Regulation of Cell Division in *Escherichia coli*: SOS Induction and Cellular Location of the SulA Protein, a Key to *lon*-Associated Filamentation and Death

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Mutations in *sulA* (*sfiA*) block the filamentation and death of *capR* (*lon*) mutants that occur after treatments that either damage DNA or inhibit DNA replication and thereby induce the SOS response. Previous *sulA-lacZ* gene fusion studies showed that *sulA* is transcriptionally regulated by the SOS response system (*lexA/recA*). SulA protein has been hypothesized to be additionally regulated proteolytically through the *capR* (*lon*) protease, i.e., in *lon* mutants lacking a functional ATP-dependent protease there would be more SulA protein. A hypothesized function for SulA protein is an inhibitor of cell septation. To investigate aspects of this model, we attempted to construct *lon*, *lon sulA*, and *lon sulB* strains containing multicopy plasmids specifying the *sulA* gene. Multicopy *sulA* plasmids could not be established in *lon* strains because more SulA protein accumulates than in a *lon* strain. When the *sulA* gene was mutated by a mini-Mu transposon the plasmid could be established in the *lon* strains. In contrast, *sulA* plasmids could be established in *lon*, *lon sulA*, and *lon sulB* strains. The *sulA*+ plasmids caused *lon sulA* and *lon sulB* cells to exist as filaments without SOS induction and to be sensitive to UV light and nitrofurantoin. Evidence implicated higher basal levels of SulA protein in these *lon* plasmid *sulA*+ strains as the cause of filamentation. We confirmed that the SulA protein is an 18-kilodalton polypeptide and demonstrated that it was induced by treatment with nalidixic acid. The SulA protein was rapidly degraded in a *lon* strain, but was comparatively more stable in vivo in a *lon sulB* mutant. Furthermore, the SulA protein was localized to the membrane by several techniques.

The major phenotypic property of cell division mutants in *Escherichia coli* is the production of long, nonseptated filamentous cells. Why these mutant filament is a major query in the understanding of cell division in *E. coli*. One cell division mutant is the *lon* (or *capR*) mutant (1, 21, 32, 35, 39, 54). *lon* strains filament as long, nonseptated cells after treatments that damage DNA (UV irradiation, nitrofurantoin [NF]) or inhibit DNA replication (nalidixic acid, thymine starvation) or even by shifting them from minimal to complex medium (14). Most of these inducing treatments correlate with the induction of a cellular response termed the SOS response and imply that the cell division defect associated with the *lon* mutation is mediated through the SOS response. This response is characterized by enhanced capacity for DNA repair and mutagenesis, prophage induction, and inhibition of cell division (filamentation). Regulation of the SOS response involves two proteins: LexA, the repressor of the SOS inducible operons, and RecA, a protease that is activated by the SOS-inducing signal and specifically cleaves the LexA protein (reviewed in reference 29).

In addition to filamentation, *lon* strains show other phenotypic effects such as the overproduction of capsular polysaccharide, i.e., mucoidy (32, 33) and decreased lysogeny of lambda (12, 14, 55) and P1 phages (53). The *lon* mutation also affects the stability of certain abnormal (6, 18, 19, 28, 29, 36, 48) and normal (11, 16, 38, 46) polypeptides.

The *lon* gene has been cloned (47, 57), and the protein has been purified to homogeneity (9, 56). It is a tetramer (8) with subunits of 94 kilodaltons (kdal) that has multiple enzymatic activities: an ATP hydrolysis-dependent protease activity (9, 10; M. F. Charette, Ph.D. thesis, University of Chicago, 1981), DNA stimulated ATPase activity (7a), and nucleic acid-binding activity (56; Charette, Ph.D. thesis). In addition, a defective CapR protein (capR9 allele) has also been purified. The CapR9 protein has retained the general nucleic acid affinity (9, 56), but has lost the protease activity (8, 9).

In *lon* mutants, second site mutations in *sul* (suppressor of *lon*) prevent the filamentation and UV sensitivity without affecting the mucoid phenotype of the cells (14, 17, 26, 27). These mutants were isolated as UV-, nitro-NF-, or methyl methanesulfonate-resistant derivatives of *lon* strains. The *sul* mutations are located at two loci on the *E. coli* chromosome; *sulA* is near *pyrD* (22 min), and *sulB* is near *leu* (2 min). *sfiA* and *sfiB* (*sfi* for suppressor of filament induction) are identical to *sulA* and *sulB*, respectively, and were isolated as spontaneous thermoresistant revertants of *tif-1* (*recA441*) *lon* strains (15, 23, 24). The *tif-1* *lon* strains filament and die at 41°C. To account for their data, George et al. (15) proposed that a division inhibitor (product of the *sulA* or *sulB* gene?) was induced by UV and that it might be more stable in *lon* strains.

By means of a Mu (amp-lac) operon fusion that linked the structural gene for β-galactosidase (*lacZ*) to the promoter of the *sulA* operon (22; A. McPartland, J. Yamashita, and M. Villarejo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K58, p. 147), it was demonstrated that *sulA* gene transcription is under control (LexA repressor) control. This finding further strengthened the hypothesis that the cell division (septation) defect is mediated through the SOS response. The induction of *sulA* (*sfiA*) by UV irradiation was identical in *lon*+ and *lon* mutant *sfiA*: *lacZ* fusion strains, indicating that *lon* does not affect the transcription of *sulA* (22). Thus,
because the Lon (CapR) protein is an ATP-dependent protease (9, 10; Charette, Ph.D. thesis) and mutations in lon affect the stability of proteins in vivo, it has been hypothesized that SulA protein is additionally regulated proteolytically via the Lon protease.

Recently, it has been discovered that sulA is closely linked to ompA on the E. coli chromosome (38), and both sulA (38) and most of ompA (5, 20) are contained on a cloned BamHI fragment (5, 20) that has been sequenced (2). Mizusawa and Gottesman (38) showed that this BamHI fragment, which they cloned into a λ phase vector, can complement sulA mutants and that the product of the sulA gene is an 18-kdal protein. Furthermore, they demonstrated that the half-life of the SulA protein in vivo is 1.2 min in a lon+ strain and 19 min in a lon mutant (38). The implications, although not specifically mentioned, were that the CapR protease directly proteolyzes the SulA protein. Based on the DNA sequence (2), the SulA protein is expected to be 18 kdal. The DNA sequence data also confirmed the lexA gene control of the sulA gene, because a LexA protein DNA-binding consensus sequence (SOS box) was found in the promoter region of the sulA gene (2, 38), although it was not recognized at the time of the original sequencing (2).

The ability to manipulate the sulA+ gene with a plasmid enabled us to (i) identify the SulA protein as a nalidixic acid-induced, 18-kdal polypeptide by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (PAGE-SDS); (ii) confirm that the SulA protein is more stable in a lon mutant; (iii) demonstrate that the SulA protein is membrane associated; (iv) test whether SulA protein is a substrate of the Lon ATP-dependent protease; and (v) demonstrate that multicycoplasms containing sulA+ cause filamentation of lon sulA+ and lon sulB strains in the absence of an inducer of the SOS response. Furthermore, the sulA+ plasmid could not be established in a lon strain in the absence of either a sulA+ or a sulB+ mutation.

**MATERIALS AND METHODS**

**Media and reagents.** The media used have been described previously (14, 47). NF, a drug that mimics UV radiation treatment (14, 22, 57), was purchased from Sigma Chemical Co. and used at a concentration of 2 μg/ml. [35S]methionine (0.015 Ci/ml, 999 Ci/mmole) was purchased from New England Nuclear Corp. Nalidixic acid, bleomycin, and phenylmethylsulfonyl fluoride were purchased from Sigma.

**Bacteria and plasmids.** The bacterial strains used are listed in Table 1. The procedures for isolation of covalently closed plasmid DNA and for transformation of bacteria with plasmids were as previously described (47).

For these studies we obtained two sulA+ -containing plasmids from U. Henning. Plasmid pTU100 has a 7.5-kilobase EcoRI fragment of E. coli DNA encoding theompA+ and sulA+ genes cloned in the vector plasmid pSC101 (5). Plasmid pTU302 has a 1.78-kilobase BamHI fragment encoding the sulA+ gene and the NH2-terminal end of an ompA amber mutant gene (ompA31 [amber mutation at position 7 of the amino acid sequence]) cloned in the multicopy vector plasmid pBR322 (2; Fig. 1A). The fragment of the ompA amber mutant gene was cloned because earlier studies had failed to clone either the entire ompA+ gene or the same NH2-terminal fragment of the gene in pBR322, presumably because such multicycoplasms were lethal to cells (2, 5). The evidence that these plasmids indeed specify the sulA gene was initially reported by S. Mizusawa and S. Gottesman (38) and is confirmed in this paper. For most of our experiments we used the high-copy plasmid pTU302.

A mini-Mu transposon constructed by M. Casadaban was used to mutate plasmid pTU302. The mini-Mu transposon used, MudII1734, is a 11.0-kilobase derivative of the MudI phase (7). Unlike MudI it confers kanamycin resistance (Kan') instead of ampicillin resistance; however, like MudI, mini-Mu could transpose into a target gene and generate a transcriptional fusion between the target gene and the lacZ gene on the transposon (by using the lacZ ribosome-binding site). Plasmid pTU302 was transformed into strain POI1734 which contained a MuCts and Mini-Mu (MudII1734, Kan'). This strain was heat induced, and the resulting phage lysate was used to infect strain RGC103-9 (capR9 sulB9). Mutated plasmid pTU302::mini-Mu were packaged with helper functions provided by MuCts. Phage transductants that conferred both original plasmid resistance (ampicillin from pTU302) and mini-Mu resistance to kanamycin contained plasmid with mini-Mu inserts. A mini-Mu insertion into plasmid-specified sulA was isolated by further selecting an NF-resistant transductant. By restriction mapping of plasmid pTU302::mini-Mu (pRGC13) the location and orientation of the insert was determined (Fig. 1B).

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Relevant genotype</th>
<th>Derivation, source, or genotype</th>
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<tbody>
<tr>
<td>MC100</td>
<td>capR+ (lon+) sulA+ sulB+</td>
<td>R. Curtiss III (his strain X-156); leu-6 proC34 purE42 thrpE38 thi-1 ara-14 lacY1 galK2 xyl-5 mit-1 tonA23 trs-67 azi-6 rpsL109 pon supE44</td>
</tr>
<tr>
<td>RGC103</td>
<td>capR9 (lon)</td>
<td>P1 transduction of MC100 (14)</td>
</tr>
<tr>
<td>RGC103-2</td>
<td>capR9 sulA2</td>
<td></td>
</tr>
<tr>
<td>RGC103-9</td>
<td>capR9 sulB9</td>
<td>(14)</td>
</tr>
<tr>
<td>DS410</td>
<td>capR6 (lon)</td>
<td>(44) minicell producer without suppressors</td>
</tr>
<tr>
<td>Lon Min</td>
<td>capR+</td>
<td>H. Adler (11) minicell producer with suppressor</td>
</tr>
<tr>
<td>X7102</td>
<td>capR+</td>
<td>proC trp ΔlacX74; strain containing no known suppressors; J. Beckwith via D. Court</td>
</tr>
<tr>
<td>RGC123</td>
<td>capR9</td>
<td>(14)</td>
</tr>
<tr>
<td>RGC123-2</td>
<td>capR9 sulA12</td>
<td>(14)</td>
</tr>
<tr>
<td>X7102-82</td>
<td>capR82</td>
<td>Spontaneous capR mutant of X7102 (47)</td>
</tr>
<tr>
<td>JMC301</td>
<td>capR82 sulA</td>
<td>Spontaneous sulA mutant of X7102-82 obtained on nitrofurantoin (47) and mapped by P1 transduction</td>
</tr>
<tr>
<td>RGC208</td>
<td>capR+ ompA</td>
<td>K3h1 (5) resistant MC100; absence of ompA product verified by PAGE-SDS</td>
</tr>
<tr>
<td>POI1734</td>
<td></td>
<td>M. Casadaban</td>
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with approximately equal quantities of total radioactivity in each gel slot (13, 47). The preparation of cells for two-dimensional gel electrophoresis has been described previously (46), as have the details for the two-dimensional gel electrophoresis used here (3, 41–43).

**Cell fractionation procedures.** Preparation of whole cell extracts, isolation of Sarkosyl-insoluble membranes, and separation of inner and outer membranes by isopycnic sucrose gradients were as described previously (13). Membranes layered on the isopycnic gradient were obtained by either French pressure cell lysis at 16,000 lb/in² (49) or sonic disruption. The quick membrane extraction procedure with NaOH precipitation, leaving a subset of inner and outer membrane proteins in the pellet, was as described previously (45).

The four pTU302-containing strains (RGC103-2, RGC103-9, MC100, and RGC208) induced with nalidixic acid for 90 min and then labeled for 1 min were fractionated as follows. A sample was immediately fractionated by NaOH precipitation (45). Another sample was chilled and sonicated, and the envelope was separated from soluble cytoplasmic proteins by centrifugation (20,000 × g for 15 min). One half of the envelope fraction was then further solubilized with 1% Sarkosyl for outer membrane localization. The proteins were examined by PAGE-SDS and autoradiography.

**RESULTS**

Effects of high sulA gene dosage on various lon strains. We attempted to construct lon, lon sulA, and lon sulB strains containing the multicopy sulA+ plasmid, pTU302 (Table 2). The ability to establish the multicopy sulA+ plasmid pTU302 in a lon mutant might be expected to depend on whether the SulA protein is more stable in the lon cell and, if so, the phenotypic effects of increased sulA+ gene dosage (i.e., whether multiple copies of sulA are lethal to the cell). All strains were grown in minimal medium because in complex medium some lon strains tend to filament and die (14). After transformation, cells were plated on minimal agar and complex agar with carbenicillin and with or without the drug NF. NF mimics the effects of UV irradiation on lon cells; it invokes the SOS response in these cells (22), leading to cell filamentation and death (14). pTU302 transformants of the lon− strain MC100 were obtained on all media with no apparent effects of the increased sulA+ gene dosage (com-

![FIG. 1. Restriction maps of pTU302, pRGC11, and pRGC13. Cloned chromosomal genes are accentuated by the heavy line. (A) pTU302 has a 1.78-kilobase (kb) BamHI fragment encoding the sulA+ (2, 38) gene and the NH2-terminal end of an ompA amber mutant [ompA31] cloned in the multicopy vector pBR322 (2, 5, 20). An enlargement of the sulA ompA DNA is indicated with the dashed lines. (B) pTU302::mini-Mu (pRGC13) has an 11-kilobase mini-Mu insertion within the sulA gene of pTU302. The mini-Mu insertion site in sulA is noted. (C) pRGC11 contains the BamHI to HaeIII DNA fragment of pTU302 containing the sulA+ gene (---) cloned into pBR322 by replacing the BamHI-PvuII restriction fragment. Restriction enzyme codes: Rl, EcoRI; PstI, PstI; B, BamHI; H, HaeIII.](image)

### TABLE 2. Characterization of a multicopy plasmid containing sulA(pTU302) by transformation

<table>
<thead>
<tr>
<th>Recipient bacterial strain</th>
<th>Plasmid pTU302 (sulA+)</th>
<th>Plasmid pBR322 (control)</th>
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<tr>
<td><strong>Transformants per ng of DNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Minimal</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>YET</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>YET-NF2</strong></td>
<td>27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td><strong>YET-NF2</strong></td>
<td>28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> All agar media also contained 25 μg of carbenicillin per ml.
<sup>b</sup> Minimal medium is M9 without CaCl₂ and without streptomycin (57).
<sup>c</sup> YET is yeast extract-tryptone (14).
<sup>d</sup> YET-NF2 is YET supplemented with 2 μg of nitrofurantoin per ml.
<sup>e</sup> The colony size of these transformants was smaller than that of comparable transformants with pBR322.
pare with and without NF), whereas pTU302 transformants of the isogenic lon strain RGC103 were not obtained on any of the media (Table 2). Strain RGC103 was transformable with the cloning vector pBR322 on both minimal and complex media in the same experiments, although the lon strain did not survive on medium with NF as expected (57). pTU302 transformants were obtained with the lon sulA and lon sulB strains (Table 2). pTU302 transformants of the lon sulA strain (RGC103-2) grew slowly, producing small colo-
nies on both minimal and complex agar compared to pBR322-transformants. In contrast pTU302-transformants of the lon sulB strain (RGC103-9) grew almost as well as the control pBR322-transformants. No pTU302 transformants of either lon sulA or lon sulB were obtained on agar containing NF, although these strains are themselves resistant to NF (14; Table 2). Since the sulA gene is inducible by SOS-
inducing treatments (22), this sensitivity of pTU302-transformed lon sul strains to NF is consistent with the interpreta-
tion that the lethal effects of a high dosage of sulA + gene after SOS induction cannot be compensated for by a single
chromosomal mutation in either sulA or sulB.

Similar transformation results with plasmid pTU302 were obtained for another set of isogenic lon +, lon, and lon sulA strains without suppressors (X7102, RGC123, RGC123-2; Table 3).

The results of transformation with plasmids pRGC11 and pRGC13 (Table 3) show that the inability to transform into a capR (lon) strain is a result of the plasmid-localized sulA + allele. The subcloned sulA +-containing plasmid pRGC11 specifies the sulA + gene plus only the first 20 amino acids of the ompA protein (still retaining the ompA31 amber mutation at position 7 of the amino acid sequence; Fig. 1C). No pRGC11 transformants were obtained with the capR (lon) strain, as with pTU302. In contrast, mutating the sulA gene of pTU302 by inserting a mini-Mu transposon, generating plasmid pRGC13 (Fig. 1B), resulted in viable transformants of the capR (lon) strain (Table 3).

We next asked the question: can we introduce the capR9 allele by P1 transduction into a capR + strain already con-
taining pTU302. The introduction of the capR9 mutation was attempted by P1 phage transduction into a capR + (X7102) strain containing plasmid pTU302 by selecting for proC + transductants and scoring for capR9 (mucoid phenotype). In such an experiment in which 380 proC + transductants were screened, no mucoid proC + transductants of X7102 (pTU302) were obtained, whereas with strains X7102 and X7102(pBR322), 10% of the proC + transductants were mucoid. Thus it appears that even when the plasmid pTU302 is stable the introduction of a capR9 allele produces a nonacceptable combination. A further characterization of this phenomenon is presented in a later section.

Phenotypic effects of the sulA + plasmid. If the SulA protein by itself is an inhibitor of cell division and is more stable in lon strains, then the introduction of a high-copy sulA + plasmid might mimic UV irradiation or other SOS-inducing treatments by causing lon cells to filament. Since pTU302-transformants of lon mutants are not viable, we examined pTU302 transformants of lon sulA (RGC103-2) and lon sulB (RGC103-9) as well as lon + (MC100) cells, pBR322 transform-
stants of these strains were also examined. All transform-
ected strains were grown in minimal medium to the midexponential phase. As a filamentation control all such cul-
ture was also treated with nalidixic acid for 120 min to induce the SOS response and filaments. The cells were examined by phase-contrast microscopy. Even without nal-
dixic acid treatment, the pTU302-transformed lon sulA and lon sulB populations (Fig. 2B and D, respectively) contained many large filamentous cells compared with the control populations transformed with pBR322 (Fig. 2A and C).

The lon sulB mutant with (Fig. 2C) or without pBR322 (data not shown) is always elongated compared with the lon sulA mutant (Fig. 2A) in the absence of an SOS-inducing treatment when salt concentration is low (17, 26) as in minimal medium. pTU302-transformed strains treated with nalidixic acid for 120 min were not substantially more filamentous than the untreated cells (data not shown), al-
though this type of treatment is lethal. Thus, the introduction of a multicopy sulA + plasmid causes cell filamentation in lon sulA and lon sulB strains without any apparent SOS re-

Identification of the SulA protein specified by plasmid pTU302. Plasmid pTU302 was transformed into the minicell-producing strain DS410 to identify the sulA product specified by pTU302. We were also able to obtain viable pTU302 transformants of a lon (capR6) strain that produces minicells (designated Lon Min) because the capR6 mutation has residual lon function (more UV resistance) as compared with the capR9 allele (34). Minicells were isolated, labeled for 10 min at 37°C with [35S]methionine, and then diluted into cold buffer containing excess l-methionine and the protease inhibitor phenylmethylsulfonyl fluoride. A short labeling time was chosen because: (i) the SulA protein is less stable in wild-type bacterial strains (38), and (ii) the SulA protein was not detected when pTU100 plasmid-containing minicells were labeled for 2 h (2, 5). In Fig. 3, the SulA protein can be identified as the major unique band specified by plasmid pTU302 (Fig. 3, compare lanes 1 and 2). It has a molecular mass of 18 kdal. The other major polypeptide bands are specified by the cloning vector pBR322 and include the β-lactamase that migrates as 31- and 28-kdal polypeptides. We found no enhacing effects of the SOS-inducing drugs nalidixic acid or bleomycin on the expression of the sulA gene in isolated minicells (Fig. 3, lanes 1, 3, and 4). The presence of PMSF during the labeling period did not increase the relative amount of SulA protein (Fig. 3, lanes 1 and 5). In addition, the SulA protein was synthesized in Lon Min minicells containing pTU302 (Fig. 3, lanes 6 and 7) and in DS410 minicells containing the pTU100 plasmid (Fig. 3, lane 8), which also specifies the complete OmpA protein, protein II*. This latter protein is visible (33 kdal) along with a slightly larger polypeptide that is the precursor of protein II* (20).

The Lon Min(pTU302) strain shows several additional polypeptide bands including two polypeptides with molecu-
lar masses of approximately 29 kdal (just above the 28-kdal β-lactamase polypeptide) and 24 kdal that are not visible in DS410(pTU302) (Fig. 3, compare lanes 5 and 6). They are

**TABLE 3. Transformation ability of sulA plasmids into capR mutants**

<table>
<thead>
<tr>
<th>Recipient bacterial strain</th>
<th>Transformation ability*</th>
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<tbody>
<tr>
<td></td>
<td>pTU302</td>
</tr>
<tr>
<td>X7102 (capR +)</td>
<td>+</td>
</tr>
<tr>
<td>RGC123 (capR9)</td>
<td>−</td>
</tr>
<tr>
<td>RGC123-2 (capR9 sulA12)</td>
<td>+</td>
</tr>
</tbody>
</table>

* All agar media contained 25 μg of carbenicillin per ml.

+ + , Approximately 100 transformants per ng of DNA; +, approximately 25 to 50 transformants per ng of DNA; −, no transformants obtained. Plasmid transformation was done with minimal medium-grown and plated cells (see Table 2, footnote b).
polypeptides resulting from suppression of the amber allele of the ompA cloned fragment because (i) the Lon Min strain contains a suppressor (11), (ii) the polypeptides are not visible in the control, Lon Min(pBR322) (Fig. 3, lane 7), and (iii) Bremer et al. reported the appearance of polypeptides with apparent molecular masses of 30 and 24 kdal in the envelopes of pTU302-transformed supD or supF strains that were absent in nonsuppressing strains (5). They also provided evidence that the larger polypeptide is a precursor of the 24-kdal polypeptide (5).

Identification of the SulA protein in whole cells. The identification of the SulA protein specified by pTU302 in minicells provided a standard to use to identify the SulA protein in whole cells. Since the sulA promoter is SOS inducible (22), SulA protein should be in high amounts in cells treated with nalidixic acid (an SOS-response inducer). We initially chose plasmid transformants of the lon sulB and lon sulA strains as the best strains for observing SulA protein because of previously cited evidence suggesting that this protein is more stable in a lon background. An exponentially growing culture in minimal medium was treated with nalidixic acid for various lengths of time between 0 and 4 h. Samples of the cultures were labeled with [35S]methionine for 10 min. Cell preparations were then examined for SulA protein, as identified by comparison with the labeled minicell preparation (Fig. 4, lane 10). SulA protein was not detected in the absence of nalidixic acid treatment of lon sulB or lon sulA strains containing the sulA+ plasmid pTU302 (Fig. 4, lanes 5 and 6; Fig. 5, lane 7). However, it was visible in these strains after the 1 h of nalidixic treatment (Fig. 4, lanes 7 through 9, lon sulB(pTU302); Fig. 5, lanes 8 and 9, lon sulA(pTU302)). SulA protein was also visible after nalidixic acid treatment of the lon sulB strain containing the other sulA+ plasmid, pTU100 (Fig. 4, lanes 11 through 14). The OmpA protein (prominent band between 24 and 45 kdal) in

FIG. 2. Effect of multicopy sulA+ plasmid on morphology of bacterial mutants grown in minimal medium. (A) RGC103-2 (lon sulA) with cloning vector pBR322; (B) RGC103-2 with sulA+ plasmid pTU302; (C) RGC103-9 (lon sulB) with pBR322; (D) RGC103-9 with pTU302.

FIG. 3. Polypeptides synthesized in plasmid-containing minicells and separated on a 15 to 30% polyacrylamide gradient in SDS. Lanes: 1, DS410(pTU302); 2, DS410(pBR322); 3, DS410(pTU302) treated with nalidixic acid (40 µg/ml) for 30 min before [35S]methionine labeling; 4, pTU302 treated with bleomycin (50 µg/ml) for 30 min before labeling; 5, DS410(pTU302) labeled in the presence of the protease inhibitor phenylmethylsulfonyl fluoride (200 µg/ml); 6, Lon Min (pTU302); 7, Lon Min (pBR322); 8, DS410 (pTU100).
SuLA protein was also observed, albeit to a lesser extent, in the nalidixic acid-treated *lon sulB* haploid strain containing only the control plasmid pBR322 (Fig. 4, lanes 2 through 4; Fig. 5, lanes 2 and 3). This observation allowed us to examine a nalidixic acid-induced, haploid *lon sulA* strain for either lack of SuLA protein or an altered SuLA protein. Although an SOS response appears to have occurred, since newly induced proteins were visible (Fig. 5, arrow), no SuLA protein was induced in this strain (Fig. 5, lanes 4 through 6). The fact that a *sulA* mutant strain lacks an inducible 18-kdal polypeptide supports the identification of the 18-kdal polypeptide as the *sulA* gene product. Whether this *sulA* mutant produces a truncated polypeptide is unknown, as we did not resolve any smaller inducible polypeptide.

**Stability of SuLA protein in vivo.** To examine the hypothesis that the SuLA protein is more stable in a *lon* background, we induced with nalidixic acid and labeled the SuLA protein in a *lon* + strain containing the *sulA* + plasmid pTU302.

By decreasing the labeling time to 1 min, the SuLA protein was indeed induced and amplified in the *lon* + strain (Fig. 6, lane 5). Furthermore, when the labeled SuLA protein in the *lon* + strain was chased for 5 min by the addition of medium containing an excess of L-methionine, very little SuLA protein was detected on the autoradiogram, and none was visible after a chase of 20 min. In contrast, a significant amount of SuLA protein was detectable in the *lon sulB* (pTU302) strain that was labeled for 1 min and chased for up to 20 min (Fig. 6, lanes 2 and 3; in the original autoradiogram the SuLA protein was visible up to 40 min). Thus SuLA protein is very unstable in a *lon* + strain as compared with a *lon* mutant.

**Cellular location of the SuLA protein.** The ability to induce SuLA protein with nalidixic acid in pTU302 transformants of the *lon sulB* strain allowed us to examine the location of the SuLA protein. Cells [RGC103-9(pTU302)] were induced with...
nalidixic acid for 60 to 90 min, and then total proteins were labeled with $[^{35}S]$methionine for 10 min. The cells were sonically disrupted, and the envelope was separated from soluble cytoplasmic proteins by centrifugation at 50,000 $\times$ g for 30 min. The majority of the SulA protein remained with the envelope fraction (Fig. 7, lanes 1 and 2). The labeled cell envelope was further fractionated by the classical membrane procedure of isopycnic sucrose gradient centrifugation (Fig. 8). In this procedure the outer membrane equilibrates at a density of 1.22 g of sucrose per ml, and the inner membrane equilibrates at a density of 1.17 to 1.16 g of sucrose per ml. Soluble proteins float on the top of the gradient. Electrophoresis of the labeled protein from such a membrane fractionation is shown in Fig. 7. Labeled SulA protein is found in the outer membrane fraction (Fig. 7, lanes 3 and 7). A small amount of SulA protein relative to the other labeled proteins was also found in the inner membrane fraction and with the proteins floating on top of the gradient (Fig. 7, lanes 4 through 6).

Nalidixic acid-induced and labeled RGC103-9(pTU302) cells were also fractionated based on alkali (NaOH) sedimentation. Proteins derived from the inner and outer membrane remain insoluble for the most part (45). Most proteins partition unambiguously (45). A large portion of SulA protein was NaOH precipitable; however, some SulA protein was also in the supernatant fraction. In no case was there less than 50% of the SulA protein in the precipitable fraction. The 18-kdal $[^{35}S]$-labeled protein band identified in both the precipitable and supernatant fractions of RGC103-9(pTU302) is unstable, since that protein band decreased compared with other protein bands after a 20-min chase (Fig. 9). This is consistent with the increased lability of the SulA protein even in a capR mutant.

SulA protein localization in the envelope was additionally confirmed in three other pTU302-containing strains (RGC103-2 [lon sulA], MC100 [lon+], RGC208 [ompA lon+]). These plasmid-containing strains were induced with nalidixic acid for 90 min and then labeled with $[^{35}S]$methionine for 10 min. The cells were disrupted as described in the text.
nine for 1 min. Approximately equal amounts of SulA protein were found in the NaOH-soluble and insoluble fractions.

Is SulA protein a substrate of the Lon ATP-dependent protease? The simplest current model of lon-sulA interaction predicts that SulA protein is a substrate of the Lon protease. Although the SulA protein was localized to the cellular membrane, we still tested its susceptibility to the soluble Lon protease in vitro. As described above, we prepared a cell envelope fraction that was enriched for the SulA protein. The envelope fraction was suspended in buffer with or without 0.3% Triton X-100 and then added to an assay along with the purified Lon protease and ATP. In the assay with Triton X-100 the final concentration was 0.03%. As a control, the envelope preparations were mixed in equal protein concentration with a lon cell extract containing radioactive protein a, an acidic 11-kdal polypeptide. This protein is degraded in a similar in vitro assay upon the addition of the purified Lon protease and ATP (46).

The assays were incubated at 37°C for 90 min, and then proteins were analyzed by two-dimensional gel electrophoresis. Only protein a (the control) was detectably degraded (data not shown). In other experiments with one-dimensional PAGE-SDS, the SulA protein that was present in the soluble fraction was also not degraded by the Lon protease, although these conditions would support proteolysis of casein by Lon protease. Since the SulA protein was not degraded under conditions in which a protein a and casein were, we concluded that SulA protein may not be a direct substrate of Lon protease. Alternatively, other conditions than we have tested may be required for Lon protease to degrade the SulA protein in addition to factors necessary for protein a or casein digestion (i.e., modification of the SulA protein, unidentified small molecule cofactors, or other protein or nucleic acid factors or absence of inhibitors). Further studies are required to decide among these possibilities.

Why can you establish the multicopy sulA + plasmid in a lon sulA strain, but not in a lon sulA + strain, when the amount of wild-type SulA protein would be expected to be approximately equivalent in the two strains? Five possibilities were considered.

Since some sulA mutations are dominant (12, 27) it is possible that a putative chromosomal SulA mutant protein is anticomplementing (8) some of the plasmid-coded SulA wild-type protein, thereby modifying the lethal effects of multiple copies of the latter. We tested seven other independent sulA mutations in two lon backgrounds (derivatives of MC100 and X7102), and all yielded transformants with pTU302. If we had found one sulA allele giving no transformants the data would have supported this interpretation. This alternative would require the unlikely assumption that a chromosomal (single-copy) allele be dominant to a wild-type allele present in multiple copies.

Another alternative is that when pTU302 (sulA +) is transformed into a capR9 sulA strain the transformants that survive are in fact homogenotes of the mutant sulA allele that arose by recombination. This explanation seems unlikely from the following data: the transformation frequency of pTU302 into the capR9 sulA2 strain is identical to that obtained with the capR9 sulB9 strain (Table 2). Furthermore the phenotype of the capR9 sulA2(pTU302) (sulA +) is easily distinguished from the capR9 sulA2(pTU302):mini-Mu(sulA inserted); the former is filamentous (Fig. 2), grows poorly on minimal medium, and is NF sensitive (Table 2), whereas the latter grows well on minimal medium and is NF resistant.

Another alternative is that chromosomal sulA mutants exert a polar effect on the following adjacent ompA gene, which is transcribed in the same direction (Fig. 1). We prepared ompA mutants by bacteriophage K3h1 selection (5) and verified that they were missing the OmpA protein membrane. When a capR9 allele was transduced into such a strain it was mucoid and NF and UV sensitive. They would have been UV resistant if sulA mutants caused UV resistance simply by blocking OmpA protein synthesis. Furthermore, pTU302 transformants could not be established in such strains. Therefore, the chromosomal ompA + allele was not required for the lethal events that occur after an SOS inducing treatment (UV) of the lon strain or that follow transformation of the lon strain with sulA + plasmid pTU302.

A fourth alternative is that there is greatly increased synthesis of the SulA (wild-type) protein in a hypothetical capR9 sulA + (pTU302) strain (resulting in lethality) as compared with a capR9 sulA12(pTU302) strain or a capR9 (pTU302) strain. We examined this possibility (indirectly) by using the plasmid pRG13 (pTU302:mini-Mu), in which β-galactosidase is transcribed from the sulA promoter. Plasmid pRG13 was transformed into the capR9 sulA12 (RGC132-2), capR9 sulA + (RGC123), capR82 sulA + (X7102-82), and capR9 + (X7102) strains. These strains are isogenic and suppressor free, and the entire lac operon is deleted from the chromosome. The specific enzymatic activity of β-galactosidase in each strain containing pRG13 was measured in the steady state (growing exponentially in minimal glucose medium supplemented with 0.5% Casamino Acids). The specific enzymatic activity in strain X7102 [capR9 + (pRG13)] was 106 U/mg of protein at 28°C under the conditions and calculations of Miller (37). The relative activities were as follows: capR9 + (pRG13), 1.0; capR9 sulA12 (pRG13), 4.0; capR9 sulA + (pRG13), 2.0; capR82 (pRG13), 1.6. There is a higher differential rate of synthesis of sulA promoter-controlled β-galactosidase in the capR9 mutants compared with the capR wild type. However, according to the stated hypothesis, the capR9 sulA + strain should have been higher than the capR9 sulA12 strain to account for lethality, and the reverse is the case. This difference; thus this hypothesis is not supported by the data. Incidentally, the differences in β-galactosidase activity reported here between capR9 + and capR9 strains are likely to reflect a difference in plasmid copy number since Huisman and D’Ari reported that with β-galactosidase inserted into the chromosomal (single-copy) sulA gene there is no difference in β-galactosidase synthesis in capR9 + and capR mutants (22).

Since none of the above hypotheses is supported by the data, another alternative we consider is the following. Another unknown protein increases in capR9 sulA mutants (in contrast to the capR9 sulA + strain) that somehow overcomes the lethality caused by plasmid-coded copies of sulA +. Further understanding of the molecular function of the SulA protein is required before it would appear worthwhile to investigate such an hypothesis.

**DISCUSSION**

Previous sulA-lacZ gene fusion studies showed that the sulA gene is transcriptionally regulated by the SOS response system (lexA/relA) (22). Our results demonstrate that SulA protein synthesis is induced by nalidixic acid. Thus, the SulA protein is induced along with the products of the other SOS genes (uvrA, uvrB, umuC, lexA, recA, etc.) under LexA repressor control (29). A hypothesis to explain the lon cell
division defect would involve the SOS response (22, 29), the sulA gene, and the SulA protein's increased stability in a lon mutant (38); i.e., after SOS induction (UV irradiation, nalidixic acid), even the Lon protein negatively regulates the concentration or activity (or both) of the SulA protein through proteolysis, and the SulA protein inhibits septation.

The question of whether multicopy sulA plasmids could be established in lon strains might be expected to depend on the effects of increased cellular concentration of the SulA protein. We found that multicopy sulA plasmid transformants could not be established when we attempted to transform lon strains, but were established in lon sulA and lon sulB strains. The data indicated that the lethal effect of increased sulA gene dosage in a lon background can be overcome by either a sulA or sulB mutation, implicating sulB as well as sulA in the lethal events. An examination of the cells of either the lon sulA or the lon sulB strains with the multicopy sulA plasmid showed that they were filamentous without any apparent induction of the SOS response. The filamentation of the lon sulA and lon sulB strains containing the sulA plasmids is very likely the result of increased basal levels of SulA protein in such strains. We measured the basal level of sulA expression by plasmid localized sulA fused to β-galactosidase (plasmid pRGC13 [sulA::lacZ]). lon strains expressed 1.6 to 4 times as much β-galactosidase as did a lon control. The wild-type β-galactosidase is stable in lon and lon strains. We attribute the difference in β-galactosidase to higher plasmid copy number of lon compared with lon strains (see above). The increased stability of the SulA protein in lon strains (38; present results) would be expected to exaggerate the difference in the cellular concentration of SulA protein and could account for the filamentation observed in lon sulA and lon sulB strains and the inviability of the lon strain containing sulA plasmids.

We were able to detect the SulA protein in whole cells of lon sulA and lon sulB strains containing a multicopy sulA plasmid after nalidixic acid induction. We confirmed the finding of Mizusawa and Gottesman (38) that the SulA protein was indeed unstable in the lon strain when compared with the lon sulB mutant in our studies. The detection of the SulA protein in the lon strain was dependent on induction of the SOS response and a short, 1-min, [35S]methionine pulse-labeling. Our ability to detect the SulA protein in a haploid lon sulA strain, but not in a haploid lon sulA strain, provided additional evidence that the 18-kdal polypeptide was indeed the SulA protein.

The instability of the SulA protein in lon as compared with lon mutant strains (Fig. 6) and the rarity of specific protein instability (39, 46) strengthens the view that the SulA protein may function chiefly as an inhibitor of septation in connection with the SOS response. There is at least one other protein that is a candidate for an inhibitor of cell division (but not of septation) that is not controlled by the SOS response, but is under apparent direct control of the Lon ATP-dependent protease (46).

A further observation that the SulA protein fractionates with the membrane, although up to half has also been found in soluble form. Furthermore, SulA protein is associated with the outer membrane by isopycnic sucrose centrifugation and, by the NaOH technique, with the membrane; the latter technique does not distinguish between inner and outer membrane proteins, but has the great advantage that the cells do not have to be broken before the fractionation (45). In other studies the membrane-associated SulA protein was insoluble in Sarkosyl (data not shown), which is usually taken as a definitive way of localizing outer membrane proteins. From the above results we might expect the SulA protein to contain a signal sequence. There is only one open reading frame in the DNA containing the sulA gene (2; Fig. 1) that could code for an 18-kdal polypeptide. Since the SulA protein molecular mass is 18 kdal (38; present results), that DNA sequence must code for the SulA protein. Other genetic data support his conclusion (38). However, there is no apparent N-terminal amino acid signal sequence specified by that sulA gene nucleotide sequence (2). The pulse-chase experiments on the instability of the SulA protein in the membrane and soluble fractions (NaOH technique) demonstrated that SulA protein was unstable in both fractions (Fig. 9). Nevertheless, there was no indication of larger intermediates in the experiments of Fig. 9 or in those where the labeling time was as short as 1 min. Thus we consider it necessary to leave open the cellular site at which the SulA protein functions.

The SulA protein may act in the membrane or soluble fraction to inhibit septation. The sulB gene has recently been reported to be an allele of ftsZ (30). Only temperature-sensitive lethal mutants of ftsZ have been described previously, and these form nonseptate filaments at high temperature and die (31). Thus the sulB (ftsZ) gene is an essential gene. The SulA protein could be functioning by inhibiting the synthesis of the SulB protein (SulA protein as a soluble repressor) or by inhibiting the activity of the SulB protein (SulA protein in the soluble or membrane fraction). Although the SulB protein is a candidate for a protein whose synthesis or activity is inhibited by the SulA protein, others are not excluded and are equally possible, for example, penicillin-binding protein 3, a membrane-localized transpeptidase and transglycosylase (25) also known to be required for septum formation (4, 50, 52).

A level of excess SulA protein that results in cell lethality was achieved in both lon sulA and lon sulB strains when pTU302 (sulA) transformants were plated on nitrofurantoin (Table 2). Under the same plating conditions the plasmid was not lethal in a lon strain. The absence of lethality in the lon strain is best explained if the lethal excess of SulA protein (which accumulates in the lon sulA and lon sulB mutants because of the nitrofurantoin-induced SOS response and multiple copies of the sulA gene) never accumulates because it is destroyed, directly or indirectly, by the Lon ATP hydrolysis-dependent protease in the lon strain. We have not excluded the possibility that other proteins induced by the SOS response might also be proteolysed by the Lon protease and could play a role in the multicopy sulA gene-dependent lethality in the absence of the Lon protease.

It may be that the lon and sulA gene products are involved in inhibiting cell division only during the SOS response and are not involved in normal cell division; the isolation of chromosomal Mu d(amp-lac) insertions in the sfiA (sulA) gene (22; McPartland et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K38, p. 147) argues that the SulA protein is dispensable; similarly, the absence of the Lon protein in some mutants (46) suggests that Lon is not absolutely dispensable. Alternatively, both the SulA and Lon proteins may participate in normal cell division, but have backup mechanisms that substitute for them as is the case with other crucial cellular processes (40). In either case it seems likely that the SulA protein is interacting to inhibit synthesis or activity of protein(s) involved in normal septation (during the SOS response) and should therefore be a useful tool to further study the biochemistry of normal cell division.
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LITERATURE CITED


