Role of the sfiA-Dependent Cell Division Regulation System in Escherichia coli

OLIVIER HUISMAN,1,2 MICHELINE JACQUES,1 RICHARD D’ARI,1 AND LUCIEN CARO2

Institut Jacques Monod, CNRS, Université Paris VII (Tour 43), 75251 Paris Cedex 05, France1; and Département de Biologie Moléculaire, Université de Genève, CH1211-Geneva 4, Switzerland2

Received 25 October 1982/Accepted 4 November 1982

Several authors have suggested that the SOS-associated (sfiA-dependent) system of division inhibition, normally induced by perturbations of DNA replication, also regulates steady-state (unperturbed) cell division. The present work shows that mean cell mass is identical in sfiA+ and sfiA mutant cultures during steady-state growth, that mass adjustment is identical after shift up, that sfiA expression is not induced by shift up, and that a sfiA mutation does not cause aberrant chromosome segregation.

Cell division in Escherichia coli is tightly coupled to DNA replication. Aberrant divisions producing DNA-less cells are rare, the frequency of such cells being about 0.1% during normal growth (12). Furthermore, unscheduled stops in DNA synthesis lead to a rapid arrest of cell division (4, 5, 7, 10). Witkin (25) suggested that DNA replication-cell division coupling involves a division inhibitor whose synthesis is induced whenever DNA synthesis is perturbed. Recent results have established the existence of this type of coupling mechanism in E. coli (15, 18). The sfiA gene product is an indispensable component of this mechanism (8), most likely the division inhibitor itself. Its synthesis, induced by DNA-damaging treatments (15, 23), is one manifestation of the pleiotropic SOS response (20).

Expression of the sfiA gene, and of the SOS response in general, is regulated by two loci, lexA and recA. The LexA protein is the repressor of a number of unlinked operons, presumably including sfiA (15–17). The RecA protein is the inducer of the SOS response. After DNA damage, the cell forms a cofactor, probably single-stranded DNA, which enables the RecA protein to cleave LexA repressor, thus derepressing the entire lexA regulon (for review, see reference 20).

The sfiA system clearly couples cell division to DNA replication whenever the latter is perturbed. Several observations suggested that the sfiA-dependent mechanism is involved in cell division regulation and chromosome segregation during normal (unperturbed) growth. First, lon mutants (11), which are hypersensitive to sfiA-dependent division inhibition, transiently stop dividing after a nutritional shift up (24); we have shown that this division arrest is sfiA dependent and have suggested that sfiA expression is induced after a shift up to facilitate the increase in cell length known to take place under these conditions (18). Second, Howe and Mount reported (13, 14) that lexA (Ind−) mutant cells, in which the LexA repressor cannot be cleaved by RecA protease, are shorter than lexA+ cells during normal growth; they suggested that this was due to the inability of these mutants to induce SOS-associated division inhibition. Third, Howe and Mount (12) reported that in cultures of a lexA (Ind−) mutant, noninducible for the SOS response, 1 to 2% of the cells do not contain DNA, compared with 0.1% in lexA+ cultures; again they suggested that this was due to the non-inducibility of the SOS-associated division inhibitor.

We have tested these hypotheses by comparing mean cell mass and DNA distribution in sfiA+ and sfiA mutant cultures. We monitored the rate of sfiA expression by means of a previously described sfiA::lac operon fusion (15, 16).

After a nutritional shift up from 63-minimal-glycerol to LB-glucose medium, no induction of sfiA expression was detected (Fig. 1). Furthermore, the mean cell mass, which increased more than fourfold during postshift growth, was indistinguishable in sfiA+ and sfiA mutant cultures before, during, and after the transition (Fig. 1). Thus the sfiA-dependent coupling mechanism is not required for the cell size increase that follows a nutritional shift up. We also conclude that the sfiA-dependent filamentation observed in lon mutants after shift up does not reflect transient induction of sfiA expression. It may have to do with the medium dependence of the Lon− phenotype, whose hypersensitivity to induction of the SOS response is only observed in complex media (11, 24).

It is noteworthy that the mean cell mass of the
sfiA mutant was not measurably different from that of wild type during steady-state growth in 63-minimal-glycerol or LB-glucose medium (Fig. 1). This was further confirmed in unshifted cultures that had been in steady-state growth for many generations in LB medium (data not shown). We concluded that the sfiA system is not required for proper division regulation during normal (unperturbed) growth.

We next examined chromosome segregation in a sfiA mutant to see whether the sfiA system normally helps prevent the accidental segregation of DNA-less cells. This hypothesis was suggested by the observation that cultures of recA (1) and, more especially, lexA (Ind⁻) mutants (12), noninducible for the SOS response, contain an abnormally high proportion of DNA-less cells. We therefore measured the frequency of DNA-less cells in wild-type and sfiA or lexA mutant cultures, using autoradiography.

The results (Table 1) show that the sfiA mutant culture does not have a higher proportion of DNA-less cells than does wild type. Analysis of 500 cells of each culture after a shorter (2.5-day) exposure gave similar results. Thus, the high proportion of DNA-less cells observed in lexA (and recA) mutant cultures cannot be accounted for by the inability of these strains to induce sfiA-dependent inhibition or division.

In the case of recA mutants, most or all of the DNA-less cells are due to the high level of spontaneous DNA degradation in these strains and do not reflect aberrant cell division (1). The lexA mutants, although exhibiting near-wild-type levels of spontaneous DNA degradation (3), are unable to induce the SOS-associated inhibition of DNA degradation (21). If 1 to 2% of the cells degraded their DNA completely, the excess degradation would probably not have been detected and could account for the DNA-less cells observed.

The present work shows that the sfiA-dependent system of division inhibition in E. coli is not involved in cell size determination or chromosome segregation during steady-state growth or in size adjustment after a nutritional shift up. In fact, the SOS response in general is probably not involved in these processes, for it is unlikely that the lexA and recA genes control a sfiA-independent mechanism of division regulation, given that sfiA mutations totally suppress SOS-associated division inhibition in the strains used (unpublished data).

Other work has shown that the SOS response,

![Graph showing the level of sfiA expression and mean cell mass in sfiA⁺ and sfiA mutant cultures after a nutritional shift up.](image)

**FIG. 1.** Level of sfiA expression and mean cell mass in sfiA⁺ and sfiA mutant cultures after a nutritional shift up. Bacteria were grown into exponential phase at 37°C in 63 medium (22) containing glycerol and thiamine. At an optical density at 600 nm (OD₆₀₀) of about 0.2, the cultures were diluted twofold into prewarmed, twice-concentrated LB medium (22) supplemented with 0.8% glucose (time zero). Samples were withdrawn periodically for OD₆₀₀ measurements and for assays of viable counts (on 63 glycerol plates before the shift up, and on LB plates after it) and of β-galactosidase activity (22), which reflects the level of sfiA expression. Cultures were diluted fourfold into prewarmed LB glucose medium whenever the OD₆₀₀ approached 0.2. β-Galactosidase measurements (Δ) were with strain GC4516, a ΔlacU169 sfiA⁺ lysogen for λ p(sfiA::lac)cl ind (16). Cell mass measurements, calculated as OD₆₀₀ per 10⁹ viable cells, were with strains GC4511 (sfiA⁺, Δ) and GC4510 (sfiA⁺, Δ). The three strains are essentially isogenic.

**TABLE 1.** Frequency of DNA-less cells in cultures of wild-type, sfiA, and lexA strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Proportion of DNA-less cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC2431</td>
<td>sfiA⁺ lexA⁺</td>
<td>2/1,000</td>
</tr>
<tr>
<td>GC2432</td>
<td>sfiA⁻ lexA⁺</td>
<td>1/1,000</td>
</tr>
<tr>
<td>GC2433</td>
<td>lexA⁻</td>
<td>12/1,000</td>
</tr>
</tbody>
</table>

*Bacteria were grown for about 18 generations at 37°C in M9 medium (22) containing glucose, Casamino Acids, thiamine, and 5 μg of [³H]thymine per ml (specific activity 1 Ci/mmol). At a concentration of 2 x 10⁸ cells per ml, they were harvested and prepared for autoradiography as previously described (2, 9), using Ilford L-4 emulsion. After a 7-day exposure (after which a single unreplicating chromosome would yield an average of 24 grains), slides were developed in Kodak D19 and analyzed; cells having fewer than 7 grains were scored as deficient in DNA.**
via the sfiA system, clearly couples cell division to DNA replication whenever the latter is interrupted. Even minor perturbations of DNA replication cause rapid induction of sfiA expression (6, 15, 23). During thymine starvation of wild-type bacteria, this induction is accompanied by a nearly immediate arrest of cell division and the formation of filamentous cells. With sfiA (18), lexA (Ind-*) (12, 13), or recA (19) mutants, unable to induce sfiA expression, there is a period of residual division before filaments form, and the ultimate population contains numerous small cells which, in the case of lexA (Ind-*) and recA mutants, have been shown not to contain DNA (12, 19).

Taken together, these data suggest that the essential utility of the sfiA-dependent system of cell division control is to prevent the formation of DNA-less cells when DNA replication is perturbed.

This work was supported in part by grants from the Institut National de la Recherche Agronomique (ATP no. 4185), the Institut National de la Sante et de la Recherche Medicale (ATP no. 72-79-104), and the Swiss National Science Foundation (no. 3.169-1.81).

LITERATURE CITED


