promoters located within or upstream of these genes contribute to *ftsZ* expression.

Surprisingly, cells containing pJW5 behaved differently

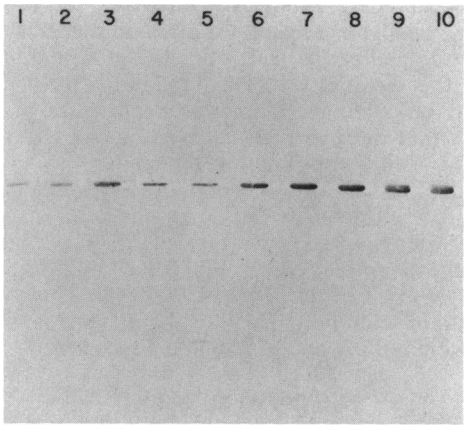

FIG. 6. Determination of FtsZ levels in strains carrying different plasmids. Samples (1 ml) of exponentially growing cultures (at the same optical density) were centrifuged, and the cells were lysed by resuspension in SDS-sample buffer. A portion of each sample was then subjected to SDS-polyacrylamide gel electrophoresis. The proteins in the gel were transferred to nitrocellulose, and FtsZ was detected by indirect immunostaining. Strain JFL125 contained the following plasmids: lane 1, pBR322; lane 2, pJW2; lane 3, pJW5; lane 4, pZAQ::28; lane 5, pZAQ::26; lane 6, pZAQ::1; lane 7, pZAQ::45; lane 8, pZAQ::14; lane 9, pZAQ::41; lane 10, pZAQ.

from cells containing the plasmids with insertions in the *ftsQ* gene. Whereas the latter plasmids were very effective at suppressing *lon*, pJW5 was very ineffective. Since these plasmids express the same genes (i.e., *ftsA* and *ftsZ*, but not *ftsQ*) this result was unexpected. Nonetheless, the slight increase in FtsZ observed with pJW5, in contrast to the relatively large increases observed with the *ftsQ*-insertion plasmids, correlates with its inability to suppress the sensitivity of *lon* to nitrofurantoin. The explanation for the different levels of FtsZ caused by these plasmids is unknown.

Examination of the kinetics of cell division after UV irradiation revealed that overproduction of FtsZ was specifically suppressing the filamentation aspect of the *lon* phenotype (Fig. 2). Inhibition of cell division after induction of the SOS response in a *lon* mutant is essentially irreversible (Maguin et al., submitted for publication); however, division in cells containing pZAQ was only transiently inhibited. Transient inhibition of cell division following UV irradiation has been previously observed in *lon sulA* and *lon sulB* strains and must be due to the alternate pathway (3, 10). Our results show that overproduction of FtsZ suppresses SulA-mediated division inhibition, but is unable to suppress the alternate pathway (*sfiC* does not appear to be present in this strain). Thus, increasing the level of FtsZ does not override the requirement for completion of chromosome replication for cell division to take place. In our investigation of the minicell phenotype caused by excess FtsZ, we also came to the conclusion that increased FtsZ cannot override the coupling of DNA replication to normal cell division (32). However, increased FtsZ can cause minicell divisions which are similar to normal divisions except for location and the requirement for completion of chromosome replication.

Increasing the level of FtsZ, like *sulB* mutations, suppressed the sensitivity of *lon* mutants to DNA-damaging agents, but did not suppress mucoidy. This suggests that the *deg* phenotype of the *lon* mutant is not suppressed and that increasing the level of FtsZ does not lead to an increase in

proteolytic activity. This is supported by the observation that, in maxicells, increasing FtsZ did not destabilize SulA, but rather stabilized it (17). Since *SulB* mutations also suppress the sensitivity of *lon* mutants to DNA-damaging agents without affecting other aspects of the *lon* phenotype, it appears that these specific alterations of FtsZ and overproduction of FtsZ are functionally similar.

The inhibition of cell division by SulA resembles the inhibition of cell division caused by the product of a gene fusion between *lacZ* and *ftsZ* (termed ZZ)(32). Inhibition by either of these proteins can be suppressed by *sulB* mutations or by an increase in the level of FtsZ suggesting a similar mechanism of inhibition. The most likely mechanism is that these proteins, SulA and ZZ, inhibit FtsZ directly.

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LITERATURE CITED

1. **Beck, E., and E. Bremer.** 1980. Nucleotide sequence of the gene *ompA* coding the outer membrane protein II* of *Escherichia coli* K-12. Nucleic Acids Res. **8**:3011–3023.
2. **Burnette, N. W.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl-sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
3. **Burton, P., and I. B. Holland.** 1983. Two pathways of division inhibition in UV-irradiated *E. coli*. Mol. Gen. Genet. **189**:128–132.
4. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon*(*capR*) protein of *Escherichia coli* K-12 Proc. Natl. Acad. Sci. USA **78**:4728–4732.
5. **Chung, C. H., and A. L. Goldberg.** 1981. The product of the *lon*(*capR*) gene is the ATP-dependent protease La. Proc. Natl. Acad. Sci. USA **78**:4931–4935.
6. **D'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156**:243–250.
7. **De Bruijn, F. J., and J. R. Lupski.** 1984. The use of transposon Tn*5* in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids—a review. Gene **27**:131–149.
8. **Gayda, R. C., L. T. Yamamoto, and A. Markovitz.** 1976. Second-site mutations in *capR* (*lon*) strains of *Escherichia coli* K-12 that prevent radiation sensitivity and allow bacteriophage lambda to lysogenize. J. Bacteriol. **127**:1208–1216.
9. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the expression of mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.
10. **Gottesman, S., E. Halpern, and P. Trisler.** 1981. Role of *sulA* and *sulB* in filamentation by Lon mutants of *Escherichia coli* K-12. J. Bacteriol. **148**:265–273.
11. **Gottesman, S., and D. Zisper.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
12. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics **49**:237–246.
13. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
14. **Huisman, O., R. D'Ari, and J. George.** 1980. Inducible *sfiA*-dependent division inhibition in *Escherichia coli*. Mol. Gen. Genet. **177**:629–636.
15. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4.
16. **Jones, C. A., and I. B. Holland.** 1984. Inactivation of essential genes, *ftsA*, *ftsZ*, supresses mutations at *sfiB*, a locus mediating division inhibition during the SOS response in *E. coli*. EMBO J. **3**:1181–1186.
17. **Jones, C. A., and I. B. Holland.** 1985. Role of the SfiB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SfiA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.
18. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
19. **Lutkenhaus, J. F.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154**:1339–1346.
20. **Lutkenhaus, J. F., H. Wolf-Watz, and W. D. Donachie.** 1980. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). J. Bacteriol. **142**:615–620.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Markovitz, A.** 1977. Genetics and regulation of capsular polysaccharide biosynthesis and radiation sensitivity, p. 415–462. *In* I. W. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press, Inc, New York.
23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Mizusawa, S., D. Court, and S. Gottesman.** 1983. Transcription of the *sulA* gene and repression by LexA. J. Mol. Biol. **171**:337–343.
25. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of SulA protein. Proc. Natl. Acad. Sci. USA **80**:358–362.
26. **Mount, D. W., A. C. Walker, and C. Kosel.** 1973. Suppression of *lex* mutations affecting deoxyribonucleic acid repair in *Escherichia coli* K-12 by closely linked thermosensitive mutations. J. Bacteriol. **116**:950–956.
27. **Schoemaker, J. M., R. C. Gayda, and A. Markovitz.** 1984. Regulation of cell division in *Escherichia coli*: SOS induction and cellular location of the SulA protein, a key to *lon*-associated filamentation and death. J. Bacteriol. **158**:551–561.
28. **Takano, T.** 1971. Bacterial mutants defective in plasmid formation: requirement for the *lon*$^+$ allele. Proc. Natl. Acad. Sci. USA **68**:1469–1473.
29. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
30. **Walker, J. R., C. L. Ussery, and J. S. Allen.** 1973. Bacterial cell division regulation: lysogenization of conditional cell division *lon*$^-$ mutants of *Escherichia coli* by bacteriophage lambda. J. Bacteriol. **113**:1326–1332.
31. **Ward, J. E., and J. F. Lutkenhaus.** 1984. A *lacZ-ftsZ* gene fusion is an analog of the cell division inhibitor *sulA*. J. Bacteriol. **157**:815–820.
32. **Ward, J. E., and J. F. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicells in *E. coli*. Cell **42**:941–949.
33. **Witkin, E. M.** 1967. The radiosensitivity of *Escherichia coli* B: a hypothesis relating filament formation and prophage induction. Proc. Natl. Acad. Sci. USA **72**:147–151.
34. **Yi, Q.-M., and J. Lutkenhaus.** 1985. The nucleotide sequence of the essential cell division gene *ftsZ*. Gene **36**:241–247.
35. **Yi, Q.-M., S. Rockenbach, J. E. Ward, and J. Lutkenhaus.** 1985. Structure and expression of the cell division genes *ftsQ*, *ftsA*, and *ftsZ*. J. Mol. Biol. **184**:399–412.