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A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*

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Cell division in *Escherichia coli* requires the products of the *ftsQ*, *ftsA* and *ftsZ* genes. It is not known how the cell regulates the cellular concentrations of these essential elements of the division system. We describe here a factor that activates cell division by specifically increasing transcription from one of the two promoters that lie immediately upstream of the *ftsQAZ* gene cluster. The *trans*-acting factor is the product of the *sdiA* gene, which was isolated on the basis of its ability to suppress the division inhibitory effect of the MinC/MinD division inhibitor. In addition, the *sdiA* gene product suppressed the action of other chromosomally encoded division inhibitors, induced minicell formation in wild type cells, and restored division activity to an *ftsZ* temperature-sensitive mutant grown under nonpermissive conditions. All of these properties were explained by the ability of the *sdiA* gene product specifically to increase transcription of the *ftsQAZ* gene cluster, resulting in an increase in cellular concentration of the FtsZ protein. The *sdiA* gene product is the first factor thus far identified that specifically regulates expression of this key group of cell division genes.

Key words: cell division/*Escherichia coli*/*ftsQAZ* cluster/*minCD* suppressor/*sdiA*/transcription activator

Introduction

Cell division involves the formation of a division septum at the proper time in the division cycle and at the proper location in the cell. Genetic studies in *Escherichia coli* have indicated that these events—formation of the division septum and localization of the division site—involve different gene products and can be viewed as two distinct processes (de Boer *et al.*, 1990a).

A number of genes that are located in the two minute region of the *E.coli* chromosome have been implicated in the process of septum formation, based on the identification of temperature-sensitive mutants that fail to septate at nonpermissive temperatures (Donachie and Robinson, 1987). Among these, the *ftsZ* gene has attracted particular interest because it behaves as a division activator that acts at an early stage of the division process. Over-expression of *ftsZ* leads to an increase in the frequency of septation events in normal cells and also suppresses the effects of a number of endogenous cell division inhibitors (Ward and Lutkenhaus, 1985; Lutkenhaus *et al.*, 1986; de Boer *et al.*, 1990b).

The *ftsZ* gene lies immediately downstream of two other essential cell division genes, *ftsQ* and *ftsA*. Transcription of the genes of the *ftsQAZ* cluster can be driven by several promoters, some located immediately upstream of *ftsQ* and others embedded within *ftsA* (Robinson *et al.*, 1984, 1986; Aldea *et al.*, 1990). Because relatively slight changes in the cellular concentration of FtsZ can have profound effects on the ability of cells to divide and on the frequency of septation events (Ward and Lutkenhaus, 1985; Dai and Lutkenhaus, 1991) it is likely that mechanisms exist to regulate the concentration of FtsZ and other essential division components in order to maintain the normal division pattern. It is not known how this is achieved.

A different subset of genes is involved in the proper placement of the division septum at midcell. This requires the coordinate expression of the three genes of the *minB* operon—*minC*, *minD* and *minE*. It has been shown that expression of *minC* and *minD* in the absence of *minE* leads to a global inhibition of cell division. The MinC/MinD-mediated division inhibition is given topological specificity by the *minE* gene product. As a result the inhibitor no longer blocks septation at the proper site at midcell, while continuing to block septation events at the cell poles which would otherwise give rise to the formation of chromosome-less minicells. In this way the normal division pattern is maintained (de Boer *et al.*, 1989). A functional relationship between the MinC/MinD division inhibitor and the *ftsZ* gene product was suggested by the ability of increased levels of FtsZ to suppress the division inhibition induced by MinC/MinD (de Boer *et al.*, 1990b; Bi and Lutkenhaus, 1990) and to induce minicell formation (Ward and Lutkenhaus, 1985), and the finding that certain *ftsZ* mutant alleles impart resistance to MinC/MinD-mediated division inhibition (Bi and Lutkenhaus, 1990).

At present, neither the mechanism of division inhibition by MinC/MinD nor the mechanism whereby MinE gives topological specificity to the process are understood. Because the MinC/MinD-dependent division inhibitor presumably interacts with the normal septation machinery, an understanding of the division inhibition reaction is likely to provide information about the normal process of septum formation as well as information required for an understanding of the site-specific modulation of the inhibition process by MinE.

We therefore attempted to identify proteins that play a role in the MinC/MinD-mediated division inhibition process by screening a chromosomal DNA plasmid library for genes that suppress MinC/MinD-mediated division inhibition when over-expressed. Among the genes that can be expected to emerge from this type of search are the gene (or genes) for the molecular target of the division inhibitor, genes for other proteins that play a role in the inhibition process and genes involved in regulating the amounts or activities of the target molecules or of MinC or MinD themselves.

In the present paper we describe one of the several classes of suppressor genes that were identified by this approach. In addition to its ability to suppress the MinC/MinD division

block, the new gene, *sdiA*, was strikingly similar to *ftsZ* in its other effects on the cell division process. These included the ability to suppress the division inhibitory activity of other chromosomally encoded division inhibitors in addition to MinC/MinD, the ability to induce minicell formation in wild type cells and the ability to restore division activity to an *ftsZ*Ts mutant grown at its nonpermissive temperature.

We further show that the *sdiA* gene product acts as a positive regulator of transcription of the *ftsQAZ* gene cluster. This leads to an increase in the cellular concentration of the FtsZ protein, thereby explaining the effects of *sdiA* over-expression. The transcriptional activation affected transcripts arising from one of the two promoters that lie immediately upstream of *ftsQAZ* but did not affect transcripts arising from internal promoters that are located within the cluster. The SdiA protein is the first factor thus far described that can regulate the division process by activating transcription of essential cell division genes. This provides a mechanism whereby the cell can modulate the levels of the division-related proteins and, by implication, the division capacity of the cell.

Results

Supressors of MinC/MinD mediated division inhibition

To identify chromosomal genes that were capable of preventing the MinC/MinD-induced division block, a library of chromosomal DNA in the low copy number vector pGB2 was introduced into strain PB114(λPB173) [Δ*minCDE*(P_{lac}::*minCD*)], in which *minCD* is expressed under P_{lac} control. Expression of *minCD* was induced by exposure to isopropyl-β-D-thiogalactopyranoside (IPTG) and IPTG-resistant transformants were identified and characterized. The DNA library was prepared from a strain in which *minCDE* was deleted, thereby avoiding the isolation of plasmids carrying the *minE* gene, which is known to suppress the MinC/MinD filamentation phenotype (de Boer *et al.*, 1989). Similarly, the absence of *minE* in the recipient strain PB114 eliminated the possibility of isolating clones that suppressed MinC/MinD filamentation by acting as positive regulators of *minE* expression.

In this way a number of recombinant plasmids were identified that prevented the MinC/MinD-induced division block. The possibility thats the suppressor plasmids acted by preventing induction of P_{lac} was excluded by showing that β-galatosidase production from the chromosomal *lac* operon of the host strain was induced to normal levels by growth of the plasmid-containing cells in the presence of IPTG (data not shown).

The suppressor clones that have been charcterized thus far fell into three classes.

Approximately 60% of the suppressor plasmids contained the wild type *ftsZ* gene, as shown by restriction mapping. These had been anticipated since we previously had shown that over-expression of *ftsZ* suppresses MinC/MinD-mediated division inhibition (de Boer *et al.*, 1990b).

Two of the suppressor plasmids contained a new gene, *sdiA*, whose properties are discussed in detail below.

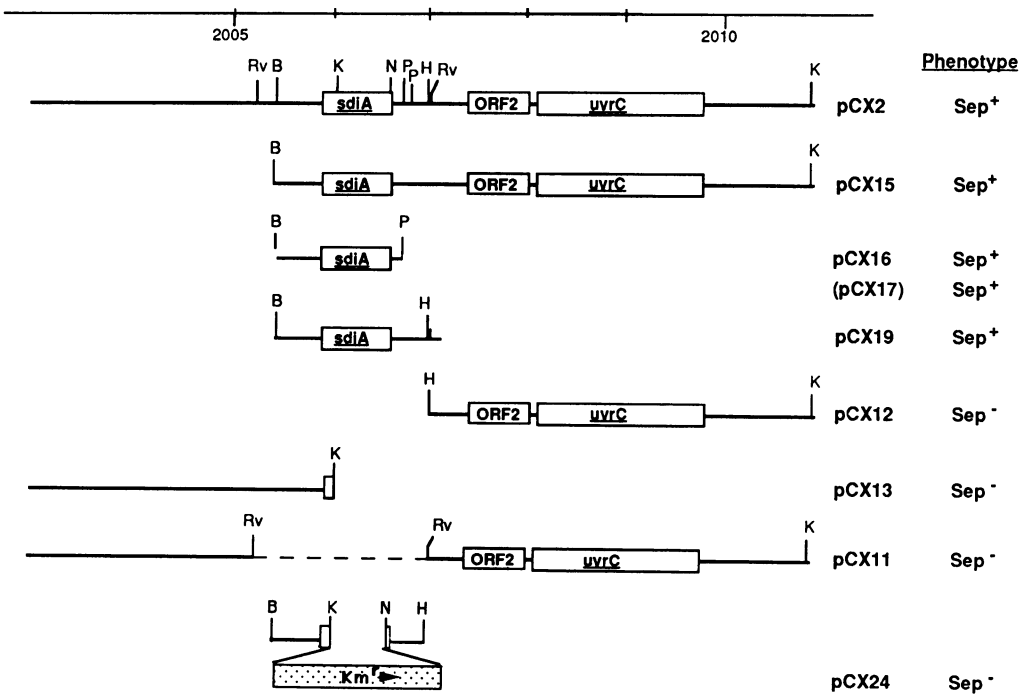

Fig. 1. Physical map of the *sdiA* region and diagram of chromosomal inserts of plasmids. The chromosomal inserts in the indicated plasmids are shown. The right column indicates the phenotypes of cells of strain PB114(λDB173) [Δ*minCDE*(P_{lac}::*minCD*)] containing each of the indicated plasmids, after growth in the presence of IPTG to induce expression of *minCD* as described in Materials and methods. Sep^+ indicates the minicell phenotype of plasmid-free PB114 or of PB114 containing vector plasmids (see Figure 2a); the minicell formation reflects the deletion of the chromosomal *minCDE* genes in this strain. Sep^- indicates a uniform population of long non-septate filaments (see Figure 2c). In all cases described here, cells grown in the absence of IPTG were Sep^+ whereas cells lacking plasmids or containing the vector plasmids were Sep^-. All plasmids were derivatives of pGB2 except for pCX24, which was derived from pMAK705, and pCX19, which was derived from pUC19. In the pGB2 derivatives other than pCX17 the *sdiA* gene is downstream from and oriented in the same direction as the *aadA* gene of the vector. pCX17 is identical to pCX16 except that the orientation of the insert is reversed. Dashed lines indicate segments that were deleted. The scale at the top of the figure shows coordinates according to the Kohara map of the *E.coli* chromosome (Kohara *et al.*, 1987). The only restriction sites shown are those relevant to the present study. Restriction sites: B, *Bam*HI; K, *Kpn*I; H, *Hin*dIII; N, *Not*I; P, *Pst*I; Rv, *Eco*RV.

A third group of suppressor plasmids contained at least two other classes of chromosomal inserts. These will not be further discussed here.

Structure of sdiA

The chromosomal insert in the original *sdiA*$^+$ clone pCX2 (Figure 1) was located between 2003 and 2011 kb on the Kohara map of the *E.coli* chromosome (~42 min on the standard genetic map), based on a comparison of its restriction map with the physical map of Kohara (Kohara *et al.*, 1987). The 2003–2011 kb segment includes the *uvrC* gene plus ~5.2 kb of upstream DNA. The map position was confirmed by showing that pCX2 was capable of correcting the UV-sensitive phenotype of strain AB1884 [*uvrC*34].

The chromosomal region corresponding to the insert in pCX2 had previously been sequenced and shown to contain two open reading frames that are located adjacent to *uvrC* (*sdiA* and ORF2 in Figure 1) (Sharma *et al.*, 1986). After further subcloning, the chromosomal fragment responsible for the suppression of MinC/MinD-induced filamentation was shown to correspond to the first open reading frame. We call the new gene *sdiA*, for suppressor of division inhibition.

The open reading frame corresponding to *sdiA* codes for a 241 amino acid protein. The predicted 28 kDa peptide had previously been demonstrated in maxicell experiments on cells containing plasmids that included the 5.4 kb region which lies to the right of the *Bam*HI site in the chromosomal insert of pCX2 (Figure 1) (Sancar *et al.*, 1981).

Sequence analysis suggests that a σ70 promoter is located ~90 bp upstream of the translational start site of *sdiA*. This promoter appears not to be involved in transcription of ORF2 or *uvrC* (Sharma *et al.*, 1986). Consistent with the presence of a functional promoter in this region, the 1.3 kb *Bam*HI–*Pst*I *sdiA* fragment which includes the putative promoter was functional both in pCX16, in which *sdiA* lies downstream of the *aadA* gene of the vector which can provide an exogenous promoter for downstream genes (J.P.Bouché, personal communication; and our unpublished data), and in pCX17, in which the orientation of the insert is opposite to that of the *aadA* promoter.

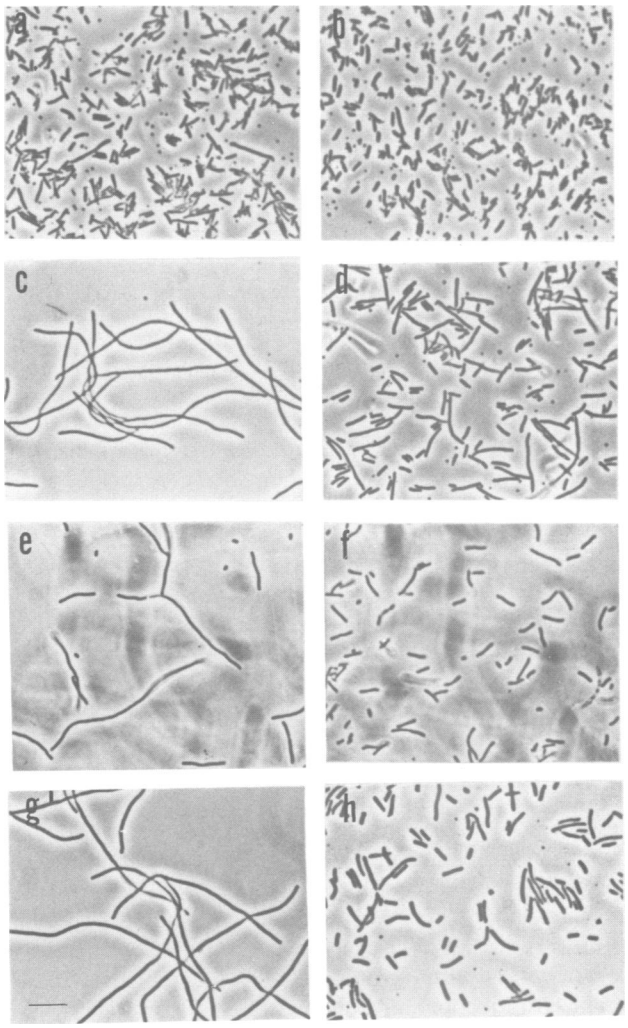

Fig. 2. Suppression of division inhibition phenotypes by *sdiA* plasmids. Cells were grown in the presence or absence of 25 μM IPTG as described in Materials and methods prior to preparation of phase contrast micrographs. (**a**) PB114(λDB173)/pGB [Δ*minCDE*(P_{lac}::*minCD*)], no IPTG; (**b**) PB114(λDB173)/pCX16 [Δ*minCDE*(P_{lac}::*minCD*)/*sdiA*], no IPTG; (**c**) PB114(λDB173) [Δ*minCDE*(P_{lac}::*minCD*)]/pGB2, +IPTG; (**d**) PB114(λDB173)/pCX16 +IPTG; (**e**) PB103/pDB192 [P_{lac}::*sfiA*]/pGB2, +IPTG; (**f**) PB103/pDB192/pCX16 [P_{lac}::*sfiA*/*sdiA*]+IPTG; (**g**) AW40/pGB2 [*ftsZ*ts], grown at 42°C; (**h**) AW40/pCX16 [*ftsZ*Ts/*sdiA*], grown at 42°C. Bar, 10 μm.

Effects of sdiA on endogenous division inhibitors

All plasmids that contained the intact *sdiA* gene suppressed MinC/MinD-mediated division inhibition (Figure 1), as illustrated for pCX16 in Figure 2c and d.

In addition to MinC/MinD, several other chromosomal genes are known whose expression leads to a global block in cell division. The best studied of these are *dicB* and *sfiA* (Huisman *et al.*, 1984; Béjar and Bouché, 1985). We have previously shown that DicB-induced filamentation requires the expression of *minC* (de Boer *et al.*, 1990b) and Labie *et al.* (1990) have shown that certain *minC* mutations suppress DicB-induced filamentation. In contrast, SfiA-mediated division inhibition, which is normally expressed as part of the SOS response to DNA damage, is independent of MinC (de Boer *et al.*, 1990b).

To determine whether *sdiA* was capable of counteracting the MinC/DicB and SfiA-associated division inhibitors, we introduced the *sdiA* plasmid pCX16 into cells in which expression of *dicB* or *sfiA* was under control of P_{lac}. In both

Table I. Effects of SdiA on filamentation induced by DicB and SfiA

Plasmids and/or phages	Phenotype	
	−IPTG	+IPTG
pGB2/λDB182 [P_{lac}::*dicB*]	Sep$^+$	Sep$^-$
pCX16 [*sdiA*]/λDB182 [P_{lac}::*dicB*]	Sep$^+$	Sep$^+$
pZAQ [*ftsQAZ*]/λDB182 [P_{lac}::*dicB*]	Sep$^+$	Sep$^+$
pGB2/pDB192 [P_{lac}::*sfiA*]	Sep$^+$	Sep$^-$
pCX16 [*sdiA*]/pDB192 [P_{lac}::*sfiA*]	Sep$^+$	Sep$^+$
pZAQ [*ftsQAZ*]/pDB192 [P_{lac}::*sfiA*]	Sep$^+$	Sep$^+$

Strain PB103 containing the indicated plasmids and/or lysogenic for the indicated phages were grown in the presence or absence of IPTG and examined by phase microscopy as described in Materials and methods. Sep$^-$ indicates a uniform population of long, non-septate filaments. Sep$^+$ cells showed no long filaments. Cultures of cells containing pCX16 or pZAQ also contained minicells. For further details see Results and Figure 2.

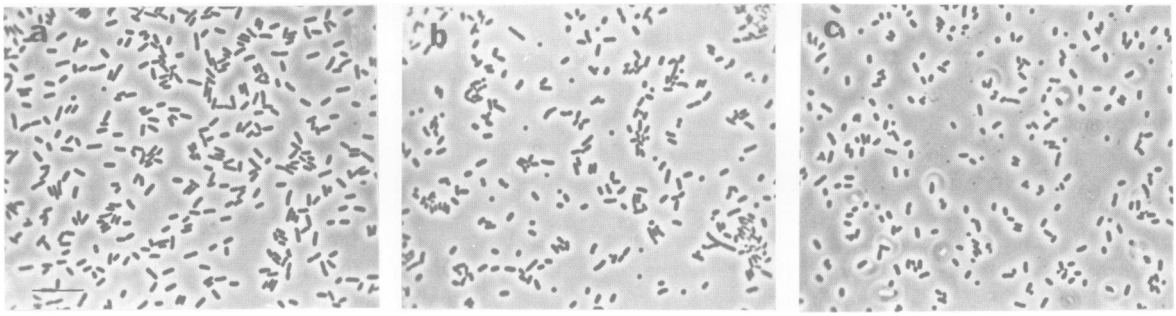

Fig. 3. Effect of *sdiA* and *ftsZ* plasmids on wild type cells. (**a**) PB103/pGB2; (**b**) PB103/pCX16 [*sdiA*]; (**c**) PB103/pDB196 [*ftsZ*]. Bar, 10 μm.

cases, the presence of the *sdiA* plasmid suppressed the IPTG-induced filamentation which occurred in the absence of the *sdiA* plasmid (Table I and Figure 2e and f).

Thus, *sdiA* is capable of counteracting the division block induced by the expression of other division inhibition genes in addition to *minC/minD*.

Effects of sdiA in wild type cells

Consistent with the finding that *sdiA* can suppress MinC/MinD-mediated division inhibition, introduction of the *sdiA* plasmids into wild type cells led to the formation of minicells (Figure 3b). This presumably reflects release of the normal MinC/MinD inhibition of septation at polar division sites (de Boer *et al.*, 1989). The cultures resembled those of wild type cells carrying multiple copies of the *ftsZ* gene (Figure 3c), which has also been shown to induce minicell formation in wild type cells (Ward and Lutkenhaus, 1985).

Deletion of the chromosomal sdiA gene

As the first step in the construction of a functional deletion of the chromosomal *sdiA* gene, we replaced the carboxy-terminal 73% of the *sdiA* coding sequence with a kanamycin resistance (Km^r) element, yielding plasmid pCX24. The *sdiA*::Km^r allele in pCX24 did not suppress the division inhibition resulting from induction of *minCD* and was also incapable of inducing minicell formation in wild type cells. This confirmed that the insertion mutation resulted in loss of *sdiA* function. The chromosomal *sdiA*$^+$ gene was then replaced by the *sdiA*::Km^r allele of pCX24 as described in Materials and methods. The absence of the intact chromosomal *sdiA* gene in the *sdiA*::Km^r strain was confirmed by Southern hybridization analysis.

The resulting *sdiA*::Km^r strain (WX2) was capable of growing and dividing normally on a variety of media in the absence of the *sdiA*$^+$ allele. Thus, a functional *sdiA* gene was not required for cell division in an otherwise normal genetic background under the conditions that were examined.

Effects of sdiA on ftsZ mutant cells

The effects of the *sdiA* plasmids—suppression of filamentation induced by the MinC/MinD, MinC/DicB and SfiA division inhibitors, and the induction of minicell formation in wild type cells—resembled the effects of over-expression of *ftsZ* (Ward and Lutkenhaus, 1985; Lutkenhaus *et al.*, 1986; de Boer *et al.*, 1990b). We therefore asked whether the *sdiA* gene product also resembled FtsZ in its ability to suppress the division defect resulting from inactivation of the *ftsZ* gene product in strains containing the temperature-sensitive *ftsZ*84 mutation. As illustrated in Figure 2g and h, introduction of the *sdiA* plasmids restored division activity to *ftsZ*84 strains grown at 42°C, as shown by the disappearance of the long non-septate filaments and the restoration of the ability to form colonies on plates. The suppression was marked but was not complete, as shown by the persistence of short filaments in the cultures. Similar results were obtained when *sdiA* was present in the pGB2 derivative pCX16 and in the pUC19 derivative pCX19.

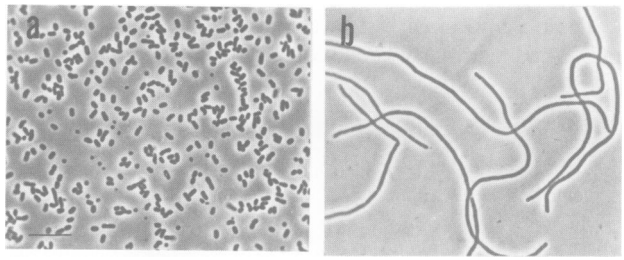

Fig. 4. Effect of chromosomal *ftsZ* frameshift mutation. Strain WX7/pCX41 [*ftsZ*/ftsZ*$^+$] was grown at 30°C with vigorous aeration in LB to ~3 × 10^7 cells/ml. Half the culture was shifted to 44°C and growth of both the 30°C and the 44°C cultures was continued for 6 h before preparation for phase contrast microscopy. The cultures were diluted once with an equal volume of LB after five generations to maintain exponential growth. (**a**) 30°C culture; (**b**) 44°C culture. Bar, 10 μm.

Functional deletion of the chromosomal ftsZ gene

Because of the conditional division-negative phenotype of cells containing the *ftsZ*84 mutation, it has been assumed, although without direct proof, that the *ftsZ* gene product is essential for the normal division process. To confirm this supposition, we introduced a frame shift mutation into the promoter-proximal portion of *ftsZ*. When the frame-shifted *ftsZ* allele (*ftsZ**) was present on a multicopy plasmid (pCX29) it failed to correct the division-negative phenotype of an *ftsZ*84 strain grown at 42°C and failed to induce minicell formation in wild type strains, thereby confirming the loss of FtsZ function. The chromosomal *ftsZ*$^+$ gene was then replaced by the *ftsZ** allele.

The resulting *ftsZ** strains were maintained at 30°C in the presence of an *ftsZ*$^+$ gene in plasmid pCX41, which can replicate at 30°C but not at 42°C. When replication of the thermosensitive *ftsZ*$^+$ plasmid in the *ftsZ** strain was prevented by increasing the temperature to 44°C, the cells stopped dividing after a lag period of 2–3 h, leading to formation of a uniform population of long non-septate filaments after 6 h (Figure 4). When the cells were shifted

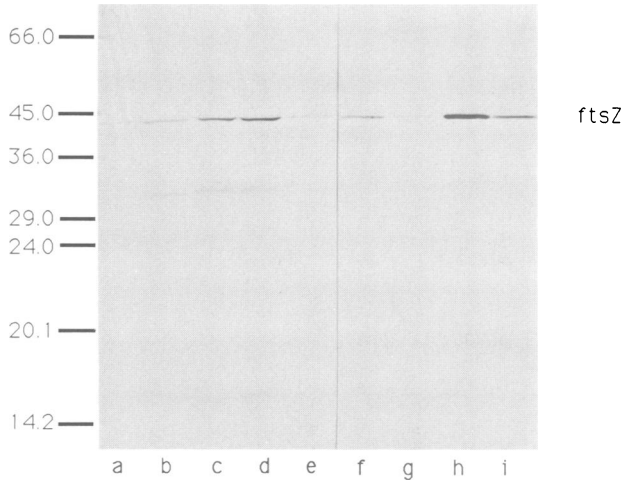

Fig. 5. Immunoblots of FtsZ protein. Immunoblots were prepared on extracts of strain PB103 containing the indicated plasmids, using phosphatase-conjugated antibody. (**a**–**d**). No plasmid. Different amounts of protein were assayed (a, 17 μg; b, 34 μg; c, 51 μg; d, 68 μg). (**e**–**i**) 17 μg protein were assayed. (e, PB103/pGB2; f, PB103/pCX16 [*sdiA*]; g, PB103/pBR322; h, PB103/pZAQ [*ftsQAZ*]; i, PB103/pDB191 [*ftsZ*]).

back to 30°C after 10 generations at 44°C, septation did not resume, suggesting that the FtsZ protein is required both at 44°C and at 30°C. This demonstrates that *ftsZ* is in fact an essential cell division gene. Similar results have been obtained by others (Dai and Lutkenhaus, 1991; Pla *et al.*, 1991).

Effect of sdiA in an ftsZ deletion strain

The ability of SdiA to mimic the effects of FtsZ on the various division inhibitors and on wild type cells, and the ability of SdiA to restore division activity to temperature-sensitive *ftsZ*84 strains, could be explained by either of two hypotheses. First, SdiA might be capable of substituting for FtsZ in the division process. This would predict that increased levels of SdiA would restore division activity to $ftsZ^{null}$ cells. Alternatively, SdiA might be a positive regulator of the amount or activity of the FtsZ protein. In this case increased levels of SdiA would not restore division activity to strains lacking the FtsZ protein.

We therefore asked whether *sdiA* was able to correct the division defect of the *ftsZ** strain. This was accomplished by introducing the *sdiA* plasmid pCX19 into strain WX7/pCX41 [$ftsZ^*/ftsZ^+$]. The WX7/pCX41/pCX19 cells were unable to divide when replication of the temperature-sensitive pCX41 [$ftsZ^+$] plasmid was blocked by growth for 2–3 h at elevated temperature, as shown by the formation of long non-septate filaments. We conclude that *sdiA* cannot restore division activity to cells which lack functional FtsZ protein, indicating that SdiA does not simply act as a surrogate for FtsZ.

Effect of sdiA on cellular concentration of FtsZ

Evidence that *sdiA* expression leads to an increase in the amount of cellular FtsZ protein was obtained by the use of a quantitative radioimmunoblotting assay. As shown in Figure 5 and Table II, introduction into wild type cells of the *sdiA* gene in the low copy number plasmid pCX16 led to a 1.7-fold increase in immunoreactive FtsZ (ranging from 1.4- to 2.1-fold in six independent measurements). Similar results were obtained when the amount of FtsZ was estimated using alkaline phosphatase-labeled second antibody. When introduced into an *ftsZ*84 mutant strain the *sdiA* plasmid increased the concentration of immunoreactive FtsZ protein by a similar amount (Table II).

Table II. Effect of *sdiA* on amount of immunoreactive FtsZ protein

Plasmid	Host	
	$ftsZ^+$	*ftsZ*84
pGB2	1.0	1.0
pCX16 [*sdiA*]	1.7	2.1
pUC19	1.0	nd
pCX19 [*sdiA*]	3.0	nd
pZAQ [*ftsQAZ*]	6.0	nd

The indicated plasmids were introduced into strain PB103 [$ftsZ^+$] or strain AW40 [*ftsZ*84]. Cells were grown either at 37°C (PB103) or at 42°C (AW40) for 2.5 h prior to immunoassay. Quantitative immunoblots were analyzed as described in Materials and methods. Concentrations are expressed relative to the values in cells containing the vector plasmids [pGB2 (lines 1 and 2) or pUC19 (lines 3–5)] and represent the means of six samples obtained from two independent experiments. The concentrations of immunoreactive FtsZ in PB103/pGB2 and AW40/pGB2 were approximately equal. nd, not done.

When the *sdiA* gene was present in a high copy number vector (in pCX19), the amount of FtsZ increased ~3-fold over wild type levels. For comparison, the well-studied high copy number pZAQ plasmid (*ftsQ ftsA ftsZ*) led to an increase in FtsZ of ~6-fold over wild type levels (Figure 5 and Table II), consistent with previous reports (Ward and Lutkenhaus, 1985).

These results demonstrate that increased gene dosage of *sdiA*, and—by implication—increased levels of the SdiA protein lead to an increase in the cellular concentration of FtsZ.

Effect of sdiA in cells that are already overproducing FtsZ

Introduction of *sdiA* plasmid pCX16 into a strain that was already overproducing FtsZ due to the presence of pZAQ led to the formation of many long filaments (data not shown). We ascribe this to a further increase in cellular FtsZ levels above the already high levels associated with pZAQ since it previously has been shown that division is blocked when the concentration of cellular FtsZ is increased >12-fold over the wild type level (Ward and Lutkenhaus, 1985).

Effects of sdiA on transcription of the ftsQAZ gene cluster

Several transcriptional promoters are associated with the *ftsQAZ* gene cluster (Figure 6) (Robinson *et al.*, 1984, 1986; Yi *et al.*, 1985; Aldea *et al.*, 1990). Four of these (P3–P6) are located within *ftsA*, leading to transcription of *ftsZ*, and two (P1 and P2) are located upstream of *ftsQ*, leading to transcription of *ftsQ*, *ftsA* and *ftsZ* (Aldea *et al.*, 1990). To determine whether the SdiA-induced increase in the cellular concentration of FtsZ might be due to transcriptional activation of one or more of the *ftsQAZ* promoters, we inserted each of the two main promoter groups upstream of a *lacZ* transcriptional probe in the mini-F plasmid pFZY (Koop *et al.*, 1987). β-galactosidase activity was then used

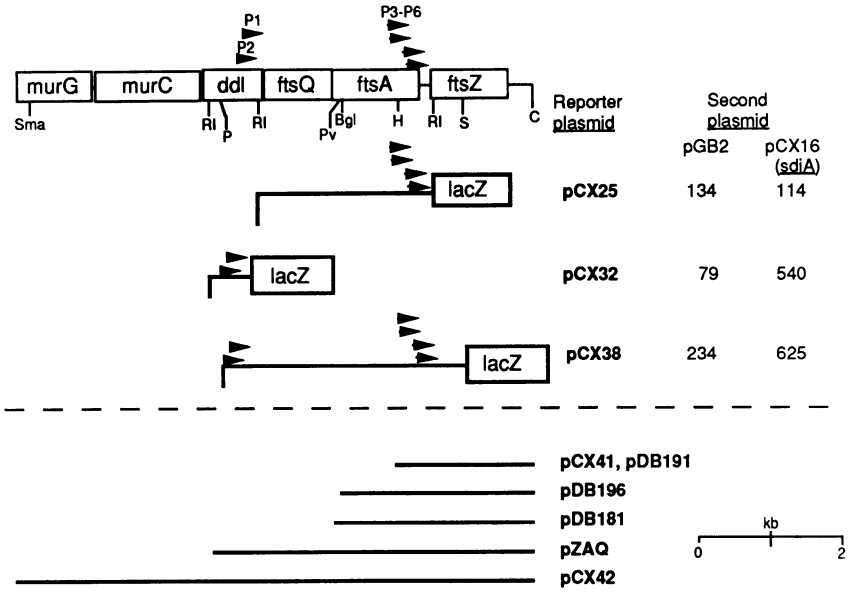

Fig. 6. LacZ expression from *ftsQAZ* transcriptional probes. The organization of the *ftsQAZ* region of the *E.coli* chromosome is shown at the top of the figure; the only restriction sites shown are those relevant to the present study. The positions of promoters P1–P6 are indicated by the arrowheads (Aldea *et al.*, 1990). The fragments that were cloned upstream of the *lacZ* transcriptional reporter in pFZY are indicated. The reporter plasmids were first introduced into strain UT481 and the second plasmid (either *sdiA* plasmid pCX16 or the pGB2 vector) was then transformed into each of the resulting strains. The two right-hand columns give the results of β-galactosidase assays performed on exponentially growing cells of each of the final strains. The lower portion of the figure shows the chromosomal inserts in other *ftsZ* plasmids used in the present study. Abbreviations: Bgl, *Bgl*II; H, *Hin*dIII; P, *Pst*I; PV, *Pvu*II; RI, *Eco*RI; S, *Sac*II; Sau, *Sau*96I; Sma, *Sma*I.

as a measure of *lacZ* expression to monitor the effects of the plasmid-borne *sdiA* gene on the *fts* promoter activities.

As shown in Figure 6, introduction of *sdiA* (in plasmid pCX16) led to a 5-fold increase in β-galactosidase from pCX32, which monitors transcription from the upstream promoters P1 and P2. In contrast, there was no detectable effect on *lacZ* expression from pCX25, which monitors transcription from promoters P3, P4, P5 and P6. Consistent with these results, the SdiA-induced increase in LacZ expression from pCX38, which monitors transcription from both promoter groups, was approximately the same as from pCX32 (391 versus 461 units). The pCX38 probe monitors transcription into *ftsZ*, thereby excluding the possibility that the regulated transcripts terminate prematurely within *ftsQ* or *ftsA*. These results showed that an increase in *sdiA* copy number leads to an increase in transcripts originating from P1 and/or P2.

Aldea *et al.* (Aldea *et al.*, 1990) have shown that the P1 promoter belongs to a class of promoters ('gearbox promoters') which are expressed at low levels in rapidly dividing cells but which respond to decreases in growth rate by proportionately increasing their activity, to a greater extent than the other *fts* promoters. In contrast, the P2 promoter is the promoter that is primarily responsible for transcription into *ftsQAZ* in actively growing cells (Aldea *et al.*, 1990).

Evidence that the SdiA-induced transcriptional activation was specific for transcripts originating from P2 came from experiments in which the two upstream promoters were cloned individually upstream of the *lacZ* transcriptional probe. Introduction of the *sdiA* plasmid pCX16 into cells containing the P2 probe (in pCX39) led to a 5- to 10-fold increase in *lacZ* expression. In contrast, there was no change in expression in cells containing the P1 probe (in pCX40) (Figure 7). Similar results were obtained from cells at all stages of growth, ranging from early exponential to late stationary phase. Thus, the *sdiA*-mediated transcriptional activation was highly specific for transcripts originating from the P2 promoter. As expected, the *sdiA* plasmids had no effect on expression from the gearbox promoter that lies upstream of the *bolA* gene (Aldea *et al.*, 1989) as measured by LacZ expression from appropriate P_{bolA}–*lacZ* transcriptional fusions (data not shown).

Effect of sdiA::Km^r mutation on ftsQAZ transcription

Introduction of the same transcriptional probes into *sdiA*$^+$ and *sdiA*::Km^r cells provided evidence that SdiA plays a role in maintaining the normal level of transcription from P2 during growth of wild type cells. Replacement of the chromosomal *sdiA*$^+$ gene of UT481 by *sdiA*::Km^r in strain WX2 led to a reproducible decrease of ~40% in expression from P2 (Table III). Expression from pCX32, which monitors expression into *ftsQAZ* from P1+P2 was decreased by a similar amount. There was no corresponding effect on *lacZ* expression from pCX40, which monitors expression from the P1 promoter alone, nor from pCX25, which monitors transcripts originating from promoters P3–P6.

Sequence similarities of sdiA to other transcriptional regulatory proteins

The carboxy-terminal portion of SdiA showed significant sequence similarity to several other bacterial proteins that are positive regulators of gene expression (Figure 8). Part of the region of similarity includes a motif resembling the helix–turn–helix DNA-binding domains that are present in certain DNA-binding proteins (Harrison and Aggarwal, 1990). Of the listed proteins (Figure 8), GerE has recently been shown to bind DNA specifically (L.Zheng, R.Halberg, L.Kroos and R.Losick, personal communication), and two of the other proteins (SigG and SigF) are sigma factors (Stragier and Losick, 1990) and thus can also be assumed to bind directly to DNA. It is not unlikely that the other

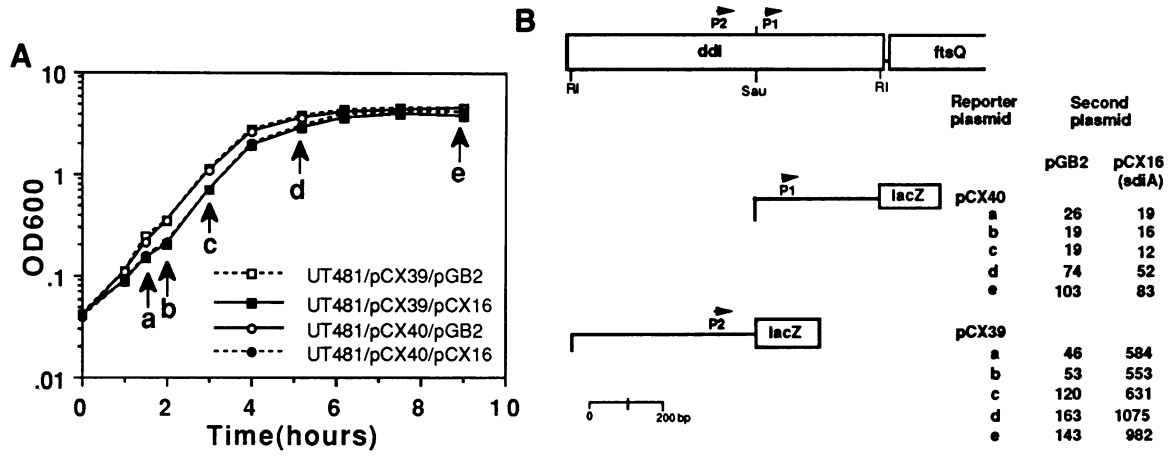

Fig. 7. LacZ expression in strains containing P1 and P2 promoter probes. (**A**) Strain UT481 containing each of the indicated plasmids was grown in LB and samples were removed for β-galactosidase assays at the points indicated by the letters a–e. Growth was followed by measuring optical density at 600 nm (OD600). (**B**) The region immediately upstream of *ftsQ* is shown, together with the relevant transcriptional probes. Samples were taken at different times (a–e) from the culture described in (A) and were assayed for β-galactosidase. See Figure 6 for further details.

proteins are also DNA binding proteins but, to our knowledge, this has not yet been directly demonstrated.

Discussion

The *sdiA* gene was isolated on the basis of its ability to counteract the global inhibition of division that results from expression of *minCD* in the absence of MinE. When cells containing the *sdiA* plasmids were further characterized, the most striking finding was that all of the observed effects of *sdiA* over-expression were identical to those induced by over-expression of *ftsZ*, including the ability to restore division activity to cells in which a mutant *ftsZ* gene product had been inactivated by temperature shift.

The possibility that SdiA is itself a division activator that can substitute for FtsZ was excluded by the inability of the *sdiA* plasmids to restore division activity to an *ftsZ* frame-shift mutant. Instead, the FtsZ-like effects of *sdiA* over-expression are explained by the ability of SdiA to increase transcription of the *ftsQAZ* gene cluster, thereby increasing the cellular concentration of the FstZ protein. The activation event should lead to an increase in *ftsQ* and *ftsA* mRNA as well as *ftsZ* mRNA. We do not yet know whether this is accompanied by an increase in concentration of the FtsQ and FtsA proteins or whether post-transcriptional controls act to maintain these two gene products at the levels that exist in normal cells.

To explain the restoration of division activity to *ftsZ*84 cells grown at 42°C, we presume that the thermoinactivated FtsZ84 protein retains sufficient residual activity so that the 1.5- to 2-fold increase in FtsZ concentration induced by the *sdiA* plasmids raises total FtsZ activity to a level sufficient to support septum formation.

The transcriptional activation was promoter-specific, affecting transcripts originating from P2 but not from P1 nor from any of the several promoters embedded within the *ftsQAZ* cluster. The P1 and P2 promoters respond to different regulator signals, P2 responding directly or indirectly to the *sdiA* gene product whereas P1 responds to an as yet undefined sensor of growth rate (Aldea *et al.*, 1990). Therefore, the presence of two upstream promoters provides a mechanism whereby multiple regulatory inputs can be funneled into the same *ftsQAZ* transcriptional unit. Other regulatory molecules that impact on the two promoters may well remain to be discovered.

Table III. β-galactoside activity from transcriptional probes in *sdiA*$^+$ and *sdiA*::Km^r cells

Plasmid	Strain		*P* value
	UT481 (*sdiA*$^+$)	WX2 (*sdiA*::Km^r)	
pCX32 (P1+P2)	78 (5.1)	45 (10.7)	0.001
pCX25 (P3–P6)	121 (15.1)	113 (3.9)	
pCX39 (P2)	102 (6.8)	62 (4.5)	<0.001
pCX40 (P1)	19 (2.8)	17 (1.3)	
pCX38 (P1–P6)	240 (24.8)	206 (13.8)	0.004

The indicated plasmid-containing strains were assayed for β-galactosidase in mid-exponential growth; the *ftsQAZ* promoters present in each of the plasmids are shown in parentheses. The values are the mean values obtained from four independent experiments. The numbers in parentheses represent one standard deviation from the mean. *P* values represent the probability that the differences between the experimental values were due to chance alone, determined by *t* test analysis. Where *P* values are not shown the values were not significant ($P > 0.4$).

The increased transcription monitored by the P2 probes could reflect an increased rate of initiation of transcription from the P2 promoter, or could result from a decrease in premature termination or an increase in stability of mRNA originating from P2. The sequence similarity between SdiA and several other bacterial transcriptional regulatory proteins suggests that SdiA may act directly on the transcriptional apparatus. Further experiments will be needed to distinguish between these and other possibilities.

Replacement of the chromosomal *sdiA*$^+$ gene by the *sdiA*::Km^r allele was associated with a decrease in expression from the P2 transcriptional probe to approximately half of the level seen in wild type cells, reducing transcription into *ftsQ* and *ftsA* by 40–50%. This confirms that the *sdiA* gene product is expressed during normal growth and plays a role in establishing the steady state level of transcription of the *ftsQAZ* gene cluster. On the other hand, *sdiA*::Km^r mutant cells showed no apparent division defect. Thus, the SdiA protein appears not to be the only factor responsible for maintaining the *fts* gene products at levels that are adequate to support septum formation under normal growth conditions, suggesting that the protein may be one of several

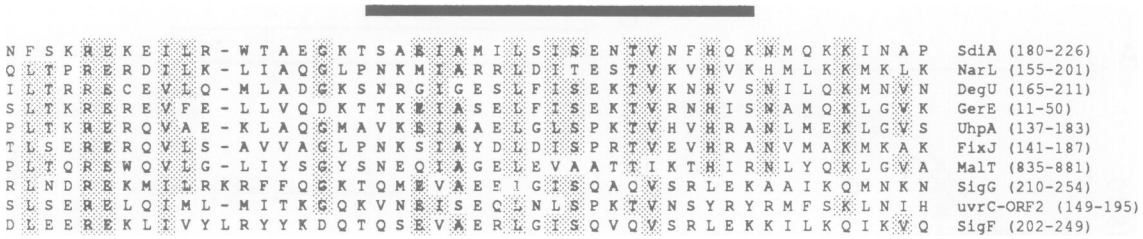

Fig. 8. Sequence homologies. Amino acid residues 180–226 of SdiA are aligned to the indicated regions of several other bacterial transcription regulatory proteins. Amino acid residues that are identical in at least five of the ten proteins are boxed. A region resembling the helix–turn–helix DNA binding domain of several known DNA binding proteins is indicated by the bar. No significant similarities between SdiA and the other proteins were found in other regions of the SdiA sequence. References: *sdiA* and *uvrC*-ORF2, Sharma *et al.* (1986); *narL*, Stewart *et al.* (1989); *degU*, Henner *et al.* (1988); *gerE*, Holland *et al.* (1987); *uhpA*, Friedrich and Kadner (1987); *fixJ*, David *et al.* (1988); *MalT*, Cole and Raibaud (1986); *sigG*, Karmazyn-Campelli *et al.* (1989); *sigF*, Yudkin (1987).

Table IV. Bacterial strains and phages

	Relevant characteristics	Source/reference
Strains		
AB1157	*ftsZ*$^+$ *leu*	H.Wu
AB1884	*uvrC*34	Howard-Flanders *et al.* (1966)
AW40	*ftsZ*84Ts	This study[a]
JFL100	*ftsZ*84Ts *leu*	Lutkenhaus *et al.* (1980)
PB103	wild type	de Boer *et al.* (1988)
PB114	Δ*minCDE* Km^r	de Boer *et al.* (1989)
SG13107	*leu*::Tn*10*	Gottesman *et al.* (1981)
UT481	Δ*lac*	G.Carmichael
WX2	as UT481 + *sdiA*::Km^r	This study
WX4	as PB114 + *ftsZ**	This study
WX6	as PB114 + *ftsZ** *leu*::Tn*10*	This study
WX7	as PB103 + *ftsZ** *leu*::Tn*10*	This study
Bacteriophages		
λDB173	P_{lac}::*minCD*	de Boer *et al.* (1989)
λDB182	P_{lac}::*dicB*	de Boer *et al.* (1990b)

[a]The temperature-sensitive *ftsZ*84 mutation was transferred from strain JFL100 into AB1157 by P1-mediated transduction, selecting for leucine prototrophy and then screening transductants for filamentation after growth at 42°C.

regulatory elements that modulate the levels and activity of the essential division proteins.

Why would the cell need to regulate the amount of these division-related proteins? We consider the following two possibilities.

First, the triggering of septation at the proper time in the cell cycle could be mediated by promoter-specific transcriptional modulators such as SdiA, which acted at defined points in the cell cycle, thereby effecting cyclical changes in concentrations of the *fts* gene products. This possibility cannot be excluded since it is not known whether the levels of the FtsQ, FtsA or FstZ proteins vary cyclically during the division cycle.

On the other hand, if septation were triggered by activation of pre-existing division proteins rather than by a change in their rate of synthesis, there would be no need to activate transcription of division-related genes cyclically. Even if this were correct, however, the amounts of the critical gene products would still require careful regulation since relatively narrow fluctuations in concentration of FtsZ can significantly alter the division pattern of the cell. A decrease of the cellular concentration of FtsZ to 30% of wild type levels results in a complete cessation of sepatation (Dai and Lutkenhaus, 1991) whereas a 1.5- to 2-fold increase in FtsZ concentration leads to minicell formation, reflecting the formation of septa at the cell poles in addition to those at midcell (Ward and Lutkenhaus, 1985; and this study). Paradoxically, still higher levels of FtsZ are again associated with a division block (Ward and Lutkenhaus, 1985; and this study). Thus, the amount of FtsZ, and probably of FtsA as well (Wang and Gayda, 1990), must be maintained within narrow limits for normal cell division to proceed. One role of the observed SdiA-mediated transcriptional regulation could be to ensure that the levels of these essential proteins are maintained within permissible limits over a wide range of physiological conditions.

Specific regulators may also act on the internal promoters that are located within *ftsA* (P3–P6 in Figure 6). Transcriptional probe experiments have shown that transcription from one or more of these promoters increases significantly when a mutant *ftsA* gene product is synthesized by the cell (Dewar *et al.*, 1989), implying that the level of functional FtsA protein feeds back on transcription into *ftsZ*. It has also been suggested that transcription from the internal promoters might be regulated by the DnaA protein, based on the presence of putative DnaA binding sites with *ftsA* (Masters *et al.*, 1989). It would not be surprising if other as yet unidentified factors existed to modulate the activity of these promoters, whose activation would specifically increase the expression of *ftsZ*.

The approach used in the present study can be expected to identify factors of this type, as well as other factors that may positively regulate the division process at the transcriptional or post-transcriptional level.

Materials and methods

Growth conditions

Bacterial strains and bacteriophages are listed in Table IV. Cells were grown in L-broth (LB) at 37°C unless otherwise indicated.

Plasmids

pGB2 is a derivative of pSC101 [copy number ~15 (G.Churchward, personal communication)] that contains the *aadA* gene imparting spectinomycin-resistance to cells containing the plasmid (Churchward *et al.*, 1984). pFZY is a mini-F derivative (average copy number 1–2 per cell) with a polycloning site upstream of a promoterless *galK'-lacZYA* reporter segment (Koop *et al.*, 1987). pMAK700 and pMAK705 are chloramphenicol-resistant derivatives of pSC101 that contain a temperature-sensitive *rep* mutation which prevents them from replicating in cells grown at 44°C (Hamilton *et al.*, 1989). pDB181 is a pBR322 derivative that contains the 2.7 kb *Pvu*II–*Cla*I fragment of pZAO (Figure 6). pDB191 is a pBR322 derivative in which the chromosomal insert of pZAQ was replaced by the same *Hin*dIII–*Cla*I fragment as pCX41 (Figure 6). pDB196 is a pBR322 derivative that contains the *Bgl*II–*Cla*I fragment of pZAQ (Figure 6) (de Boer *et al.*, 1990b). pDB192 is a pMLB1113 derivative which contains *sfiA* under control of P_{lac} (de Boer *et al.*, 1990b). pCX41 is a pMAK700 derivative carrying the 1.9 kb *Hin*dIII–*Cla*I fragment which includes that intact *ftsZ* gene (Figure 6), isolated as one of the resolved plasmids obtained during isolation of the *ftsZ** chromosomal mutation (see below).

pCX2 (Figure 1) and pCX42 (Figure 6) are recombinant plasmids that impart resistance to MinC/MinD, isolated as described below. pCX11, pCX12 and pCX13 were prepared from pCX2 by deletion of segments of the chromosomal insert, as indicated in Figure 1. pCX15: the 5.5 kb *Bam*HI–*Eco*RI fragment from pCX2 was ligated into pGB2 that had been digested with *Eco*RI and *Bam*HI. pCX16: the 1.3 kb *Pst*I fragment from pCX15 was ligated into the *Pst*I site of pGB2. pCX17: same as pCX16 but in opposite orientation. pCX19: the 1.5 kb *Bam*HI–*Hin*dIII fragment from pCX2 was ligated into pUC19 that had been digested with *Bam*HI and *Hin*dIII.

fts::lacZ transcriptional fusions. (Figures 6 and 7). pCX25: the 2.3 kb *Eco*RI fragment from pZAQ was ligated into *Eco*RI-digested pFZY. pCX32: the 0.84 kb *Eco*RI fragment from pCX42, containing the entire *ftsZ* gene and the promoter-distal portion of *ftsA*, was ligated into *Eco*RI-digested pFZY. pCX38: the 3.4 kb *Pst*I–*Sac*II fragment from pZAQ was treated with T4 DNA polymerase to remove the 3′ overhangs. The resulting blunt-ended fragment was then ligated into pFZY which had previously been digested with *Bam*HI and treated with the Klenow fragment of *E.coli* polymerase I in the presence of nucleoside triphosphates. pCX39: the 0.84 kb *Eco*RI fragment from pCX42, containing the *ftsQAZ* P1 and P2 promoters (Figure 6), was digested with *Sau*96I and treated with Klenow fragment in the presence of dGTP and dCTP, yielding 0.35 kb and 0.49 kb fragments with one blunt end and one *Eco*RI overhang. The two fragments were separately ligated into *Eco*RI–*Eco*RV-digested pBluescriptKS (Stratagene), yielding pCX35 (containing the *ftsQAZ* P2 promoter) and pCX36 (containing the *ftsQAZ* P1 promoter). The 0.51 kb *Bam*HI–*Hin*dIII fragment from pCX35 was then ligated into *Bam*HI–*Hin*dIII-digested pFZY, yielding pCX39. pCX40: the 0.4 kb *Bam*HI–*Kpn*I fragment from pCX36 (see above) was ligated into *Bam*HI–*Kpn*I-digested pFZY.

Construction of sdiA::Km^r plasmids. pCX21: the 1.7 kb *Bam*HI fragment from pDB102::T_{mk}17 (de Boer *et al.*, 1988), which includes the kanamycin-resistance element of T_{mk} (Way *et al.*, 1984), was ligated into the *Bam*HI site of pBluescriptKS (Stratagene). This positioned the Km^r element between the *Kpn*I and *Not*I sites in the polycloning region of pBluescript. pCX22: to destroy the *Kpn*I site in pMAK705, the plasmid was digested with *Kpn*I, treated with mung bean nuclease to remove the 3′ overhang and religated by treatment with DNA ligase. pCX23: the 1.5 kb *Bam*HI–*Hin*dIII fragment from pCX2, which includes the entire *sdiA* gene, was ligated into pCX22 that had been digested with *Bam*HI and *Hin*dIII. The chromosomal insert was identical to that of pCX19. pCX24: the 1.7 kb *Kpn*I–*Not*I fragment from pCX21, which contains the Km^r element, was ligated to the 6.5 kb *Kpn*I–*Not*I fragment from pCX23, thereby replacing most of the *sdiA* gene with Km^r. The chromosomal insert in the resulting pMAK705 derivative retains 195 bp from the 5′ end of *sdiA* and 30 bp from the 3′ end, separated by the Km^r element (Figure 1).

Cloning of chromosomal genes that suppress MinC/MinD-induced filamentation

Chromosomal DNA from strain PB114 was subjected to incomplete digestion with *Sau*3A. DNA fragments of ~6–9 kb were eluted from an agarose gel and ligated to *Bam*HI-digested and phosphatase-treated DNA from pGB2 (Churchward *et al.*, 1984). The total ligation mixture was transformed into strain PB114 [Δ*minCDE*] that was lysogenic for λDB173 [P_{lac}-*minCD*]. In this strain, growth in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG) leads to the prompt cessation of septation. To select transformants resistant to MinC/MinD-induced filamentation, the transformation mixture was grown for 60 min in LB and then plated on LB plates containing 0.25 mM IPTG. This concentration of IPTG was shown in preliminary experiments to kill >99% of untransformed cells. Recombinant plasmids were isolated from the IPTG resistant cells and the ability of the plasmids to suppress IPTG-induced filamentation was confirmed by retransforming them into a new PB114(λDB173) recipient. The resulting transformants were capable of dividing in the presence of IPTG whereas, as expected, exposure to IPTG led to filamentation in cells transformed with the vector plasmid pGB2.

Construction of chromosomal sdiA::Km^r mutant

The chromosomal *sdiA* gene was replaced by the *sdiA*::Km^r allele from pCX24 [*sdiA*::Km^r Cm^r] by the method of Hamilton *et al.* (Hamilton *et al.*, 1989), using the chromosomal sequences that flank the Km^r element in pCX24 as sites for homologous recombination into the *sdiA* gene. The method makes use of the temperature-sensitive replication defect of pMAK derivatives such as pCX24 to permit selection of cells in which the plasmid has integrated into the chromosome. To select for cells in which the *sdiA*::Km^r plasmid had integrated into the chromosome, pCX24 was introduced into strain UT481 and transformants were grown at 44°C in the presence of kanamycin and chloramphenicol. These cells presumably contained one copy of the chromosomal *sdiA*$^+$ gene and one copy of the integrated plasmid containing the *sdiA*::Km^r allele. Survivors were purified and grown for 48 h in LB in the presence of kanamycin and chrloramphenicol at 30°C to permit replication of the chromosomally integrated plasmid. Cells that contained resolved plasmids that had been excised from the chromosome were identified by screening for colonies that were chloramphenicol-resistant at 30°C but chloramphenicol-sensitive at 44°C because of the inability of the plasmid to replicate at 44°C.

Approximately half of these isolates were expected to be identical to the original pCX24 plasmid (*sdiA*::Km^r), leaving an intact *sdiA*$^+$ allele in the chromosome. These were identified on the basis of their kanamycin resistance at 30°C and kanamycin sensitivity at 44°C. The presence of the predicted *sdiA*::Km^r plasmid was confirmed by restriction mapping. These isolates were not further studied. The other half were expected to contain the *sdiA*$^+$ gene on the plasmid, leaving the *sdiA*::Km^r allele in the chromosome. This class was presumptively identified on the basis of their resistance to kanamycin at both 30°C and 44°C. As predicted, the plasmids isolated from these cells were identical to pCX23(pMAK705::*sdiA*$^+$). Plasmid-free clones were then isolated by growing the cells at 44°C and screening for cells that were chloramphenicol-sensitive at both 30°C and 44°C. The loss of the chromosomal *sdiA*$^+$ gene and the presence of the chromosomal *sdiA*::Km^r allele were confirmed by Southern hybridization. The resulting *sdiA*::Km^r strain was named WX2. P1 transduction was used to transfer the *sdiA*::Km^r allele from WX2 into other strains, selecting for resistance to kanamycin.

Construction of chromosomal ftsZ frameshift mutation

Four bases were inserted at the *Eco*RI site located 108 bp downstream of the translational start of the *ftsZ* gene in pDB181 by digesting the plasmid with *Eco*RI, filling in the overhangs by treatment with Klenow fragment and nucleoside triphosphates and religating the resulting fragment, yielding pCX29. This leads to a translational frame shift at the previous *Eco*RI site, generating a TAA termination codon 36 bp further downstream. It also generates a new *Xmn*I site within the filled in segment. The loss of *ftsZ* function was confirmed by the inability of pCX29 to correct the temperature-sensitive division defect of strain AW40 [*ftsZ*84Ts] or to induce minicell formation in wild type cells. The 2.3 *Hin*dIII fragment from pXC29, which extends from the *Hin*dIII site near the 5′ end of *ftsA* (see Figure 6) to the *Hin*dIII site in the vector, was then ligated into *Hin*dIII-digested pMAK700, yielding pCX30. The frameshifted *ftsZ* allele is referred to as *ftsZ**.

The strategy for replacement of the chromosomal *ftsZ* gene by the mutant *ftsZ** allele from pCX30 was similar to that described above for the chromosomal insertion of the *sdiA*::Km^r allele. The host strain was PB114 [Δ*minCDE*] lysogenic for λDB173 [P_{lac}-*minC minD*]. Because of the absence of MinE, the low levels of MinC and MinD that are present in this strain even in the absence of IPTG give a selective advantage to cells containing *ftsZ*$^+$ plasmids, thereby facilitating the selection for the desired cells at the plasmid resolution stage as described below. Cells in which pCX30 had integrated into the chromosome were isolated and purified by growth at 44°C in the presence of chloramphenicol. Cell containing resolved plasmids were selected by growth at 30°C for 48 h in the presence of chloramphenicol. Survivors were then screened to identify colonies that were chloramphenicol-resistant at 30°C and chloramphenicol-sensitive at 44°C. About half of these isolates contained the desired *ftsZ*$^+$ plasmid (pCX41

in Figure 6). This implied that the *ftsZ** gene from the original pCX30 plasmid had replaced the chromosomal *ftsZ*$^+$ allele. This was confirmed by Southern hybridization analysis which showed loss of the *Eco*RI site and appearance of the new *Xmn*I site at the predicted location. This yielded strain WX4(λDB173)/pCX41 [*ftsZ**(P_{lac}::*minCD*)/*ftsZ*$^+$]. The remaining isolates contained plasmids that were indistinguishable from pCX30 and were not further studied.

To facilitate transfer of the *ftsZ** mutation from WX4 to other strains, a closely linked *leu*::Tn*10* allele was introduced into WX4/pCX41 by P1-mediated transduction from SG13107, yielding WX6/pCX41. The *ftsZ** and *leu*::Tn*10* alleles were then cotransduced from WX6 into PB103/pCX41 [*ftsZ*$^+$/*ftsZ*$^+$]. Tetracycline-resistant transductants were selected at 30°C and were screened for clones that failed to divide and formed filaments when grown at 44°C. This yielded strain WX7 [*ftsZ**]/pCX41.

LacZ assays

Overnight culutres were diluted 100-fold into LB containing the appropriate antibiotics, and were grown at 37°C with vigorous aeration to mid-exponential growth (optical density at 600 nm ~0.7). Samples were assayed for β-galactosidase as described by Miller (Miller, 1972). Values from three or four independent experiments were averaged to give the values shown in the figures and tables.

Immunoassays

Unless otherwise noted, cells were grown in LB at 37°C and harvested in mid-exponential growth at an optical density at 600 nm of ~1.0. When *ftsZ*84 cells were studied the cultures were grown at 30°C to an optical density at 600 nm of ~0.1 and then shifted to 42°C for 2.5 h before harvesting. Cells were suspended in 4% SDS, 0.125 M Tris–HCl (pH 6.8) to a density of ~1.6×10^{10} cells/ml and heated in a boiling water bath for 10 min. Protein concentration was determined using the Bio-Rad Protein Assay reagent (Bradford, 1976) after diluting an aliquot of the suspension to <0.2% SDS. The samples were then subjected to SDS gel electrophoresis and electrophoretically transferred to a nitrocellulose filter. Samples (17, 34, 51 and 68 μg protein) prepared from wild type strain PB103 were analyzed on the same gel to construct a standard curve, permitting the results to be expressed relative to the concentration of FtsZ in wild type cells. The filter was then successively exposed to a rabbit polyclonal antiserum against FtsZ (a gift from J.Lutkenhaus), to rabbit anti-OmpA antiserum (a gift from M.J.Osborn) and to [^{125}I]protein A. The positions of the FtsZ and OmpA bands were determined from autoradiographs, the bands were cut from the filters and radioactivity in each band was determined in a gamma counter. C.p.m. in the FtsZ band were expressed relative to c.p.m. in the OmpA band to normalize for possible variations in the amount of material applied to the different lanes. In some experiments anti-OmpA was omitted and goat anti-rabbit IgG coupled to alkaline phosphatase was used in place of [^{125}I]protein A. The intensity of the stained bands was determined densitometrically. In both the [^{125}I]protein A and phosphatase-labeled antibody assays the graph of protein concentration versus intensity of the FtsZ band was linear over the concentration range used in the experiments.

Other methods

For IPTG induction of strains containing P_{lac}::*minCD*, P_{lac}::*dicB* or P_{lac}::*sfiA*, the cells were grown overnight at 37°C and then diluted 100-fold into LB either in the absence or presence of 25 μM (for P_{lac}::*minCD* and P_{lac}::*dicB*) or 50 μM (for P_{lac}::*sfiA*) IPTG and grown with vigorous shaking for 4 h at 37°C (to an OD_{600} of ~1.6) before examination by phase contrast microscopy. Cell lengths and cell morphology were determined from phase contrast micrographs of glutaraldehyde-fixed cells (Cook *et al.*, 1986). Sensitivity of strains to ultraviolet light was tested as previously described (Gottesman *et al.*, 1981). Other procedures were performed as described by Maniatis (Maniatis *et al.*, 1982) and Miller (Miller, 1972). Proteins having sequence similarity to SdiA were identified by searching the NBRF Protein Identification Resource data base, release 15.0 (George *et al.*, 1986).

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