Reversibility of SOS-Associated Division Inhibition in *Escherichia coli*

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In *Escherichia coli* the SOS response, induced by DNA-damaging treatments, includes two systems of cell division inhibition, SfiA and SfiC, which are thought to prevent cell division by interacting with the division protein FtsZ. It is shown here that SfiA-mediated division inhibition is readily reversible, even in the absence of de novo protein synthesis, suggesting that functional FtsZ molecules can be recovered from SfiA-FtsZ complexes. The action of SfiC, on the other hand, is essentially irreversible; induction by expression of the recA (Tif) mutation for 60 min results in division inhibition that continues for at least 180 min after the end of the induction period. An excess of the presumed target molecule FtsZ, furnished by a multicopy plasmid, suppresses the action of SfiA but not SfiC. Simultaneous induction of SfiA and SfiC results in irreversible division inhibition, showing that SfiC is epistatic to SfiA. The irreversibility of SfiC action is most readily accounted for by assuming that the SfiC product, unlike SfiA, is stable. The reversibility of SfiA action is slowed in a *lon* mutant, in which the SfiA protein is partially stabilized. From the kinetics of division resumption in the absence of protein synthesis, we estimated the in vivo half-life of the SfiA protein to be 10 min in a *lon* strain and 170 min in a *lon* mutant.

Perturbations of DNA replication in *Escherichia coli* induce the SOS response, a pleiotropic set of manifestations that includes a rapid inhibition of cell division with the formation of aspartate filaments (30). The induction of this response occurs through activation of the RecA protein, which, under conditions of replication perturbation, acquires the ability to mediate proteolytic cleavage of the LexA protein, repressor of most SOS functions (15, 26). Induction can also be observed without replication perturbation in strains carrying the *recA441* (Tif) mutation, which results in spontaneous activation of the RecA protein at high temperatures, or a *lexA(Ts)* mutation, which makes the LexA repressor thermolabile (15, 26, 30).

Among the SOS functions is a division inhibitor, the SfiA (or SulA) protein. The *sfiA* gene is directly repressed by LexA (21), and a high concentration of SfiA protein is sufficient to block cell division (11). The SfiA division inhibitor is an unstable protein which is partially stabilized in the absence of the Lon protease (22, 24). Several lines of evidence suggest that its target of action is the FtsZ protein, which is thought to play a role in cell division (6, 17, 23, 27–29): (i) *sfb* (or *sulB*) mutations, located in the *ftsZ* gene (12, 16), prevent the action of SfiA (4, 5) without affecting its induction (8), and (ii) the SfiA protein is stabilized in the presence of an excess of FtsZ protein, but not of FtsZ (SfiB) mutant protein (13). This suggests a direct SfiA-FtsZ interaction.

A second SOS-associated system of division inhibition was discovered when certain *recA* (Tif) *sfiA* strains were observed to stop dividing at high temperatures. This Tif-induced SfiA-independent filamentation is due to the *sfiC* locus (3). By analogy with *sfiA*+, strains that exhibit this filamentation were designated *sfiC*+, although the nature and activity of the *sfiC* gene product are unknown. SfiC-dependent division inhibition is regulated positively by RecA, because it is induced by *recA* (Tif) expression; but unlike SfiA, it is not induced in *lexA(Ts)* strains and thus is not negatively controlled by *lexA* (3). We previously suggested that it may be part of a defective prophage (3); recent results have shown that it is part of the excisable element ε14 (E. Maguin, R. D’Ari, H. Brody, and C. Hill, manuscript in preparation). SfiC-dependent division inhibition is also suppressed by *ftsZ* (SfiB) mutations (3), suggesting that SfiA and SfiC have the same target of action, FtsZ.

SfiA- and SfiC-dependent division inhibition differ in their effect on cell survival. After 2 h of Tif expression in liquid medium, SfiA causes essentially no loss of viability, whereas the SfiC mechanism allows only 0.1% survival. In addition, inactivation of the Lon protease decreases this survival 20,000-fold for SfiA but only 13-fold for SfiC (3). This difference may reflect an intrinsic stability of SfiC, even in *lon*+ strains.

In this study we examine the reversibility of SfiA- and SfiC-dependent division inhibition. The results indicate that SfiA action is fully reversible because essentially all divisions inhibited by SfiA can take place when SfiA is removed. Furthermore, this reversibility does not require protein synthesis, suggesting that active FtsZ molecules can be recovered from FtsZ-SfiA complexes. SfiC action, on the other hand, is not reversible.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** All strains used in this study were derivatives of *E. coli* K-12 and are described in Table 1. GC2597 is a Tet'-tif+ transductant of a Kan'-Str+ recombinant of GC2472 obtained from a cross of GC2472 and GC2465 (3). JFL 126 is a lon-100 Tet' derivative of DM511 constructed by P1 transduction.

The plasmid pGC165 *sfiA*+, carrying the *sfiA*+ gene downstream of the *lac* promoter and conferring ampicillin resistance, has been described previously (11). The plasmid pZAQ is a 4.5-kilobase-pair *PstI-ClaI* fragment carrying the *ftsZ*,

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fisA, and fisQ genes cloned into pBR322 (29). Techniques for rapid plasmid DNA extraction and transformation were carried out as described previously (18).

Media. M63 medium (20) supplemented with 0.4% glucose and 0.4% Casamino Acids (Difco Laboratories, Detroit, Mich.) was used for isopropyl-β-D-thiogalactopyranoside (IPTG) induction experiments. For Tif expression 0.01% adenine was also added. LB broth (20) and N medium (10) were used for experiments with lexA(Ts) and pZAO-bearing strains; suppression of the lon phenotype was assessed on LB plates containing nitrofurantoin (2 μg/ml).

IPTG (5 × 10⁻³ M), ampicillin (100 μg/ml), and tetracycline (10 μg/ml) were used.

Particle counts and volume determination. Particles were counted with a Coulter counter (model ZB) equipped with a 30-μm orifice and a 50-μl manometer. Volume distributions were determined with a C1000 channelizer. Bacteria were suspended in filtered Formol saline (3).

RESULTS

SfiA action. SfiA-dependent division inhibition was first analyzed in a lexA(Ts) strain, in which the SfiC system is not induced (3). When an exponentially growing culture was transferred from 30 to 42°C, cell division slowed down immediately, with only a 28% increase in cell number in the first 1 h and essentially no further division up to 4 h (Fig. 1). Cell mass continued to increase, and long filaments were produced (Fig. 1 and 2). If the culture was returned to 30°C after 60 or 120 min, cell division resumed rapidly.

The reversal of SfiA action could involve release of de novo synthesis of the target molecule FtsZ. To distinguish between these possibilities, we examined whether SfiA action could be reversed in the absence of protein synthesis. After 115 min, protein synthesis was inhibited by the addition of chloramphenicol (100 μg/ml), and at 120 min the culture was returned to 30°C. During the incubation at 42°C the mean cell mass (optical density/particle concentration) increased 7.4-fold. When the temperature was returned to 30°C, the cell concentration increased 6.6-fold within 40 min, despite the lack of protein synthesis (Fig. 1); and the cell volume decreased correspondingly (Fig. 2). This procedure did not cause any loss of viability, and colony counts increased in parallel with particle number, showing that essentially all division products were viable.

The results presented above clearly show that SfiA action is fully reversible in the absence of de novo protein synthesis, strongly suggesting that active FtsZ protein is released from FtsZ-SfiA complexes.

Similar results were obtained when SfiA was induced by 60 min of Tif expression in the recA (Tif) sfiC strain QC2480, although division did not begin until 15 min after the temperature was returned to 30°C in the presence of chloramphenicol (data not shown).

Because chloramphenicol also inhibits SfiA synthesis in these experiments, it should not be necessary to return the culture to 30°C to restore cell division in a lexA(Ts) strain. Indeed, the addition of chloramphenicol at 115 min at 42°C produced a 3.6-fold increase in particle concentration at 42°C (data not shown). In the recA (Tif) sfiC strain, however,
chloramphenicol did not stimulate cell division at 42°C after 55 min of SfiA induction. We cannot say at present whether this is due to the persistence of activated RecA protein under the latter conditions.

Degradation of the SfiA protein, which is normally quite rapid, is slowed down about 16-fold in lon mutants (22). If the reversibility of SfiA-mediated division inhibition results from SfiA degradation, one would expect division to resume more slowly in lon mutants. When a lexA(Ts) lon strain was shifted to 42°C and chloramphenicol was added 100 min later, cell number remained constant for about 50 min and then slowly started to increase (Fig. 3B). In contrast, under the same conditions with a lexA(Ts) lon+ strain, the cell number started to increase 15 min after the addition of chloramphenicol and rose 2.7-fold by 50 min (Fig. 3A).

These results suggest that the reversibility of SfiA action does indeed reflect SfiA instability. The fact that SfiA action nevertheless remained reversible in the lon mutant is compatible with the observation that the SfiA protein is still unstable in these strains (22, 24) with an estimated half-life of 19 min (as measured in UV-irradiated cells [22]). The data presented in Fig. 3 show that cell division resumes much faster in a lon+ strain than in the lon mutant strain. If it is assumed that the rate-limiting step in the resumption of cell division is the disappearance of the SfiA inhibitor, these data can be used to arrive at an estimate of the in vivo half-life of SfiA. The half-life was calculated from a plot of the logarithm of the fraction of unused division sites versus time after the addition of chloramphenicol (Fig. 3C). After an initial lag, presumably the time needed for degradation of excess SfiA and cell separation, the number of unused sites decayed by first-order kinetics. The calculated

FIG. 2. Recovery of normal cell volume in the absence of protein synthesis after SfiA-mediated division inhibition. The lexA(Ts) strain GC4158 was shifted to 42°C at time zero; at 115 min chloramphenicol was added, and at 120 min the culture was returned to 30°C (data are from the experiment shown in Fig. 2). The cell volume distribution, as determined in a Coulter counter channelizer, is shown for the culture at time zero (A, solid line), 120 min (A, broken line), 150 min (B), and 210 min (C).

FIG. 3. Kinetics of resumption of cell division in lon+ and lon cells following SfiA-mediated division inhibition. At time zero the lexA(Ts) strain DM511 (A) and the lexA(Ts) lon strain JFL126 (B), growing exponentially in LB medium at 30°C, were divided into two portions; one was kept at 30°C (O) and the other was transferred to 42°C (x). At 100 min (arrowhead) a portion of the culture at 42°C was supplemented with chloramphenicol (100 μg/ml), and incubation continued at 42°C (O). (C) The data obtained from the chloramphenicol-treated cultures were treated as follows. First, the number of potential divisions (PD) at the time of chloramphenicol addition was calculated by subtracting the particle number of the culture at 30°C at 100 min from the particle number of the culture at 42°C at 100 min. Second, the number of actual divisions (AD) was calculated by subtracting the particle number at 42°C at 100 min from the particle number at each time point after chloramphenicol addition. The fraction of unused division sites at any time after treatment with chloramphenicol could then be calculated as follows: $1 - \frac{(AD)}{(PD)}$. The data for the lexA(Ts) lon+ (O) and lexA(Ts) lon (O) cultures were plotted versus time after chloramphenicol addition.
half-life of SfiA in lon+ cells is 10 min and in lon cells is 170 min.

The reversal of SfiA action does not require induction of any other SOS function, as shown by the following experiment. We introduced F' lacF and pGC165sfiA+ into strain GC2597. The plasmid pGC165sfiA+ has the sfiA gene under control of the lac promoter (11). The resulting strain is filamentous in the presence of the lac operon inducer IPTG, although other SOS functions are not induced (11). After 115 min of growth in the presence of IPTG, the average cell mass (optical density/particle concentration) had increased 3.3-fold. The addition of chloramphenicol (100 μg/ml) at this time induced a 2.6-fold increase in cell number, with recovery of essentially normal cell volume.

SfiC action. Expression of the SfiC system in a recA (Tif) sfiA strain caused an immediate slowdown of cell division, although the cell number continued to increase slightly, reaching 1.5 times its initial value after 4 h at 42°C (Fig. 4). Analysis of the cell volume distribution revealed a progressive increase of the average volume (data not shown), with no distinct size classes emerging during the experiment, indicating that the residual division occurred randomly in the filamenting cells.

To test the reversibility of SfiC action, the culture described above was returned to 30°C after 60 min of Tif expression. Cell survival at this time (colony-forming ability at 30°C) was 8% of its value at time zero. For the length of the experiment (180 min) the bacteria continued to increase in length with little additional cell division (Fig. 4). If chloramphenicol (100 μg/ml) was added 5 min before the return to 30°C, cell number still remained constant for at least 180 min, indicating that the division inhibition that was observed did not require the continuation of protein synthesis. Thus, SfiC-dependent division inhibition is essentially irreversible.

Combined SfiA and SfiC action. Although SfiA and SfiC both seem to inhibit cell division via the FtsZ protein, the results presented above show that SfiA action is reversible, whereas SfiC action is not. We next analyzed the effect of simultaneous induction of SfiA and SfiC with the recA (Tif) strain JM12, which is sfiA+sfiC+. After 60 min of Tif expression at 42°C, there was essentially no recovery of cell division on return to 30°C; the division inhibition under these conditions is irreversible, which is characteristic of SfiC expression. Because we have previously shown that SfiA expression is induced in JM12 at 42°C (8), SfiC must be epistatic to SfiA.

Effects of FtsZ overproduction. When the accumulation of SfiA protein passes a certain threshold, cell division is inhibited, apparently via direct interaction between SfiA and the division protein FtsZ. SfiC-dependent division inhibition is thought to follow a similar pathway, because sfiB mutations, which are located in the ftsZ gene (12, 16), suppress both SfiA- (4, 5) and SfiC-dependent (3) inhibition. If, indeed, division inhibition results from direct protein-protein interactions, then an increase in the FtsZ pool might be expected to raise the threshold level of inhibitor required to block division. Therefore, we examined the effect of FtsZ overproduction on SfiA- and SfiC-dependent division inhibition.

The level of FtsZ was increased by introducing the multicopy plasmid pZAQ, which carries the genes ftsZ, ftsA, and ftsQ. This plasmid increases the level of FtsZ sevenfold (29). In the lexA(Ts) strain DM511 the presence of pZAQ suppressed division inhibition in LB medium at 42°C, and the efficiency of colony formation at 42°C was essentially 100%, compared with 10−4 for DM511 containing the control plasmid pBR322. Similar results were found in a lon mutant, in which the SfiA protein is partially stabilized (22, 24) and mild SOS inducers, such as nitrofurantoin, cause SfiA-dependent lethality (4). The presence of the plasmid pZAQ in the lon strain RGC103 permitted the strain to grow with 100% efficiency on LB plates containing 2 μg of nitrofurantoin per ml, whereas the same strain containing pBR322 had a plating efficiency of 10−3. Thus, the plasmid completely suppressed SfiA-dependent division inhibition and lethality.

To study the effect of FtsZ overproduction on SfiC-dependent division inhibition, we had to resort to recA (Tif) strains to induce SfiC. In these strains bearing the plasmid pZAQ, bacterial mass stopped increasing within 30 min under standard conditions of Tif expression (42°C in M63 medium plus glucose, Camasino Acids, and adenine). In medium N plus adenine, growth continued for about 120 min before it stopped. Under these conditions the recA (Tif) sfiA strain GC2467, with or without the plasmid pZAQ, continued dividing for 60 min at 42°C and then started to become filamentous. If the cultures were returned to 30°C at 120 min, cell mass continued to increase but cell division remained inhibited for at least 120 min, even in the presence of the plasmid. Thus, FtsZ overproduction from the plasmid pZAQ does not detectably affect SfiC division inhibition.

DISCUSSION

The SOS response in E. coli, which is induced when DNA replication is perturbed (15, 26, 30), includes two mechanisms of cell division inhibition, SfiA (7) and SfiC (3). In this study we examined the reversibility of SfiA- and SfiC-
dependent division inhibition. The SfiC system was found to be irreversible. After 60 min of induction (by expression of the recA [Tif] mutation), division remained inhibited for over 180 min under noninducing conditions.

The SfA mechanism, on the other hand, was completely reversible. When induction of SfA was stopped, the filaments that had formed divided and the mean cell mass returned to normal. Furthermore, this division occurred even if protein synthesis was inhibited, suggesting that functional FtsZ can be recovered from the SfA-FtsZ complex.

A further difference between SfA and SfC was observed in the presence of a multicopy plasmid carrying the ftsZ, ftsA, and ftsQ genes. This plasmid completely suppressed the division inhibition caused by SfA but did not suppress that caused by SfC. This extends our earlier observation that a doubling of the ftsZ gene dosage delayed the onset of division inhibition caused by SfA (16). Thus, sevenfold overproduction of the FtsZ protein, the presumed target molecule for both division inhibitors, can titrate out the inhibitory effect of SfA but not SfC.

These differences between SfA and SfC can be accounted for by various hypotheses. The irreversibility of SfC action might reflect a stability of the induced state: sfC* is part of the element e14 (Maguin et al., in preparation), and it is possible that induction results in a stable immune state (25), unlike LexA repression, which is rapidly reestablished when induction ceases and DNA is repaired (1, 19). However, the fact that SfC-mediated division inhibition remained irreversible, even in the absence of ongoing protein synthesis, argues against this hypothesis.

By far the simplest hypothesis to account for the irreversibility of SfC action is that the SfC system of division inhibition is intrinsically stable, even in lon strains. This could explain the irreversibility of SfC action, the lack of suppression of SfC by FtsZ overproduction, and the similar (high) SfC lethality in lon+ and lon strains (3).

SfA-mediated division inhibition remained fully reversible when the mean cell mass had increased nearly eightfold, equivalent to three generations of division inhibition. The inhibited divisions took place rapidly when SfA induction ceased, even in the absence of de novo protein synthesis: a 7.2-fold increase in cell number was observed in the presence of chloramphenicol (Fig. 1). This result indicates a remarkable stability in the cell division machinery, which resumes activity rapidly after the disappearance of SfA.

Division is presumably blocked by SfA at the FtsZ stage, which is thought to carry out an early step in septation, perhaps initiation (27). Thus, during the period of division inhibition all proteins that act after FtsZ must have accumulated in sufficient quantity to synthesize over six septa per cell. In addition, any such proteins must be stable.

As expected, recovery from SfA-mediated division inhibition was considerably delayed in a lon mutant. From analysis of the kinetics of division recovery, we estimated the in vivo half-life of SfA to be 10 min in a lon+ strain and 170 min in a lon mutant. These estimates are considerably larger than those arrived at by measuring SfA degradation in UV-irradiated cells (22), although the ratio of the half-lives is the same. In addition, our estimate of the half-life in lon+ cells agrees well with the measurement of the SfA half-life in maxicells that also contain increased FtsZ (13), presumably reflecting the degradation of SfA in SfA-FtsZ complexes.

It has been reported that some essential septation protein is normally synthesized at the time of replication termination, shortly before division, and that without this synthesis the pool of the protein is too low to carry out septation (14). Our results suggest that if there is a division protein which is synthesized at a specific point in the cell cycle and is not accumulated, it must act earlier than FtsZ.

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LITERATURE CITED


