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Cell Division Inhibitors SulA and MinCD Prevent Formation of the FtsZ Ring

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Immunoelectron microscopy was used to assess the effects of inhibitors of cell division on formation of the FtsZ ring in *Escherichia coli*. Induction of the cell division inhibitor SulA, a component of the SOS response, or the inhibitor MinCD, a component of the *min* system, blocked formation of the FtsZ ring and led to filamentation. Reversal of SulA inhibition by blocking protein synthesis in SulA-induced filaments led to a resumption of FtsZ ring formation and division. These results suggested that these inhibitors block cell division by preventing FtsZ localization into the ring structure. In addition, analysis of *min* mutants demonstrated that FtsZ ring formation was also associated with minicell formation, indicating that all septation events in *E. coli* involve the FtsZ ring.

In *Escherichia coli*, cell division occurs near the midpoint of the long axis of the cell shortly after completion of chromosome replication (11). This temporal and spatial regulation of the cell division event is responsible for the narrow cell length distribution observed with exponentially growing cultures. In addition, the coordination of the cell division event with DNA segregation is responsible for the virtual absence of DNA-less cells in cultures. Under some conditions, however, this coordination between these two events is altered, leading to either inhibition of division or misplacement of the division event. Such disturbances of the coordination between the division event and DNA segregation lead to filamentation and/or production of anucleate cells, including minicells.

Inhibition of cell division following interruption of DNA replication is due in part to the induction of *sulA*, a member of the SOS regulon (13, 14). SulA is thought to inhibit division by direct interaction with FtsZ, inhibiting its essential division activity (3, 16, 18). The inhibition is readily reversible, and division activity is restored as soon as SulA is removed, even without additional FtsZ synthesis (20).

Proper placement of the division event is in part due to the *min* system, which is thought to prevent old sites (the cell poles) from being reused (25). In the absence of the *min* system, the poles become accessible to the division machinery, resulting in minicell production. The *min* system utilizes a bipartite division inhibitor, MinCD, and an additional gene product, MinE, that appears to confer topological specificity to the inhibitor (9). The target of the MinCD inhibitor appears to be FtsZ. Overproduction of FtsZ can induce minicell formation in wild-type cells (27) and suppress the inhibitory effect of excess MinCD (4, 10). In addition, mutations in *ftsZ*, selected for resistance to *sulA*, confer resistance to MinCD, and one of these, which confers the greatest resistance to MinCD (*ftsZ2* [Rsa]), leads to a minicell phenotype, even when it is not overexpressed (4, 6).

Recently, immunoelectron microscopy was used to localize FtsZ in a ring-like structure at the leading edge of the septum (5). The results indicated that formation of this structure preceded the initiation of septation, suggesting that this is a very early step in the division process. The FtsZ ring decreased in diameter during septation and disassembled following completion of septation. It was suggested that the FtsZ ring may play a cytoskeletal role in the division process, marking the division site and activating the division machinery. This suggestion is supported by the altered septal morphology induced by several *ftsZ* alleles (6). In this study, we examine the effect of SulA and MinCD on formation of the FtsZ ring structure.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The *E. coli* K-12 bacterial strains used in this study were derivatives of MC4100 (5) and BS100 (19). For examination of the effect of *sulA*, an MC4100 derivative containing plasmids pJF118EH (12) and pUGM470 (obtained from S. Gottesman) was used. The pJF118EH provides the *lac* repressor, and the pUGM470 has the *sulA* gene downstream of the *lac* promoter. BEF51 is an MC4100 derivative carrying a *min* deletion P1 transduced from PB114 (9). To examine the effects of *minCD* induction, BS100 containing λDB173, a transducing phage containing the *minCD* genes downstream of the *lac* promoter (9), was used.

Culture conditions. Strains were grown at 37°C in L broth supplemented with chloramphenicol (17 μg/ml) and ampicillin (100 μg/ml). To block protein synthesis, spectinomycin was added at 150 μg/ml. To induce *sulA* or *minCD*, isopropylthiogalactopyranoside (IPTG) was added at 0.5 to 1 mM.

Immunoelectron microscopy. Immunoelectron microscopy was performed as previously described (5) except that the secondary antibody contained 15-nm conjugated gold particles instead of the 5-nm conjugated gold particles used previously. The larger gold particles resulted in a decrease in the number of particles per cell but were easier to detect in long filaments.

RESULTS

Effect of SulA on formation of the FtsZ ring. To assess the effect of SulA on FtsZ ring formation, we used MC4100 containing a plasmid with *sulA* under the control of the *lac*

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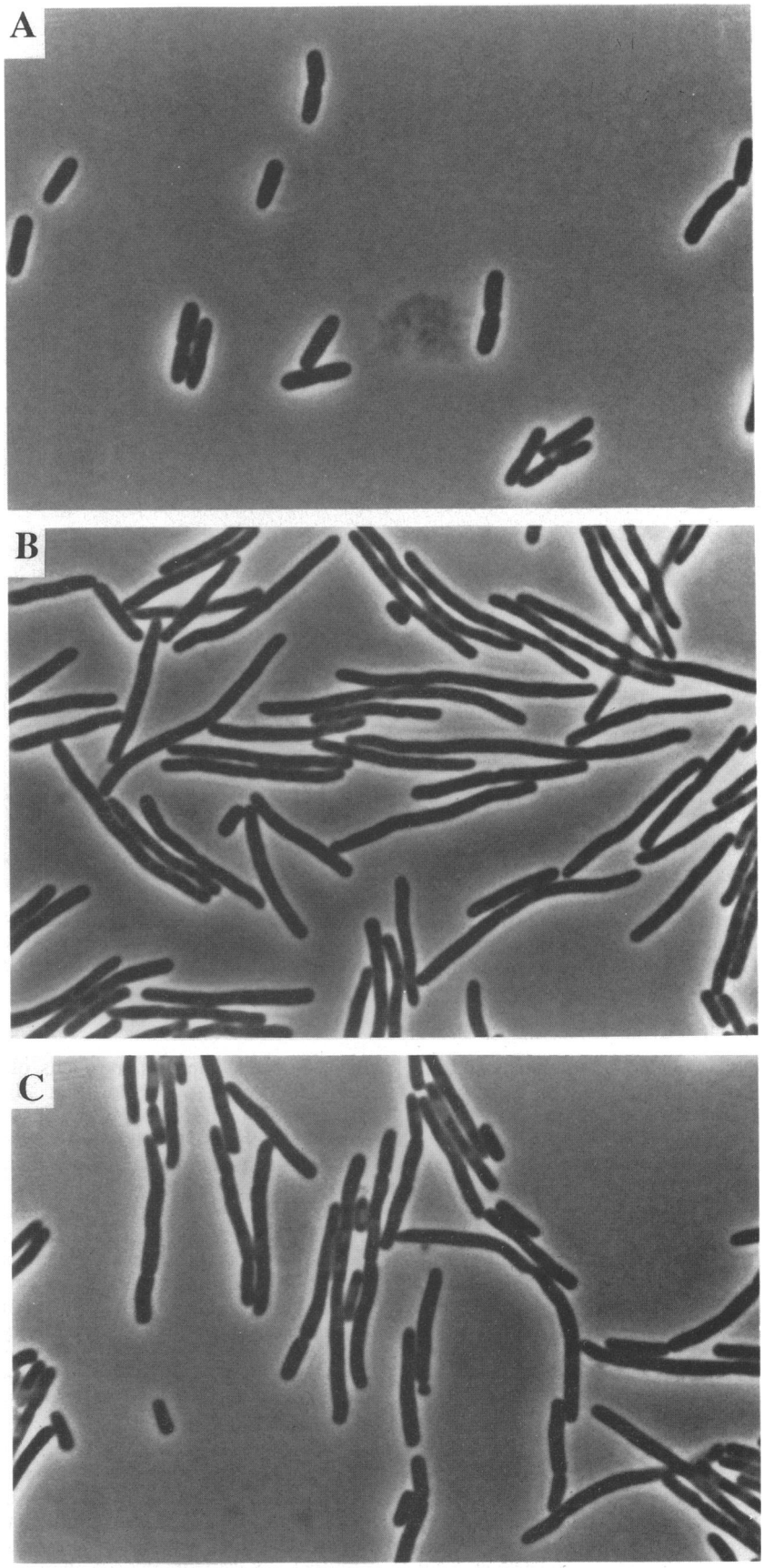

FIG. 1. SulA-induced filamentation and recovery. (A) An exponential-phase culture of MC4100(pUGM470, pJF118EH); (B) the culture 1 h after induction of SulA; (C) the culture shown in panel B 35 min after addition of spectinomycin to block protein synthesis, which results in the disappearance of SulA.

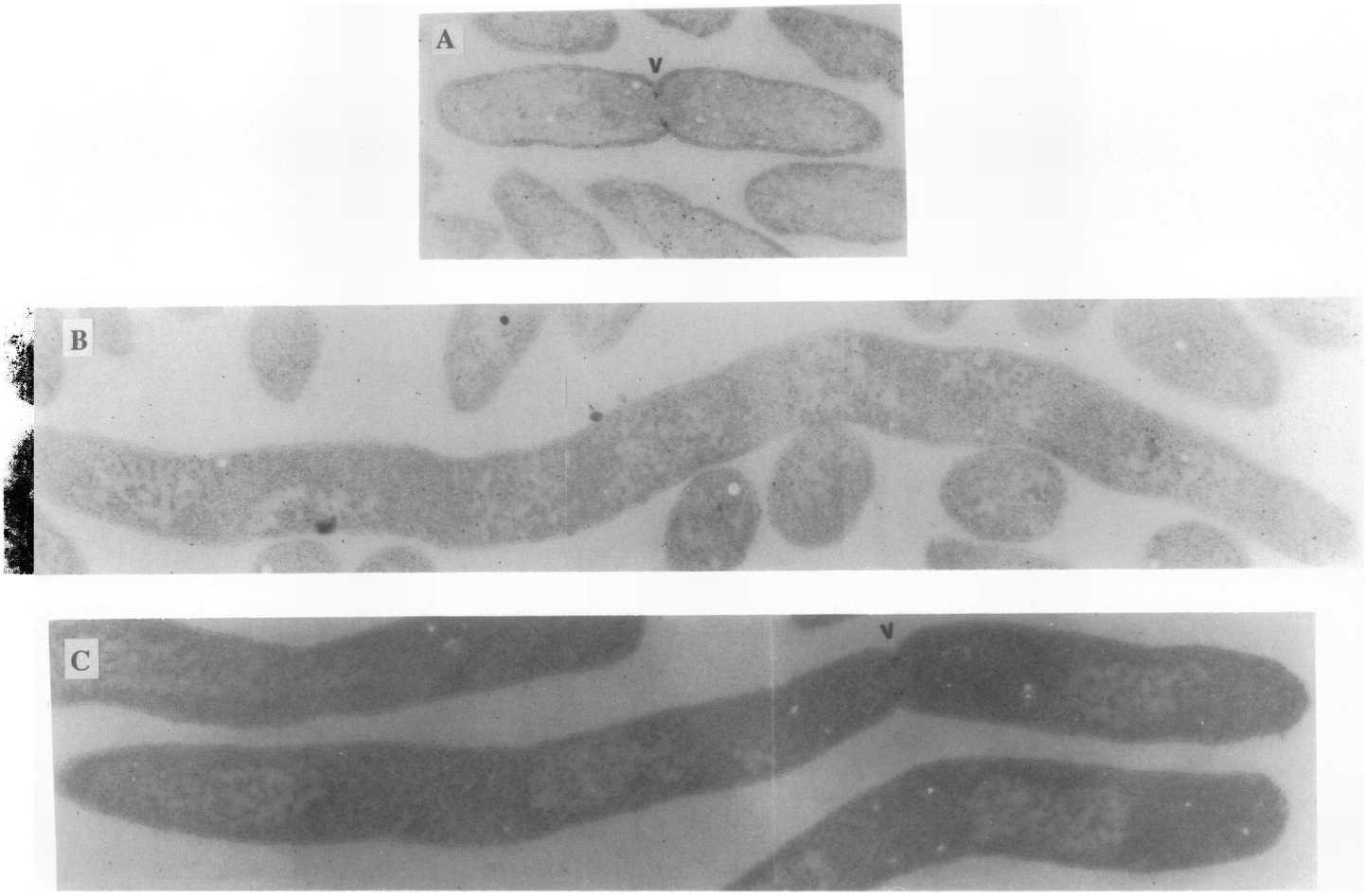

FIG. 2. Immunolocalization of FtsZ following SulA induction and recovery. The cultures photographed in Fig. 1 were processed for immunoelectron microscopy to determine the location of FtsZ. (A) An exponential-phase culture of MC4100(pUGM470, pJF118EH); (B) the culture 1 h after induction of SulA; (C) the culture shown in panel B 35 min after addition of spectinomycin to block protein synthesis, which results in the disappearance of SulA.

promoter. This strain also contained an additional plasmid to provide sufficient *lac* repressor to repress *sulA* expression. MC4100 containing these two plasmids had a normal morphology (Fig. 1A). Upon induction of *sulA* by the addition of IPTG, division rapidly ceased and cells started to filament (Fig. 1B [1 h after induction]). To determine the effect of *sulA* induction on FtsZ localization, a sample was taken 1 h after addition of IPTG and prepared for immunoelectron microscopy. For a control, a sample was taken before induction. The location of FtsZ in these cells was determined by incubating thin sections of fixed cells with affinity-purified anti-FtsZ antibodies and then treating them with a gold-conjugated secondary antibody (5). Analysis of the sample taken before induction revealed that in dividing cells, the gold label was primarily localized to the leading edge of the septum, indicating that FtsZ was localized there as previously described (Fig. 2A). Also, in most nondividing cells in this sample, the gold label was randomly distributed in the cytoplasm; in a few cells, however, the label was at the midpoint of the cell at the cytoplasmic membrane. Since these cells were approximately twice the length of the smallest cells, they were presumably at a very early stage of cell division. These results are the same as those obtained for MC4100 containing no plasmids (5). In contrast, in the SulA-induced filaments, no signs of any clustering of the gold label were observed (Fig. 2B). Of more than 100 filaments examined, none were observed to have any label symmetrically positioned at the cytoplasmic membrane. Thus, SulA appears to block cell division by preventing the localization of FtsZ to potential division sites.

The inhibition of cell division by SulA is readily reversible in *lon*$^+$ strains (20). Blocking SulA synthesis results in its rapid disappearance from the cell, since it is rapidly degraded by the *lon*-encoded protease (21). In addition, the reversibility is observed even if protein synthesis is blocked, indicating that the division machinery is intact and undamaged by SulA action (20). To observe the effect of SulA removal on FtsZ localization, spectinomycin was added to the culture 1 h after SulA induction. By 35 min after spectinomycin addition, filaments were beginning to divide as previously shown (20) (Fig. 1C). A sample was taken at this time and processed for immunoelectron microscopy. The addition of spectinomycin had a dramatic effect on FtsZ localization (Fig. 2C). Most of the filaments had at least one constriction that was decorated with gold label. Thus, the removal of SulA from the cell allows FtsZ to localize and cell division to proceed.

FtsZ localization in the *min* mutant. In the *min* mutant,

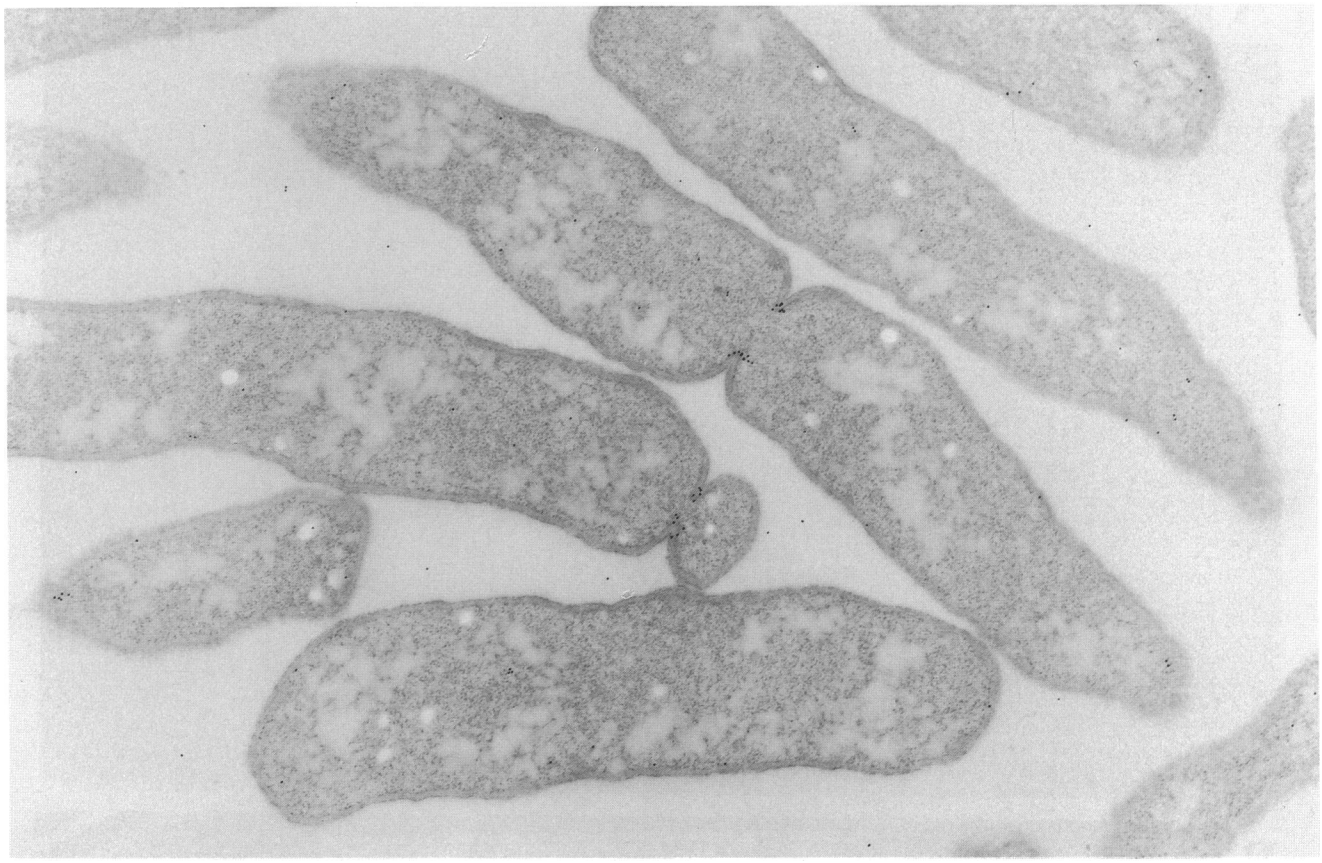

FIG. 3. Localization of FtsZ in a *min* mutant. An exponential-phase culture of BEF51 (Δ*min*) was processed for immunoelectron microscopy to locate FtsZ.

minicells are produced from the cell ends. It is thought that this is due to the division machinery operating at polar sites that are normally masked by the *min* system (9, 25). Part of the evidence for this mechanism is the observation that mutations in cell division genes, such as *ftsZ*, that block internal septation events also block minicell formation (17, 27). The involvement of FtsZ in minicell formation is also supported by studies showing that overproduction of FtsZ leads to minicell formation (27) and by the observation that certain *ftsZ* mutations, i.e., those that confer the most resistance to MinCD, also give a minicell phenotype (3, 4). To determine the effect of *min* on FtsZ localization, an exponential culture of BEF51 (Δ*min*) was processed for immunoelectron microscopy. The results were very clear (Fig. 3). All septa, polar and internal, were clearly decorated with the gold label. It should be noted that poles were not labeled with gold unless they were involved in division, indicating that once FtsZ is localized to a pole, division quickly ensues, and upon completion of division, FtsZ is rapidly disassembled. These results demonstrated that in the absence of *min*, FtsZ ring formation could form at internal sites or at one of the cell poles. In addition, it demonstrated that the FtsZ ring is involved in septation events regardless of their location in the cell.

Effect of MinCD on FtsZ localization. MinCD in the absence of MinE has no division site selectivity and acts as a nonspecific inhibitor of cell division (9). To determine the effect of MinCD on FtsZ localization, strain BS100(λDB173) was used. In this strain, the *minCD* genes, but not *minE*, are carried downstream of the *lac* promoter on λDB173 (9). Induction of *minCD* by the addition of IPTG led to a rapid cessation of cell division and consequent filamentation (Fig. 4). Seventy-five minutes after the addition of IPTG to a culture of BS100(λDB173), a sample was taken and processed for immunoelectron microscopy. By this time, the cells had increased noticeably in cell length and contained no visible sign of septa when examined in the light microscope or with the electron microscope. Immunostaining of the filaments revealed no clustering of the gold label, only a random distribution in the cytoplasm (Fig. 5B). In contrast, cells examined before induction showed the gold clustering at division sites (Fig. 5A). These results demonstrate that overproduction of MinCD blocks FtsZ localization.

The results obtained in this study are summarized in Fig. 6. In response to a cell cycle signal, FtsZ assembles at the internal division site since the old sites (the poles) are blocked by the *min* system. In the absence of the *min* system, the FtsZ ring is formed at any of the possible sites, although there is only sufficient potential to form at one of the available sites per cell cycle. In the presence of excess FtsZ, the FtsZ ring can form at internal sites and polar sites, since excess FtsZ can override the *min* inhibition at the poles. In this case, more than one division event can happen per doubling of cell mass, although as noted previously, these events occur sequentially and not simultaneously (27). Expression of *sulA* or an excess of MinCD blocks cell division by preventing formation of the FtsZ ring.

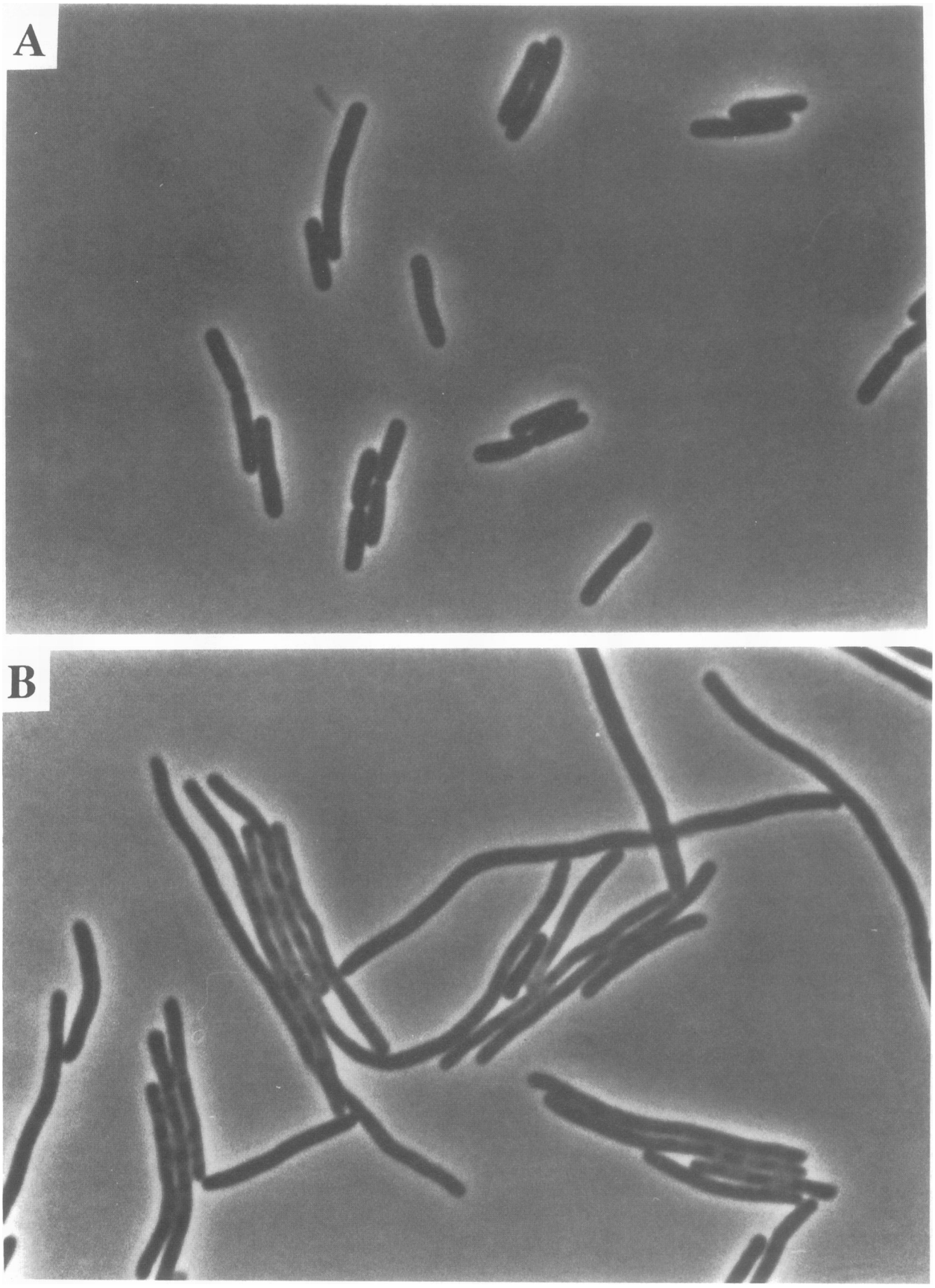

FIG. 4. MinCD-induced filamentation. (A) An exponential-phase culture of BS100(λDB173); (B) BS100(λDB173) 1 h after induction of MinCD.

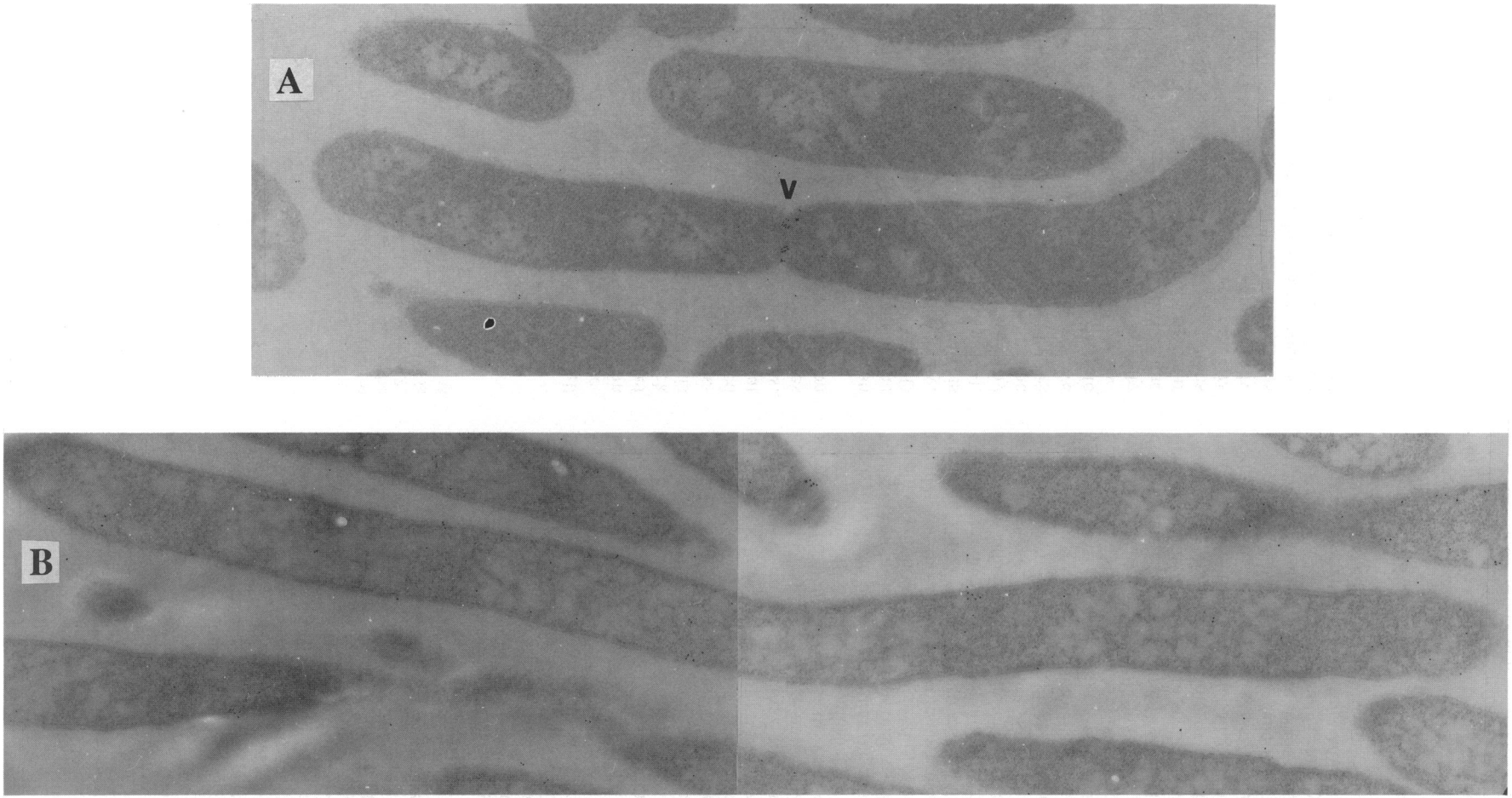

FIG. 5. Immunolocalization of FtsZ following MinCD induction. The cultures photographed in Fig. 3 were processed for immunoelectron microscopy to determine the location of FtsZ. (A) An exponential-phase culture of BS100(λDB173); (B) BS100(λDB173) 1 h after induction of MinCD.

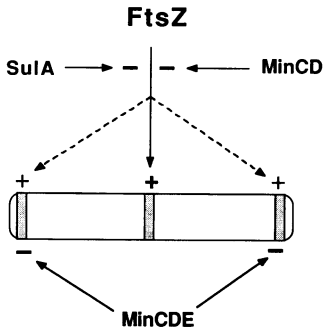

FIG. 6. Model for FtsZ localization. In wild-type cells, FtsZ is localized to the nascent division site, as MinCDE functions to exclude polar (old) sites. In the absence of *min* function, in the presence of excess FtsZ, or in the presence of a Min^r allele of *ftsZ*, FtsZ can localize to either internal or polar sites. When SulA is present or there is excess MinCD, the localization of FtsZ is prevented and cells filament.

DISCUSSION

Previous results have shown that FtsZ, an essential cell division protein (8, 24, 26), is localized in a ring-like structure at the future division site immediately before division occurs (5). In this study, we have found that two inhibitors of cell division, SulA and the bipartite MinCD inhibitor, block the formation of the FtsZ ring at potential division sites. This common ability of these two inhibitors explains their capacity to block cell division. Furthermore, we have observed that FtsZ is localized at division sites whether they are at midcell between replicated nucleoids or at the cell poles. These observations along with previous genetic studies support a critical role for the FtsZ ring in the cell division process.

The signal for localization of FtsZ to the future division site is unknown, as is the mechanism for its localization. The results presented here demonstrate that SulA and MinCD can block this localization and can do so independently. Previously, we have shown that the action of SulA is independent of MinCD and that the reverse is also true (4). Also, it is clear that both SulA and MinCD inhibit division by preventing FtsZ ring formation and not by blocking the existing ring structure from functioning. If the inhibitors blocked progression of the ring after its formation, then we would have expected to see clusters of FtsZ at discrete distances along the lengths of the filaments. Despite observing numerous filaments, we never observed symmetrically positioned clusters of gold label in any filament formed due to induction of either SulA or MinCD. This result is similar to what we observed with filaments produced by mutations in *ftsZ*, whereas filaments produced by mutations in other *fts* genes (*ftsA*, *ftsI*, and *ftsQ*) do contain some symmetrically positioned clusters of gold label along their length (2). It is possible that SulA and MinCD can block division in progress and cause disassembly of FtsZ rings already formed. In the light microscope, a small fraction of the filaments observed following SulA induction contained a partial constriction at midcell. These partial constrictions could be due to SulA blocking divisions in progress when it is first induced, while all subsequent divisions are blocked at an earlier step. If so, SulA must have caused the disassembly of FtsZ, since clusters of gold particles were not observed in any of the filaments.

Our results show that the removal of SulA from the SulA-induced filaments results in the localization of FtsZ to division sites and resumption of cell division even in the absence of protein synthesis. This result indicates that both the localizing signal and FtsZ are stable. SulA and MinCD must block the localization signal from reaching FtsZ. Genetic and biochemical evidence support a direct interaction between FtsZ and SulA, favoring the possibility that SulA binds to FtsZ and thus prevents FtsZ from receiving the signal. Less is known about the possible mechanism of MinCD action.

Although little is known about the mechanism of FtsZ localization, it is known that FtsZ is a GTP/GDP-binding protein with GTPase activity (10a, 22, 24a). Characteristics of the interaction of FtsZ with guanine nucleotides resemble tubulin's interaction with guanine nucleotides, raising the possibility that FtsZ utilizes GTP for assembly into a structure. Thus, the critical step for FtsZ ring formation may be a nucleation step followed by a rapid self-assembly of FtsZ into the ring. The action of SulA and MinCD could be to block the nucleation event. Interestingly, one *ftsZ* mutation (*ftsZ3* [Rsa]), which was selected on the basis of resistance to *sulA* and also shows resistance to *minCD*, results in an FtsZ mutant protein that has decreased GTPase activity (22). This finding raises the possibility that SulA acts by increasing the GTPase activity of FtsZ but is unable to do so in mutant proteins that are SulA resistant.

It has been suggested that the function of the *min* locus is to prevent division from occurring at old sites (the cell poles) (24) and that this occurs through the action of an inhibitor, MinCD, and a topological specificity factor, MinE (9). The present results, as well as previous results (5), show that FtsZ is not localized to cell poles in wild-type cells; upon completion of division, the FtsZ ring must be rapidly disassembled, since small cells that have FtsZ at the cell pole are not observed. Inactivation of the *min* locus results in division taking place at either internal or polar sites. In this study, we have determined that the FtsZ ring is associated with both polar and internal divisions (Fig. 3). It should be emphasized, however, that in the *min* mutant, FtsZ was localized at poles only if they were actively engaged in division. This result demonstrates that although the poles are available for division in a *min* mutant, FtsZ is localized there only when a division signal is generated and then is localized at only one of the available sites. Alternatively, it was possible that FtsZ was present at the poles in the *min* mutant (FtsZ could assemble at all available sites or, alternatively, did not disassemble from the previous division) but was activated at only one site. Our observations are in agreement with observations for wild-type cells that suggested that division ensues as soon as the FtsZ ring is formed, indicating that formation of the FtsZ ring is the limiting step in division.

An underlying assumption of the hypothesis for *min* function is that the process leading to minicell formation is a normal septation event that is just abnormally located. This assumption is supported by genetic evidence that showed that minicell formation required the function of cell division genes (17, 27). It is further supported by the demonstration in this study that the FtsZ ring, an essential component of the division machinery, is also involved in minicell formation.

More recently, the function of the *min* locus has been questioned since the *min* mutations have an effect on DNA segregation (1, 15, 23). This finding raises the possibility that the effect of *min* mutations on division may be indirect and result from a disturbance of DNA segregation. If this is the case, it indicates that *min* may act only indirectly on FtsZ

and that it is DNA segregation which influences the formation and location of the FtsZ ring. It is certainly well documented that interfering with DNA replication and/or segregation interferes with division even in the absence of *sulA* (reviewed in reference 7). It may be that localization of FtsZ into a ring structure is a sensitive first step in cell division that is subjected to a variety of controls and is vulnerable to inhibition by a variety of insults to the cell. Our results show that the FtsZ ring is involved in all division events regardless of their location within the cell and that the inhibitors SulA and MinCD can block FtsZ ring formation.

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