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Genetics

Central role for the *Escherichia coli minC* gene product in two different cell division-inhibition systems

(minicells/*minB*/*dicB*/*ftsZ*/*sfiA*)

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ABSTRACT **In *Escherichia coli*, selection of the proper division site at midcell requires the specific inhibition of septation at two other potential division sites, located at each of the cell poles. This site-specific inhibition of septation is mediated by the gene products of the minicell locus (the *minB* operon) that includes three genes, *minC*, *minD*, and *minE*. In this paper we show that one of the components of this division-inhibition system, the *minC* gene product, is also an essential component of another division-inhibition system, which is induced by derepression of the *dicB* gene and leads to inhibition of septation at all potential division sites. The two *minC*-dependent division-inhibition systems could be functionally distinguished by their different responses to the *minE* gene product. The results suggest a model in which a common mechanism, mediated by MinC, is responsible for the division block in a class of division-inhibition systems that can be independently activated by different proteins that determine the specific properties of these systems.**

Cell division in *Escherichia coli* is a complex process that must be regulated at several levels. Temporal regulation is required to ensure that septum formation not occur before chromosome replication is completed, and topological regulation is required to ensure that the septum is formed at the midpoint of the cell to permit the equipartition of cytosolic components into daughter cells. One way in which this is accomplished is by the controlled production of endogenous cell division inhibitors.

It has been shown (1) that such an inhibitor acts during normal cell growth to ensure that septation is limited to the proper site at midcell. This site-specific inhibition system is a product of the minicell genetic locus (the *minB* operon) that includes three genes, *minC*, *minD*, and *minE* (*minCDE*). Under normal conditions, coordinate expression of *minC* and *minD* leads to formation of a potent cell division inhibitor that is given topological specificity by MinE. As a result, septation is permitted at midcell but is blocked at two other potential division sites that are located at the cell poles. It has been suggested that the polar sites are remnants of division sites that were present at midcell during preceding cell cycles (1, 2). When MinE is absent or when *minC* and *minD* are overexpressed, septation is inhibited at all potential division sites, leading to filamentation. In the absence of *minC* or *minD* expression or in the presence of excess MinE, septation is not prevented at the polar sites, resulting in the formation of anucleate minicells. Therefore, the balanced expression of the *minCD* division inhibitor and the *minE* gene product are necessary to maintain the normal division pattern (1, 3).

Other known proteins that lead to inhibition of cell division in *E. coli* are only produced or activated under special circumstances. The best known of the inducible cell division inhibitors is SfiA. This protein is induced as part of the SOS response to DNA damage. As a result, cell division is delayed until the damage has been repaired (for reviews, see refs. 4 and 5).

Recently, Béjar and Bouché (6) identified another cell division-inhibition gene, *dicB*. Derepression of *dicB* leads to inhibition of septation at all potential division sites, resulting in formation of long nonseptate filaments (6, 7).

A possible relationship between the *dicB* division-inhibition system and the *minB* locus was suggested by the observation of Labie *et al.* (8) that some mutations that suppress the ability of *dicB* to cause division inhibition confer a minicell phenotype upon the host and map in or near *minB*. This suggested that the *minCD* and the *dicB* division-inhibition systems might share some common component.

In the present paper, we demonstrate that the *minC* gene product is an essential component of the *dicB* division-inhibition system. Division inhibition by both MinCD and MinC/DicB were suppressed by high levels of expression of the cell division gene *ftsZ*, and in neither case was the division inhibition mediated by the SOS response-linked SfiA protein. Nevertheless, the MinCD and MinC/DicB systems could be distinguished by their different responses to the *minE* gene product. The results suggest a model in which MinC is the effector of the division-inhibition process in both systems, with the MinD and DicB proteins serving as independent activators of the MinC-mediated inhibition mechanism. Therefore, the *minC* gene product defines a group of cell division-inhibition systems—unrelated to the well-studied SOS-mediated division-inhibition system—in which a single effector protein can respond to several unrelated activators. The activator protein, in turn, determines the specific properties of the system, leading to either a global inhibition of division at all potential sites or to the topologically restricted inhibition of septum formation at polar sites.

MATERIALS AND METHODS

Media and Strains. Cells were grown in LB medium at 37°C. *E. coli* K-12 strains used were N100 (*pro recA*), GC579 (*sfiA11 thr leu pro his gal rpsL*), JS279 (Δ*lacIZYA*X74 *hsdRrpsL* Δ*dicABC* P_{malP}::lacI^q Sm^r Spc^r, where *P* = promoter and Sm^r and Spc^r = resistance to streptomycin and spectinomycin, respectively), PB103 (*minB*$^+$ *dadR trpE trpA tna*) (3), PB114 [as PB103 but Δ*minCDE* and kanamycin-resistant (Km^r)] (1), PB117 (as PB103 but Δ*dicABC* Sm^r and Spc^r), and PB128 (as PB114 but Δ*dicABC* Sm^r and Spc^r). PB117 was constructed by phage P1-mediated transduction of Δ*dicABC* from JS279 into PB103. PB128 was constructed by transducing Δ*minCDE* from PB114 into PB117.

Phages. λDB156, λDB164, and λDB170–λDB175 (see Table 2) have been described (1). λDB182 (*bla*$^+$ *lacI*^{q} P_{lac}::*dicB*$_s$

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Abbreviations: *dicB*$_s$, 3′ part of the *dicB* gene; *P*, promoter; IPTG, isopropyl β-D-thiogalactoside.

lacY$^+$)—the 271-base-pair (bp) *Hin*dIII/*Eco*RI fragment from pKC17, which represents the 3′ part of the *dicB* gene ($dicB_s$) and encodes the 7-kDa C-terminal peptide $DicB_s$ (7, 8)—was ligated to *Hin*dIII/*Eco*RI-digested pMLB1115 (1). This yielded plasmid pDB182 (not shown). The P_{lac}::$dicB_s$ region was subsequently crossed from pDB182 onto phage λNT5 as described (1).

Plasmids. *pGB2.* Vector plasmid pGB2 is a pSC101 derivative that contains a polylinker sequence downstream of the *aadA* gene, which confers resistance to spectinomycin and streptomycin (9). Significant transcription, presumably stemming from *aadA* transcription, occurs through the polylinker region.

pDB183. The 382-bp *Hin*dIII/*Eco*RI fragment from pDB151 (1) containing the complete *minE* coding sequence was ligated to *Hin*dIII/*Eco*RI-digested pGB2, thereby placing *minE* downstream of *aadA*.

pDB184. The same 271-bp *Hin*dIII/*Eco*RI $dicB_s$ fragment from pKC17 that was used to construct λDB182 (see above) was ligated to *Hin*dIII/*Eco*RI-digested pGB2, putting $dicB_s$ downsteam of *aad*.

pZAQ. Plasmid pZAQ (*ftsQ ftsA ftsZ*) has been described (10).

pDB109. The 2284-bp *Eco*RI fragment of plasmid pZAQ was deleted, thereby removing the whole of *ftsQ* and *ftsA* and the 5′ part of *ftsZ*.

pDB196. The ≈1600-bp *Pst* I/*Bgl* II fragment of pZAQ was replaced with the 14-bp *Pst* I/*Bam*HI polylinker fragment of pUC13, thereby removing the whole of *ftsQ* and the 5′ part of *ftsA*.

pDB192. The ≈850-bp *Eco*RI/*Hin*dIII fragment from pGC165sfiA$^+$ (11) containing the whole *sfiA* open reading frame was ligated to *Eco*RI/*Hin*dIII-digested pMLB1113 (1), thereby placing *sfiA* expression under control of P_{lac}.

Determination of Cell Phenotype. Cells were grown in LB medium until the cultures untreated with isopropyl β-D-thiogalactoside (IPTG) reached early stationary growth phase (six to seven cell doublings, OD_{600} = 1.5–2.0). Cell phenotype was determined as described (1).

RESULTS

Division Inhibition by $DicB_s$ Requires the *minB* Locus. Evidence that *dicB*-induced division inhibition was mediated by components of the minicell locus was obtained by studying the effects of *dicB* expression in *minB*$^+$ cells and in cells deleted for the entire *minB* operon. In these experiments, *dicB* expression was induced by IPTG treatment of Δ*dicB* strains containing either single or multiple copies of the same P_{lac}::$dicB_s$ transcriptional fusion. The fusion links P_{lac} to an internal, in-phase translation start codon in *dicB*, resulting in synthesis of the 7-kDa C-terminal peptide, $DicB_s$, that appears to be responsible for the *dicB*-induced filamentation phenotype (7, 8).

Expression of $dicB_s$ from a single copy of P_{lac}::$dicB_s$ in a *minB*$^+$ strain [PB117 (λDB182)] resulted in the formation of long nonseptate filaments, confirming the observations of Cam *et al.* (7) that the $dicB_s$ gene product acts as a division inhibitor in wild-type strains. In contrast, induction of $dicB_s$ failed to induce filamentation in strains containing a complete deletion of the *minB* locus [PB128 (λDB182)] (Table 1). The minicell phenotype of the Δ*minB* host was unaffected even when $dicB_s$ was expressed at high levels from the high-copy-number plasmid pKC17 (P_{lac}::$dicB_s$) by growth in the presence of saturating concentrations of IPTG (3 mM).

To exclude the possibility that the failure to observe filamentation reflected changes in the P_{lac}::$dicB_s$ plasmid, plasmid DNA was isolated from the pKC17 transformants and reintroduced into the *minB*$^+$ strain (PB117). In all cases the transformants again displayed the IPTG-dependent filamentation phenotype, confirming the continued presence of a functional $dicB_s$ gene on the plasmid.

Table 1. Effects of *dic* and *minB* deletions on $DicB_s$- and MinCD-induced division inhibition

Strain	Relevant markers	Phenotypes − IPTG	Phenotypes + IPTG
PB117 (λDB182)	Δ*dicB* (P_{lac}::$dicB_s$)	wt	Sep$^-$
PB128 (λDB182)	Δ*dicB* Δ*minCDE* (P_{lac}::$dicB_s$)	Min$^-$	Min$^-$
PB114 (λDB173)	Δ*minCDE* (P_{lac}::*minCD*)	Min$^-$	Sep$^-$
PB128 (λDB173)	Δ*dicB* Δ*minCDE* (P_{lac}::*minCD*)	Min$^-$	Sep$^-$

Cells lysogenic for the indicated phages (first column, in parentheses) were grown in LB medium containing either 0 or 0.1 mM IPTG. The relevant genetic markers of the host and phage (in parentheses) are indicated in the second column. wt, Wildtype; Min$^-$, minicell phenotype; Sep$^-$, filamentation.

The inability of $dicB_s$ to induce filamentation in the absence of the *minB* locus indicated that the *dicB*-induced division block is dependent on expression of one or more of the three gene products (MinC, MinD, and MinE) encoded by the *minB* operon.

The converse is not true—that is, the *minCD*-induced division block is not dependent on the presence of a functional *dicB* locus. This was shown by the observation that Δ*dicB* cells and *dicB*$^+$ cells were equally sensitive to the *minCD*-induced division block (Table 1, lines 3 and 4).

Inhibition of Cell Division by $DicB_s$ Is Dependent on Expression of *minC*. To determine what part of the *minB* locus was required for the $dicB_s$-induced division inhibition, we studied the effects of $dicB_s$ expression in strains in which one or more of the *minB* genes were expressed under P_{lac} control (Table 2). In these experiments, $dicB_s$ was expressed constitutively from a plasmid (pDB184) in which $dicB_s$ was placed downstream from the *aadA* gene of the pSC101 derivative pGB2. In constructs of this type, transcriptional read-through, presumably from the *aadA* promoter, results in significant levels of expression of downstream genes. The level of $dicB_s$ expression from this plasmid was sufficient to block cell division, as indicated by our inability to transform the plasmid into *minB*$^+$ strains (data not shown) and as shown more definitively by the results presented below.

The presence of the *aadA*::$dicB_s$ plasmid in PB114 (λDB170) [Δ*minCDE* (P_{lac}::*minCDE*)] caused filamentation only when expression of *minCDE* was induced by the addition of IPTG to the growth medium (Table 2). This confirmed the dependence of the $DicB_s$ phenotype on expression of one or more components of the minicell locus. As shown in Table

Table 2. $DicB_s$-induced division inhibition is dependent on coexpression of *minC*

Phage	Phenotype of host: pGB2 (vector) − IPTG	pGB2 (vector) + IPTG	pDB184 (*aadA*::$dicB_s$) − IPTG	pDB184 (*aadA*::$dicB_s$) + IPTG
λDB170 P_{lac}::*minCDE*	Min$^-$	wt	Min$^-$	Sep$^-$
λDB171 P_{lac}::*minC*	Min$^-$	Min$^-$	Min$^-$	Sep$^-$
λDB164 P_{lac}::*minD*	Min$^-$	Min$^-$	Min$^-$	Min$^-$
λDB156 P_{lac}::*minE*	Min$^-$	Min$^-$	Min$^-$	Min$^-$
λDB173 P_{lac}::*minCD*	Min$^-$	Sep$^-$	Min$^-$	Sep$^-$
λDB174 P_{lac}::*minCE*	Min$^-$	Min$^-$	Min$^-$	Sep$^-$
λDB175 P_{lac}::*minDE*	Min$^-$	Min$^-$	Min$^-$	Min$^-$

Strain PB114 (Δ*minCDE*), lysogenic for the indicated phages (first column) and carrying either a plasmid from which *dicB* is constitutively expressed (pDB184) or a control plasmid (pGB2), was grown in the presence (0.1 mM) or absence of IPTG. The relevant genetic markers of the phages are indicated. wt, Wild type; Min$^-$, minicell phenotype; Sep$^-$, filamentation.

2 and Fig. 1*b*, *minC* (expressed from λDB171) was as effective as the entire *minB* locus in rendering Δ*minCDE* cells competent to respond to the $dicB_s$ division-inhibition activity. Similar results were obtained when *minC* was expressed in combination with *minE* (from λDB174). In contrast, in the absence of *minC*, the expression of *minD*, *minE*, or *minDE* (from λDB164, λDB156, and λDB175, respectively) did not change the minicell phenotype of the Δ*minCDE*/*aadA*::$dicB_s$ host. As expected (1), expression of *minCD* (from λDB173) caused filamentation whether or not $dicB_s$ was expressed. We conclude that the *minC* gene product is a required component of the *dicB* division-inhibition system and is the only product of the *minB* operon that is required.

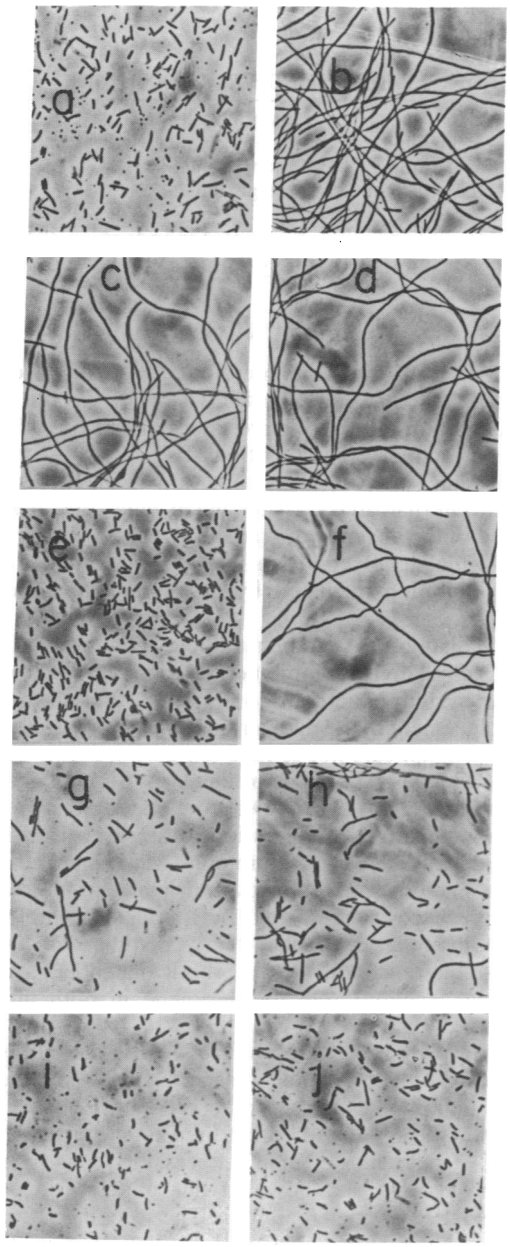

FIG. 1. Phase micrographs showing cell division phenotypes. (*a* and *b*) Effect of *minC* expression on $DicB_s$-induced filamentation. Strain PB114 (λDB171) [Δ*minCDE* (P_{lac}::*minC*)] containing pDB184 (*aadA*::$dicB_s$) was grown in the absence of IPTG (*a*) or in the presence of 0.1 mM IPTG (*b*). (*c–f*) Effects of *minE* expression on $DicB_s$- and MinCD-induced filamentation. Cells were grown in the presence of 0.05 mM IPTG. (*c* and *e*) Strain PB114 (λDB173) [Δ*minCDE* (P_{lac}::*minCD*)] containing either pGB2 (*c*) or pDB183 (*aadA*::*minE*) (*e*). (*d* and *f*) Strain PB103 (λDB182) [*minCDE*$^+$ (P_{lac}::$dicB_s$)] containing either pGB2 (*d*) or pDB183 (*aadA*::*minE*) (*f*). (*g–j*) Effects of *ftsZ* overexpression. Cells were grown in the presence of 0.05 mM IPTG prior to examination. (*g* and *i*) Strain PB114 (λDB173) [Δ*minCDE* (P_{lac}::*minCD*)] containing either pDB196 (*ftsZ*) (*g*) or pZAQ (*ftsQ ftsA ftsZ*) (*i*). (*h* and *j*) PB103 (λDB182) [*minCDE*$^+$ (P_{lac}::$dicB_s$)] containing either pDB196 (*ftsZ*) (*h*) or pZAQ (*ftsZ ftsA ftsZ*) (*j*).

Inhibition of Cell Division by MinC/$DicB_s$ Is Resistant to MinE. The results described above demonstrated that expression of *minC* together with either *minD* or $dicB_s$ leads to cessation of cell division. We had shown previously that the *minE* gene product is capable of preventing the *minCD*-induced filamentation phenotype (1). Therefore, to determine whether the *minC*/$dicB_s$ division-inhibition system could be functionally distinguished from the *minCD* system, we compared the sensitivity of the two systems to expression of *minE*.

The sensitivity of the *minCD* division inhibitor to expression of *minE* was confirmed by an experiment in which expression of *minCD* was induced in a Δ*minCDE* strain in which *minE* either was absent or constitutively expressed from a resident plasmid pDB183 (*aadA*::*minE*). Expression of *minE* from this plasmid was high enough to induce minicell formation in wild-type strains. The presence of pDB183 completely prevented filamentation in PB114 (λDB173) [Δ*minCDE* (P_{lac}::*minCD*)] (Table 3 and Fig. 1*e*) even when *minCD* expression was maximally induced. Under these conditions *minE* expression prevented filamentation without leading to minicell formation. This is consistent with the previous evidence that the *minE* gene product can relieve the MinCD-mediated inhibition of septation at internal sites while permitting the inhibitor to continue to block septation at polar sites (1).

To determine if the *minC*/$dicB_s$-mediated cell division inhibitor was also sensitive to MinE, a single copy of P_{lac}::$dicB_s$ (in λDB182) was integrated at the att^{λ} site in a *minB*$^+$ strain, permitting the conditional expression of the $dicB_s$-dependent division-inhibition activity under IPTG control. As shown in Table 3 and Fig. 1*f*, $DicB_s$-induced filamentation was unaffected by the high-level constitutive expression of *minE* from plasmid pDB183 (*aadA*::*minE*). This plasmid was incapable of modifying the division block even when $dicB_s$ was induced at IPTG concentrations as low as 25 μM, the lowest concentration seen to induce filamentation.

We conclude that, in contrast to the *minCD*-dependent division block, the *minC*/$dicB_s$-mediated inhibition of cell division is resistant to MinE.

Table 3. Effects of *minE* or *ftsZ* expression on $DicB_s$- and MinCD-dependent division inhibition

Plasmid		Phenotype of host			
		PB114 (λDB173)		PB103 (λDB182)	
		− IPTG	+ IPTG	− IPTG	+ IPTG
pGB2	Control	Min$^-$	Sep^{-*}	wt	Sep$^{-\dagger}$
pDB183	*aadA*::*minE*	Min$^-$	wt*	Min$^-$	Sep$^{-\dagger}$
pDB109	Control	Min$^-$	Sep$^-$	wt	Sep$^-$
pZAQ	*ftsQAZ*	Min$^-$	Min$^-$	Min$^-$	Min$^-$
pDB196	*ftsZ*	Min$^-$	Min$^{-\ddagger}$	Min$^-$	Min$^{-\ddagger}$

Lysogens PB114 (λDB173) [Δ*minCDE* (P_{lac}::*minCD*)] and PB103 (λDB182) [*minCDE*$^+$ (P_{lac}::$dicB_s$)] carrying the indicated plasmids were grown in LB medium in the presence (0.05 mM) or absence of IPTG. wt, Wild type; Min$^-$, minicell phenotype; Sep$^-$, filamentation.
*Similar results were obtained with 0.1, 0.5, and 1.0 mM IPTG.
†Similar results were obtained with 0.025, 0.1, 0.5, and 1.0 mM IPTG.
‡Cells carrying plasmid pDB196 were somewhat longer than cells carrying pZAQ.

Both Types of Division Inhibition Are Sensitive to *ftsZ* Expression. The *ftsZ* gene product is thought to be an essential component of the cell division machinery, since growth of an *ftsZ* temperature-sensitive mutant at 42°C leads to the formation of nonseptate filaments (for review, see ref. 4). It has also been shown that overexpression of *ftsZ* leads to a minicell phenotype (10).

The finding that *ftsZ* overexpression can induce minicell formation suggests that the *ftsZ* gene product can overcome the effect of the *minCD* division inhibitor at cell poles. It has been proposed that MinCD-induced filamentation is a manifestation of the same activity that normally blocks septation at polar sites (1). Therefore, if the current model is correct, the *ftsZ* gene product should also be capable of preventing the *minCD* division block at internal division sites and thereby be able to suppress the *minCD*-induced filamentation phenotype. To test this prediction, we studied the effects of *ftsZ* overexpression on the ability of MinCD to cause filamentation.

Two *ftsZ*$^+$ plasmids were used, pDB196 (*ftsZ*) and pZAQ (*ftsQAZ*; see ref. 10). The level of *ftsZ* expression from these plasmids was sufficiently high in both cases to induce minicell formation in wild-type strains. Both of the *ftsZ* plasmids rendered a host strain containing P_{lac}::*minCD* [PB114 (λDB173)] resistant to *minCD*-induced division inhibition as shown by the suppression of the filamentation phenotype (Table 3 and Fig. 1 *g* and *i*). In contrast, a control plasmid (pDB109) that only carries the 3′ part of *ftsZ* failed to prevent the *minCD*-mediated filamentation.

We also investigated the sensitivity of the *minC*/*dicB*$_s$ division inhibition to FtsZ by studying the effects of pDB196 and pZAQ on *dicB*$_s$-induced filamentation in PB103 (λDB182) [*minCDE*$^+$ (P_{lac}::*dicB*$_s$)]. Elevated levels of FtsZ also rendered the cells resistant to *minC*/*dicB*$_s$-mediated cell division inhibition as shown by a dramatic decrease in number and length of filaments (pDB196) or the complete disappearance of filaments (pZAQ) in the plasmid-containing strains (Table 3 and Fig. 1 *h* and *j*).

Thus, both the *minCD* and the *minC*/*dicB*$_s$ division-inhibition mechanisms can be reversed by high levels of the *ftsZ* gene product. The fact that pZAQ appeared more effective than pDB196 in suppressing the filamentation phenotype probably reflects a higher level of *ftsZ* expression from pZAQ (10), although an effect of *ftsA* and/or *ftsQ* in these experiments cannot be excluded.

SfiA and MinC-Mediated Cell Division Inhibition Are Not Interdependent. It had been shown previously that high levels of FtsZ can suppress the cell division block imposed by induction of the *sfiA* gene (12, 13). Therefore, the present finding that both of the *minC*-mediated cell division-inhibition systems are also counteracted by high levels of *ftsZ* expression was consistent with the possibility that the *sfiA*- and *minC*-mediated division blocks might be functionally interrelated. To address this question, we studied *minCD*- and *minC*/*dicB*$_s$-mediated division inhibition in strains N100 (*recA*$^-$), in which *sfiA*-mediated division inhibition cannot be induced as part of the SOS response, and GC579 (*sfiA*$^-$). Both λDB173 (P_{lac}::*minCD*) (see also ref. 1) and λDB182 (P_{lac}::*dicB*$_s$) readily induced a filamentation phenotype in both strains in the presence of low concentrations of IPTG (Table 4). From this we conclude that neither one of the *minC*-mediated division blocks is dependent on SfiA.

We also investigated the possibility that, conversely, *sfiA*-induced division inhibition is dependent on MinC. To this end, the ability of SfiA to provoke division inhibition was compared in strains PB103 (wild type) and PB128 (Δ*minCDE* Δ*dicB*) containing plasmid pDB192 (P_{lac}::*sfiA*). The results showed that IPTG induction of *sfiA* expression led to filamentation in both strains (Table 4). We conclude that the *sfiA*-induced division block is not dependent on the MinC-mediated division-inhibition systems.

Table 4. Independence of MinC- and SfiA-mediated division-inhibition systems

Strain	Relevant markers	Phenotype − IPTG	Phenotype + IPTG
N100 (λDB173)	*recA*$^-$ (P_{lac}::*minCD*)	wt*	Sep$^-$
N100 (λDB182)	*recA*$^-$ (P_{lac}::*dicB*$_s$)	wt	Sep$^-$
GC579 (λDB173)	*sfiA*$^-$ (P_{lac}::*minCD*)	wt*	Sep$^-$
GC579 (λDB182)	*sfiA*$^-$ (P_{lac}::*dicB*$_s$)	wt	Sep$^-$
PB103/pDB192	wt/P_{lac}::*sfiA*	wt	Sep$^-$
PB128/pDB192	Δ*dicB* Δ*minCDE*/P_{lac}::*sfiA*	Min$^-$	Sep$^-$

Cells (either lysogens or transformants, see first column) were grown in LB medium containing 0, 0.1 mM (λDB173 and λDB182 lysogens), or 0.5 mM (pDB192 transformants) IPTG.
*Small numbers of minicells were present.

DISCUSSION

We have shown (1) that coexpression of the *minC* and *minD* genes leads to inhibition of cell division in *E. coli*. Under normal conditions (i.e., in the presence of normal levels of the *minE* gene product), the inhibition of septation is limited to polar division sites, whereas septation is blocked at both polar and internal division sites when *minC* and *minD* are expressed in the absence of *minE*. The present paper shows that the *minC* gene product also plays an essential role in division inhibition mediated by another division inhibitor gene, *dicB*$_s$.

Several distinctions can be made between the *minCD* and the *minC*/*dicB*$_s$ division-inhibition systems. First, the *minCD* inhibition system is active during normal growth to ensure correct placement of the division septum, whereas the *minC*/*dicB*$_s$ system appears not to function during normal growth. Second, whereas MinCD-dependent division inhibition is suppressed by MinE, MinC/DicB$_s$-mediated division inhibition is not sensitive to expression of *minE*. Third, the two systems depend on different proteins, MinD and DicB$_s$, that share no significant homologies in primary structure (1, 7). These differences suggest that DicB$_s$ does not simply act as a MinD homolog. Although clearly distinct, the two cell division-inhibition pathways are both dependent on *minC* suggesting a central role for this gene in both inhibition processes.

Labie *et al.* (8) have observed that certain mutations in the *minB* locus render the cell insensitive to *dicB*$_s$-mediated division inhibition, whereas others do not. Based on the present findings, we predict that the first group are likely to be *minC*$^-$, since loss of *minC* expression leads to minicell formation (1) and, as shown in this paper, also prevents *dicB*$_s$-mediated division inhibition. The second group of *minB* mutants are likely to be *minC*$^+$, since *dicB*$_s$-induced filamentation is unaffected. In this group, minicell formation is likely to result from a mutation leading either to overproduction of MinE or to loss of activity of the *minD* gene product. An example of the latter class is the classical *minB1* mutant (14), which has retained sensitivity to DicB$_s$-mediated division inhibition (ref. 8 and our unpublished results) and is *minC*$^+$ *minD*$^-$ (unpublished observations).

Chromosomal deletions that include the *dicB* gene do not affect cell viability, and the *dicB* operon appears to be silent during normal cell growth. This and the fact that expression of the *dicB* operon is subject to a complex regulatory circuit suggest that the DicB$_s$-dependent division-inhibition system is invoked only under special circumstances (6, 15). As such, it appears to be part of a growing class of cell division inhibitors, including SfiA (SulA), SfiC, CcdB (LetD) (4), and

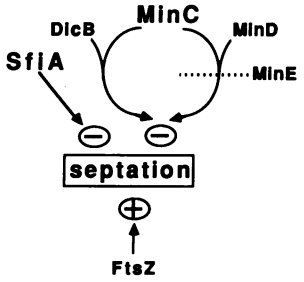

FIG. 2. Positive and negative effectors of cell division.

PemK (16), that are not functional during normal growth but can be activated under particular conditions.

The present study showed that the *ftsZ* gene product can suppress the action of the MinCD and the MinC/DicB$_s$ division inhibitors. However, it is unlikely that the sole role of the FtsZ protein in the cell is to inactivate the MinC-related division inhibitors because we have shown that growth of a *ftsZ* temperature-sensitive mutant at 42°C leads to filamentation even in *minCD*$^-$ strains (unpublished results). Moreover, although both the loss of *minCD* function and the overexpression of *ftsZ* lead to minicell formation, they differ in that *ftsZ* overexpression leads to an increased number of septa per cell mass when compared with wild-type cells (10).

FtsZ has been suggested to be the direct target of the SfiA (17, 18) and SfiC (19) division-inhibition systems. Similarly, one possible explanation for the present observations is that the FtsZ protein is also the target of the MinC-mediated division-inhibition systems. However, in none of these cases has an interaction between the putative division inhibitor and the FtsZ protein been directly demonstrated. Determination of the actual targets of these division inhibitors must await more direct evidence. This is of importance, since detailed studies on the mode of action of division inhibitors and their molecular targets should yield valuable information on the molecular mechanisms that underly the division process.

A model that is consistent with the present observations is shown in Fig. 2. In this model, MinC is the effector of the MinCD- and DicB$_s$-mediated division-inhibition processes. The activation of MinC requires the presence of MinD or DicB$_s$, and the nature of the activator protein in turn determines the other specific properties of the system. Thus, in contrast to DicB$_s$, MinD renders the system sensitive to MinE, which then allows the division inhibitor to act differentially at only the polar potential division sites, depending on the level of *minE* expression. Finally, it is the balance between negative effectors of septation, such as the MinCD, MinC/DicB$_s$, and SfiA division inhibitors, and positive effectors, such as the *ftsZ* gene product, that determines whether or not septation occurs.

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