JOURNAL OF BACTERIOLOGY, Nov. 2007, p. 8044–8052 Vol. 189, No. 22
0021-9193/07/$08.00+0 doi:10.1128/JB.00773-07

Role of SufI (FtsP) in Cell Division of *Escherichia coli*: Evidence for Its Involvement in Stabilizing the Assembly of the Divisome▽

Harish Samaluru,† L. SaiSree,† and Manjula Reddy*

Centre for Cellular and Molecular Biology, Hyderabad 500007, India

Received 17 May 2007/Accepted 23 August 2007

The function of SufI, a well-studied substrate of the TatABC translocase in *Escherichia coli*, is not known. It was earlier implicated in cell division, based on the finding that multiple copies of *sufI* suppressed the phenotypes of cells with mutations in *ftsI* (*ftsI23*), which encodes a divisomal transpeptidase. Recently, *sufI* was identified as both a multicopy suppressor gene and a synthetic lethal mutant of *ftsEX*, which codes for a division-specific putative ABC transporter. In this study, we show that *sufI* is essential for the viability of *E. coli* cells subjected to various forms of stress, including oxidative stress and DNA damage. The *sufI* mutant also exhibits *sulA*-independent filamentation, indicating a role in cell division. The phenotypes of the *sufI* mutant are suppressed by factors that stabilize FtsZ ring assembly, such as increased expression of cell division proteins FtsQAZ or FtsN or the presence of the gain-of-function *ftsA (FtsA R286W) mutation, suggesting that SufI is a divisomal protein required during stress conditions. In support of this, multicopy *sufI* suppressed the divisional defects of mutants carrying the *ftsA12*, *ftsQ1*, or *ftsK44* allele but not those of mutants carrying *ftsZ84*. Most of the division-defective mutants, in particular those carrying a Δ*ftsEX* or *ftsI23* allele, exhibited sensitivity to oxidative stress or DNA damage, and this sensitivity was also abolished by multiple copies of SufI. All of these data suggest that SufI is a division component involved in protecting or stabilizing the divisomal assembly under conditions of stress. Since *sufI* fulfils the requirements to be designated an *fts* gene, we propose that it be renamed *ftsP*.**

The process of cell division in bacteria involves the formation of a septum at the midcell, with concerted invagination of all three layers of the cell envelope, i.e., the cytoplasmic membrane, the peptidoglycan wall, and the outer membrane. In *Escherichia coli*, it requires the coordinated assembly of at least 15 proteins, namely, FtsZ, ZipA, ZapA, FtsA, FtsE/X, FtsK, FtsQ, FtsL/B, FtsW, FtsI, FtsN, AmiC, and EnvC, at the division site in an interdependent and sequential pathway to form a divisome that facilitates septum formation (1, 12, 18, 35, 47). The divisome assembly is initiated by localization of FtsZ, a eukaryotic tubulin homolog, at the site of division followed by the formation of a circumferential ring of FtsZ (Z ring) around the inner surface of the cytoplasmic membrane. The FtsZ ring is stabilized by other division proteins, including FtsA, ZapA, and ZipA (31). Once the ring is established, the proteins FtsE/X, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI, FtsN, AmiC, and EnvC are recruited in a more or less linear fashion to the division site and coordinate the formation of the septum, leading to the generation of two daughter cells. Of these, FtsQ, FtsL, and FtsB are known to assemble into a complex, which is believed to connect the Z ring scaffold (comprising FtsZ-FtsA-ZipA-ZapA) to the septal peptidoglycan synthesis machinery that consists of FtsW and FtsI (18, 43). FtsN is an essential division protein of unknown function, which in multicopy suppresses the defects of most division mutations, such as *ftsA12*, *ftsK44*, *ftsQ1*, *ftsI23*, Δ*ftsK*, and Δ*ftsEX* mutations, but not *ftsZ84* mutation (11, 14, 38). AmiC and EnvC are septal murein hydrolases that facilitate the separation of daughter cells but are not essential for growth (5, 6). FtsK is a bifunctional protein; its amino-terminal region is involved in the essential division process, and the dispensable carboxy-terminal domain facilitates chromosomal partitioning (30).

Most of the division proteins listed above are essential for cell viability. However, a gain-of-function mutation in FtsA (FtsA R286W) can bypass the requirement for ZipA, FtsK, or FtsN, suggesting the existence of functional redundancy between various cell division proteins (4, 14, 15). A *trans*-envelope Tol-Pal complex was recently shown to facilitate the invagination of the outer membrane during division (17). Chaperone proteins, such as DnaK, GroE, HscA, and trigger factor, are also implicated in division because they are known to affect the folding of one or more division proteins but are not specific for this process (13, 21, 32, 36, 41).

Recently, it was shown that FtsEX, a putative ABC transporter located in the inner membrane, is essential for growth and division only under low-osmolarity conditions and that under conditions of high osmotic strength, the viability of *ftsEX* deletion mutants is dependent on the presence of a periplasmic protein, SufI (38, 40). In addition, SufI overexpression could substitute for the deficiency of FtsEX, leading to the suggestion that FtsEX and SufI functions could be redundant (38). Previous evidence also implicated SufI in cell division, as multiple copies of *sufI* suppressed the thermosensitivity of an *ftsI23* mutant, but the gene was shown to be dispensable for normal cell growth (25). SufI has been well studied as a prototype substrate of the TatABC (twin-arginine translocase) system, which is a Sec-independent transport pathway that translocates proteins in native conformation from the cytosol into the

* Corresponding author. Mailing address: S-107, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India. Phone: 91-40-27192523. Fax: 91-40-27160951. E-mail: manjula@ccmb.res.in.

† H.S. and L.S. contributed equally to the study.

▽ Published ahead of print on 31 August 2007.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or features	Source or reference
Strains		
MC4100	Wild type; Δ*lacU169*	Lab collection
MG1655	Wild type	Lab collection
HME63	λ*cI857*Δ(*cro-bioA*) *mutS*::Amp	D. L. Court
MR2	MG1655 Δ*lacI*	38
MR22	MR2 Δ*sufI222*::Kan	This study
MR23	MR2 *tatB*::Tn*10*dKan	38
MR24	MR2 *ftsZ84 leuB82*::Tn*10*	This study
MR25	MR2 *ftsA12 leuB82*::Tn*10*	This study
MR26	MR2 *ftsK44 zbj-1230*::Tn*10*	This study
MR27	MR2 *ftsQ1 leuB82*::Tn*10*	This study
MR28	MR2 *ftsI23 leuB82*::Tn*10*	This study
MR29	MR22 *ftsA* leuB82*::Tn*10*	This study
MR30	MR22 *leuB82*::Tn*10*	This study
MR31[a]	MR2 *sulA211*	This study
MR32	MR31 *sufI222*::Kan	This study
Plasmids		
pACYC184	p15A-based; Cam^r Tet^r	Lab collection
pCL1920	pSC101-based; Spc^r	29
pBAD18	pMB1-based; Amp^r; arabinose-regulated	22
pMU2385	IncW-based; Tp^r; promoterless *lacZ* gene	45
pUC4K	pMB1-based; Amp^r; source of Kan^r cassette	Lab collection
pMN8	pCL1920-*ftsQAZ*	38
pMN14	pCL1920-*ftsN*	38
pMN16	pCL1920-*sufI*	38
pMN58	pBAD18-*sufI*	This study
pMN59	pMU2385 with promoter of *sufI*	This study
pMN60	pACYC184-*dapE*	This study

[a] Strain MR31 was constructed by two sequential transductions. Strain MR2 was initially made PyrD$^-$ by transducing a *pyrD*::Kan allele (from our lab collection), and it was subsequently transduced with a P1 lysate prepared on a *pyrD*$^+$ *sulA211* strain (obtained from CGSC), as *pyrD* is 75% linked to *sulA* in P1 transduction. PyrD$^+$ transductants were selected on minimal glucose plates followed by screening for Kan^s colonies.

periplasm across the inner membrane (2, 3, 28, 49). Most Tat substrates bind redox cofactors, such as iron-sulfur clusters and molybdopterin nucleotides, and play important roles in energy metabolism and cell wall biosynthesis. These also include two murein hydrolases, AmiA and AmiC, that facilitate daughter cell separation during cytokinesis (5, 6, 24).

Because the functions of SufI are not well understood, a genetic analysis of *sufI* was undertaken in this study. The results show that SufI is a cell division protein required for the viability of *E. coli* cells under a variety of stressful conditions, implicating a role for it in the stabilization of the divisomal assembly.

MATERIALS AND METHODS

Bacterial strains and phages. All strains used in this study are derivatives of *E. coli* K-12 and are listed in Table 1. Strain MR2 (MG1655 Δ*lacI*) was used as the wild-type strain (38). The construction of the *sufI* deletion strain is described below. The division-defective *ftsZ84*, *ftsA12*, *ftsK44*, *ftsQ1*, and *ftsI23* mutations were introduced by phage P1-mediated transduction into other strain backgrounds, with the aid of linked antibiotic markers from strains DRC14, EC290, TOE44, JOE86, and LMG64 (obtained from the laboratory of Jon Beckwith), respectively. *sodA sodB* mutants (10) were obtained from the Coli Genetic Stock Centre (CGSC), and *sodC* mutants (19) were obtained from the laboratory of James Imlay. Phage P1*kc* was obtained from a laboratory stock.

Plasmids. pBAD18, an Amp^r, ColEI-based, arabinose-inducible vector (22), was used to clone *sufI* under the control of the *ara* promoter. The *sufI* gene was amplified with primers sufIFP and sufINRP (5′-GGAATTCCATGGAGCAAA TATGTCACTC and 5′-GGAATTCGAAGATTACGGTACCGGATTG, respectively, with EcoRI restriction sites underlined), using high-fidelity *Pfx* polymerase (Invitrogen), digested with EcoRI, and cloned at the same site in pBAD18 to create pMN58. Plasmid pMU2385, a Tp^r, very-low-copy-number IncW derivative carrying a promoterless *lacZ* reporter gene (45), was used for the construction of a *sufI-lac* promoter fusion. A 230-bp fragment encompassing the putative promoter region of *sufI* was amplified with sufFP4 (5′-CGGGATC CGCCAATTGACGTCAGTC) and sufRP4 (5′-GGAATTCTGACGCCGACT GAGTGACA) (restriction sites are underlined), digested with BamHI and EcoRI, and cloned at the corresponding sites in pMU2385 to create pMN59.

Growth media and conditions. Unless otherwise indicated, Luria-Bertani (LB) medium (with 1% NaCl) was used (33), and the growth temperature was 30°C. LBON medium is the same as LB, but without NaCl. The culture media were obtained from BBL-Difco (Madison, WI). Supplementation of LBON with osmolytes (i.e., glucose, sucrose, glycerol, or NaCl) was done at 0.4 M. H_2O_2 (30% [wt/vol]) was used at 0.5 mM. Paraquat (PQ; methyl viologen) and mitomycin C (MMC) were used at the indicated concentrations. L-Arabinose or D-glucose was used at 0.2% for induction or repression of *sufI* cloned into the pBAD18 vector. Sodium ascorbate was used at 15 mM. The following antibiotics were used at the indicated concentrations: ampicillin (Amp), 50 μg/ml; kanamycin (Kan), 50 μg/ml (in LB) and 10 μg/ml (in LBON); tetracycline (Tet), 15 μg/ml; chloramphenicol (Cam), 30 μg/ml; spectinomycin (Spc), 50 μg/ml; and trimethoprim (Tp), 60 μg/ml.

Construction of a chromosomal *sufI* deletion-insertion mutant. A complete deletion of the *sufI* locus was made on the chromosome by recombineering as described previously (50). A pair of primers having homology at the 5′ end to the flanking region of the *sufI* locus and at the 3′ end to the sequence of the kanamycin resistance gene cassette (sufIFPKan [5′-TGCGGGGAACACTTTC CTGCACGGTATTACTTTAGCCAGTTTTACATGGATTGTGTCTCAAAA TCTCTGAT-3′] and sufIRPKan [5′-GCCCTCCTCGGGCGAGTATGAAGAT TACGGTACCGGATTGACCAACAGTTAGAAAAACTCATCGAGCATCA AATG] [underlined sequences are homologous to the Kan^r gene of plasmid pUC4K]) were employed to amplify a 0.9-kb Kan^r cassette of pUC4K. This linear PCR product with flanking homologous sequences of *sufI* was electroporated into strain HME63, and Kan^r transformants were obtained at 30°C. The putative *sufI*::Kan deletion-substitution was transferred to a fresh background by P1 transduction, and subsequently, the presence of the deletion was confirmed by sequencing the PCR product amplified with the flanking primers (sufIFP3 [5′-CGGAATTCGGCAATCTGTATTTTTGC] and sufIRP3 [5′-CGGAATTCGC CCTCCTCGGGCGAGT] [EcoRI restriction sites are underlined]). This deletion removed the entire *sufI* gene.

Construction of *ftsA derivative of strain MR22.** The *ftsA** mutation was transferred into the *sufI* mutant MR22 from strain WM1659 (obtained from William Margolin) with the aid of the *leuB82*::Tn*10* marker, which is approximately 50% linked to the *ftsA* locus. Initially, strain WM1659 was transduced to Tet^r with a P1 lysate prepared on a strain carrying the *leuB*::Tn*10* marker. The presence of the *ftsA** mutation in the Tet^r transductants was examined by preparing P1 lysates on six of these and checking for the ability to suppress the temperature sensitivity of an *ftsK44* mutant. Of the six Tet^r transductants examined, two were shown to have the *ftsA** allele, and subsequently, the P1 lysates made with these two strains were used to transduce strain MR22. The presence of the *ftsA** allele in these *sufI* Tet^r transductants was tested by preparing P1 lysates on six of them and transducing them again into the *ftsK44* mutant strain. Of the six *sufI* Tet^r colonies, three were shown to carry the *ftsA** allele, and one of these was designated MR29. The isogenic Tet^r transductant that retained the wild-type *ftsA* locus was designated MR30.

LBON-Ts phenotype of *sufI* mutant. For most of the experiments, the growth of the *sufI* mutant was examined on LBON plates prepared from BBL-Difco Laboratories medium, on which growth was significantly inhibited at high temperatures (LBON-Ts phenotype). However, on LBON plates prepared with medium components sourced indigenously (Hi-Media, Mumbai, India), the growth inhibition was very severe, and therefore we used this medium for the isolation of multicopy suppressor plasmids as described below.

Screening for multicopy plasmids that suppress the phenotype of the *sufI* mutant. A multicopy plasmid library carrying approximately 3- to 5-kb *E. coli* genomic DNA fragments generated by partial Sau3A digestion and cloned at the BamHI site in a p15A-based plasmid, pACYC184, was a gift from M. Radman's laboratory. This plasmid pool was introduced into the *sufI* mutant MR22, and

transformants were plated on LBON-Cam plates at 42°C. Plasmids were isolated from colonies that grew to different extents, and their ability to suppress was reconfirmed after an additional round of transformation. Subsequent restriction analysis and sequencing with the vector primers (184TetA [5′-CGCCGAAACA AGCGCTCATGAGCC] and 184TetB [5′-CTATGCGCACCCGTTCTCGGAG CAC]) allowed the identification of various classes of plasmids that restored the viability of the *sufI* mutant on LBON medium. One such suppressor plasmid, pMN60, obtained in this screen carried the complete *dapE* gene and the C-terminal part of the adjacent *acrD* gene. Deletion of *dapE* from pMN60 eliminated the ability to suppress the phenotype of MR22, indicating that *dapE* was responsible for the multicopy suppression.

Other techniques. Standard protocols were followed for experiments involving recombinant DNA and plasmid manipulations (39). Transpositions, transductions, P1 phage preparations, and β-galactosidase assays were performed using standard methods, as described previously (33).

Growth and viability measurements. The viability of each strain was measured by applying 8-μl aliquots of various dilutions (10^{-2}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) of overnight cultures to appropriate plates and incubating them for 20 to 36 h. The relative plating efficiency and growth were determined for each strain based on comparison with the control strain on the same plate.

Microscopy. The strains to be examined by microscopy were diluted into appropriate medium from fresh overnight cultures and processed after 6 to 8 h of growth. They were fixed in 2% formaldehyde for 1 h at 37°C, washed twice with phosphate-buffered saline, and suspended in the same buffer containing 50% glycerol. For DAPI (4′,6′-diamidino-2-phenylindole dihydrochloride) staining of nucleoids, fixed cells were treated with 0.25 μg/ml DAPI for 15 min at room temperature and mounted on slides. Differential interference contrast (DIC) and fluorescence images were taken using a Zeiss Axioplan fluorescence microscope and were processed in Adobe Photoshop.

RESULTS

A *sufI* deletion mutant is sensitive to oxidative stress and DNA damage at high temperature. In order to study the phenotypes of a *sufI* mutant, a complete deletion of the *sufI* locus was made in the chromosome of strain MR2 as described in Materials and Methods. This mutant, designated MR22 (MR2 Δ*sufI222*::Kan), grew quite well on LB plates at all temperatures and on LBON plates at 30°C; however, on LBON at 37°C or 42°C, growth was significantly inhibited (Fig. 1A). This growth inhibition (LBON-Ts) was osmoremedial, as supplementation with any of a variety of osmolytes, such as glucose (Fig. 1A), sucrose, glycerol, or NaCl (data not shown), completely rescued the growth of MR22. The LBON-Ts phenotype of the *sufI* mutant was seen only on medium exposed to good aerobic conditions; the generation of partially anaerobic conditions by tightly sealing the plate with Parafilm alleviated the LBON-Ts phenotype (Fig. 1A). Furthermore, the addition of a chemical reductant, ascorbate, to the growth medium eliminated the LBON-Ts phenotype (Fig. 1A). Since these observations suggested that molecular oxygen or oxidative stress could be toxic to the *sufI* mutant, the effect of reactive oxygen radicals on the growth of this mutant was examined by adding either PQ, a redox cycling agent that generates superoxide radicals (O_2^-), or H_2O_2, which generates hydroxyl radicals (OH·). The results showed that the *sufI* mutant was extremely sensitive to PQ (PQ^s) at 37°C or 42°C (Fig. 1A), indicating that superoxide-generated stress is lethal at high temperatures. It was only slightly sensitive to hydrogen peroxide up to 0.5 mM (data not shown).

We also examined the effect of DNA damage on the growth of the *sufI* mutant strain MR22 by exposing it to the DNA-damaging agent MMC. As shown in Fig. 1A, this mutant was extremely sensitive to MMC at a concentration of 0.3 μg/ml at 42°C. It is known that treatment of *E. coli* with DNA-damaging agents such as MMC elicits a strong SOS response, thereby inducing SulA (44), an inhibitor of FtsZ polymerization (34). In order to examine whether the MMC sensitivity (MMC^s) was mediated by SulA, a mutation in *sulA* was introduced into MR22, and it was shown that the *sufI sulA* double mutant (strain MR32) was insensitive to MMC treatment, clearly demonstrating that this process occurs by SOS-mediated induction of SulA (Fig. 1A). Likewise, the introduction of a mutation in *lon* that causes elevated levels of SulA (because *lon* encodes a protease whose normal substrate is SulA) (44) into strain MR22 resulted in extreme sickness at high temperature, which could be alleviated by a mutation in *sulA* (data not shown).

However, both the LBON-Ts (data not shown) and PQ^s phenotypes of MR22 (Fig. 1A) were not suppressed by *sulA* mutation, indicating that sensitivity to oxidative stress is not due to the SOS response caused by DNA damage. On the other hand, strain MR22 was not sensitive to alkylating agents, such as MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) and EMS (ethyl methane sulfonate) (data not shown). An insertion mutation in *tatB* that affects the formation of a functional TatABC translocase, thereby abolishing the export of SufI into the periplasm, also conferred sensitivity to PQ or MMC at high temperatures; however, the *tatB* mutant required slightly higher concentrations of these agents for its sensitivity than did the *sufI* mutant (data not shown).

The *sufI* mutant is filamentous at high temperatures. Although the growth of strain MR22 was appreciably inhibited on LBON plates at 42°C, the cultures grown in LBON broth showed only a minor decrease in absorbance (A_{600}) values (and also a corresponding reduction in the number of CFU) in the exponential phase and almost no difference in values in the stationary phase compared to those for the wild-type strain, MR2 (Fig. 1B). Likewise, the growth rate of MR22 was also not altered by the addition of PQ (up to 40 μM) to the broth cultures at 42°C (Fig. 1B).

We examined the cell morphology of the *sufI* mutant by taking aliquots from the cultures growing in LBON at 42°C at various time points. As shown in Fig. 1C, the cells from early exponential phase appeared to be significantly longer and were heterogeneous in size. Nevertheless, the filamentation of these mutants slowly disappeared as the culture entered into stationary phase. At an A_{600} value of around 1, the cells started regaining their normal shape, and by an A_{600} value of 1.5, the wild-type and mutant cells were almost indistinguishable, though the latter were slightly elongated (Fig. 1C). However, a few exceptionally long filaments could be seen at a very low frequency (approximately 1 in 10^4 cells) in the stationary-phase cultures of the *sufI* mutant (inset in Fig. 1C). A similar pattern of filamentation was seen in cultures grown in either LB or minimal medium (with 0.2% glucose as the C source) at 42°C, demonstrating that cell elongation is a function of temperature and does not depend on the growth rate in the medium (data not shown). Likewise, the presence of PQ in the growth medium had no effect on the filamentation of the *sufI* mutant; it neither enhanced nor allowed filamentation to persist in the stationary phase (data not shown). Conversely, MMC treatment greatly enhanced the filamentation of the *sufI* mutant (because of SulA-mediated inhibition of FtsZ), and as expected, this increase was abolished in the *sufI sulA* double mutant, MR32 (data not shown). Nevertheless, strain MR32

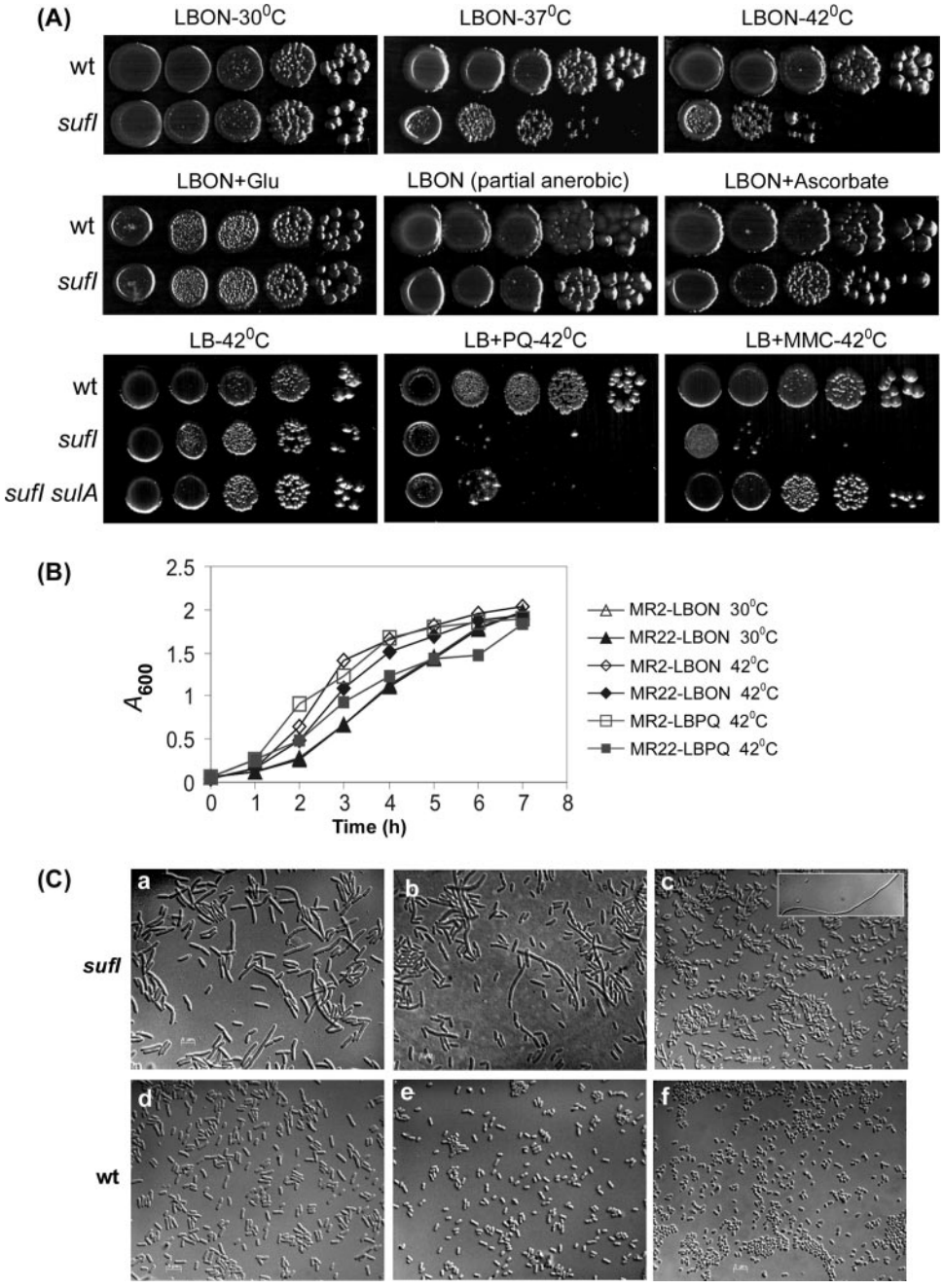

FIG. 1. Growth and filamentation of *sufI* mutant. (A) Growth of MR2 (wt) and MR22 (*sufI*) on LBON plates at 30, 37, and 42°C and on LBON plates (at 42°C) with 0.4 M glucose, tightly sealed with Parafilm (to create partial anaerobic conditions), or with 15 mM ascorbate. (Bottom) Growth of wild-type, *sufI*, and *sufI sulA* (MR32) strains on LB plates supplemented with either PQ (20 μM) or MMC (0.3 μg/ml) at 42°C. (B) Growth curves for wild-type and *sufI* strains at 30 or 42°C in LBON broth or LB broth plus PQ (40 μM; LBPQ) at 42°C. (C) DIC micrographs of *sufI* (a, b, and c) and wild-type (d, e, and f) cells taken out after 90 min, 3 h, or 6 h of growth in LBON at 42°C. The inset in panel c shows a very rare and exceptionally long *sufI* mutant filament.

was as filamentous as MR22, clearly indicating that the filamentation seen in the *sufI* mutant (Fig. 1C) was not a consequence of SOS induction and that SufI as such is required for some step in the process of cell septation.

Visualization of the nucleoids by DAPI staining followed by fluorescence microscopy revealed that they were normal and regularly spaced, without any significant chromosomal aberrations or partition defects (data not shown).

Identification of multicopy suppressors of *sufI*. To understand the basis of *sufI* phenotypes, we identified multicopy suppressor plasmids that restored viability to the mutant on LBON at 42°C as described in Materials and Methods. One major class of suppressor plasmids was found to carry the complete *ftsN* gene. Since multicopy *ftsN* is known to suppress the defects of many division mutants (11, 14, 38), we tested the effect of plasmid pMN14, a medium-copy-number vector with

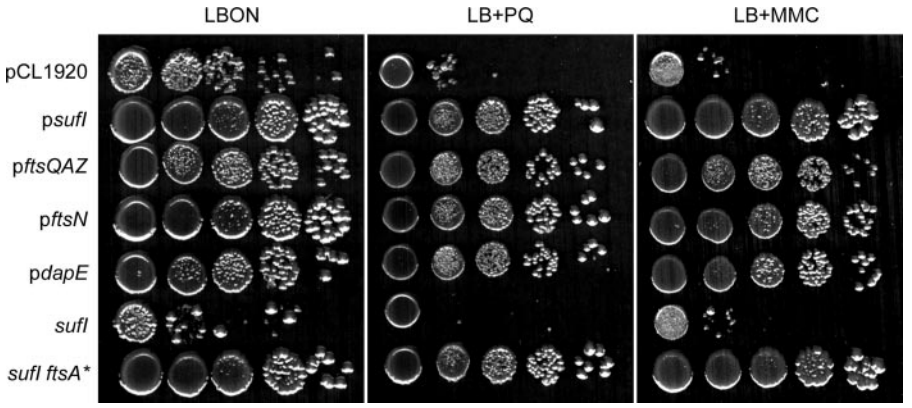

FIG. 2. Suppressors of *sufI*. Cultures of MR22 carrying the plasmid pCL1920, its derivatives with cloned *sufI*, *ftsQAZ*, or *ftsN*, or pMN60 (pACYC184-*dapE*) and strains MR29 (*sufI ftsA* leuB*::Tn*10*) and MR30 (*sufI leuB*::Tn*10*) were grown at 30°C in LB, and various dilutions (10^{-2}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) were applied to LBON, LB-plus-PQ (20 μM), or LB-plus-MMC (0.4 μg/ml) plates and grown for 24 h at 42°C.

cloned *ftsN* (38), on the growth of the *sufI* strain MR22. The results showed that multicopy *ftsN* was able to suppress the LBON-Ts phenotype of MR22 very efficiently (Fig. 2). Another set of plasmids conferring multicopy suppression of *sufI* carried the *sdiA* locus, which encodes a positive regulator of the *ftsQAZ* operon (46). Thereafter, the effect of increased *ftsQAZ* expression was directly examined by introducing plasmid pMN8 (38), and it was also found to confer viability to MR22 on LBON medium. However, plasmids carrying *ftsQ*, *ftsA*, *ftsQA*, and *ftsAZ* (38) did not restore growth to MR22 on LBON.

Another multicopy suppressor plasmid, pMN60, carrying the complete *dapE* gene, also suppressed the defects of MR22 (Fig. 2). *dapE* encodes *N*-succinyl-L-diaminopimelic acid desuccinylase, an enzyme that catalyzes the last step in the synthesis of L-diaminopimelic acid, which is an essential structural component of the peptidoglycan layer (7). It was earlier identified as a multicopy suppressor of a temperature-sensitive allele of the heat shock gene *grpE* (*grpE80*), which codes for a cochaperone of the DnaK-DnaJ system (48). DapE was also shown to function as a Mn^{2+}-dependent aspartyl peptidase in *Salmonella enterica* serovar Typhimurium (8); however, it is not very clear which activity of *dapE* is responsible for the suppression of SufI phenotypes. Multicopy *dapE* was also identified as a suppressor of *ftsEX* (M. Reddy, unpublished results).

In addition to conferring growth on LBON at 42°C, all of the above-described plasmids relieved both the PQ^s and MMC^s phenotypes of strain MR22 (Fig. 2). The filamentation of MR22 carrying these plasmids was also completely reduced (data not shown).

The *ftsA allele alleviates the defects of the *sufI* mutant.** *ftsA** is a gain-of-function allele that encodes a mutant FtsA protein (R286W) which is capable of suppressing the defects of *zipA* (15), *ftsK* (14), and *ftsN* (4) deletion mutants. The *ftsA** allele was introduced into strain MR22 by phage P1-mediated transduction with the linked *leuB*::Tn*10* marker (as described in Materials and Methods), and it was observed that the transductants carrying the *ftsA** allele were not sensitive to either PQ or MMC and showed no growth defects on LBON at 42°C (Fig. 2). The double mutants also regained their normal cell shape at high temperatures (data not shown).

Multicopy SufI suppresses the division defects of *ftsA12*, *ftsQ1*, and *ftsK44* mutants but not that of an *ftsZ84* mutant. It was shown earlier that multicopy *sufI* could partially suppress the thermosensitivity of an *ftsI23* mutant (25). In addition, it restored the viability of Δ*ftsEX* mutants under low-osmolarity conditions (38). Here the ability of multicopy *sufI* to suppress the defects of other division mutants was examined by introducing the plasmid pCL1920 (control vector) or pMN16 (pCL1920 with cloned *sufI*) into strains MR24, MR25, MR26, and MR27, which carry *ftsZ84* (Ts), *ftsA12* (Ts), *ftsK44* (Ts), and *ftsQ1* (Ts) mutations, respectively. As shown in Fig. 3A, strain MR26/pMN16 grew well at 42°C on both LB (Fig. 3A) and LBON (data not shown) plates, and correspondingly, its filamentation was notably decreased (Fig. 3B). Strains MR25/pMN16 and MR27/pMN16 also grew well at 42°C on LB plates (Fig. 3A) but not on LBON plates (data not shown). The filamentation of these strains was not significantly decreased at 42°C, although the filaments appeared to be slightly shorter and healthier (data not shown). However, the filamentation of both of these strains was considerably reduced when they were grown at 37°C (Fig. 3B). The plasmid pMN16 did not suppress the temperature sensitivity of strain MR24 on either LBON (Fig. 3A) or LBON supplemented with 0.5% NaCl (data not shown), and likewise, the filamentation remained unchanged (data not shown).

Multicopy SufI relieves the MMC and PQ sensitivity of division mutants. We examined the sensitivity of other division mutants (carrying the *ftsZ84*, *ftsA12*, *ftsK44*, *ftsQ1*, *ftsI23*, Δ*ftsEX*, or Δ*sufI* allele) to DNA damage or oxidative stress by exposing them to either MMC or PQ at 30°C. Compared to the wild-type strain MR2, all of the division mutants, excepting the *ftsZ84* mutant, were sensitive to both agents at a range of concentrations. Of all the mutants, strains MR28 (*ftsI23*) and MR10 (Δ*ftsEX*) were extremely sensitive to MMC (at 0.4 μg/ml) and PQ (at 15 μM), and this sensitivity was abolished by overexpression of SufI. Plasmid pMN58, a derivative of pBAD18 with *sufI* placed under the control of the arabinose-regulated promoter, conferred a growth ability on MR28 and MR10 upon the addition of 0.2% arabinose, whereas the addition of glucose had no effect (Fig. 4).

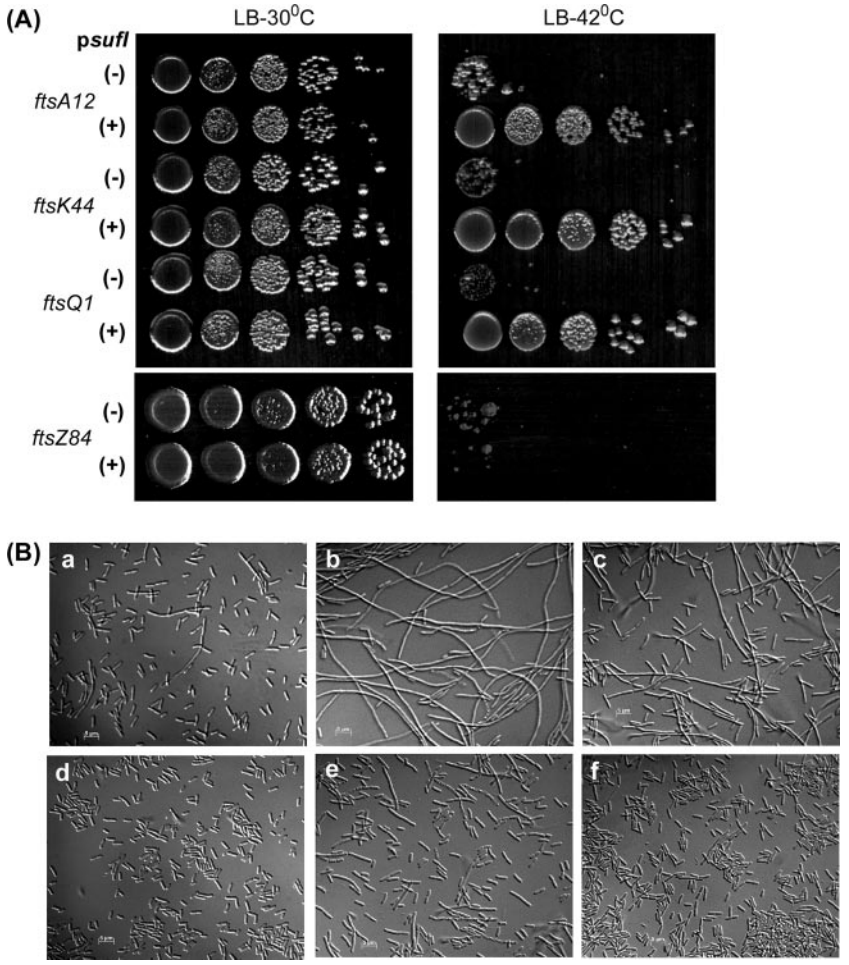

FIG. 3. Effects of multicopy *sufI* on growth and filamentation of division mutants. (A) Viability of division mutants carrying the vector, pCL1920 (−), or pMN16 (+) on LB plates at 30 or 42°C. *ftsZ84* mutants were plated on LBON. (B) DIC micrographs of *ftsA12*, *ftsK44*, and *ftsQ1* strains carrying either pCL1920 (a, b, and c) or pMN16 (d, e, and f). Cells were grown to mid-exponential phase in LB at 37°C (*ftsA12* and *ftsQ1* cells) or 42°C (*ftsK44* cells) and then taken for microscopy.

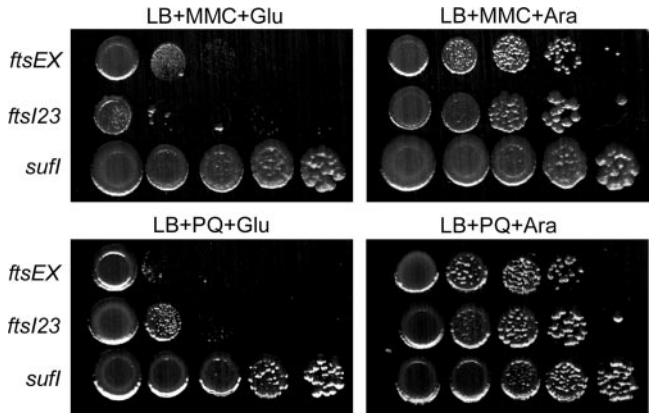

FIG. 4. Effects of multicopy *sufI* on MMC and PQ sensitivities of division mutants. Cultures of Δ*ftsEX*::Kan, *ftsI23*, and Δ*sufI* mutants carrying plasmid pMN58 (pBAD18-*sufI*) were grown, and dilutions were applied to LB-MMC (0.4 μg/ml) or LB-PQ (15 μM) plates supplemented with either 0.2% L-arabinose or D-glucose and incubated for 24 h at 30°C.

SufI is indispensable for viability of the *ftsI23* mutant. To check the effect of *sufI* deletion on the growth and viability of other division-defective mutants, we attempted to introduce the *sufI*::Kan deletion directly by phage P1-mediated transduction. It could be introduced into strains MR24 (*ftsZ84*) and MR25 (*ftsA12*) but not into MR26 (*ftsK44*), MR27 (*ftsQ1*), or MR28 (*ftsI23*). The reciprocal transductions were also done by using the linked *leuB*::Tn*10* marker for selection in the case of mutations in MR24, MR25, MR27, and MR28 and the *zbj*::Tn*10* marker in the case of mutation in MR26, and similar results were obtained (data not shown). However, the *sufI* deletion could be introduced into strains MR26 and MR27 in the presence of increased concentrations of osmolytes (i.e., on LB plates supplemented with 0.2 M glucose or NaCl), but not into MR28 (although very tiny transductants appeared after 48 h of incubation, they failed to grow upon further purification [data not shown]). Even in the presence of multicopy plasmids carrying *ftsN*, *ftsQAZ*, or *dapE*, the *sufI* deletion could not be introduced into strain MR28 (data not shown), suggesting that *sufI* is absolutely required for the viability of the *ftsI23* mutant. It was shown earlier that a *sufI ftsEX* double mutant is

also inviable (38). All of these observations indicate that the absence of SufI causes sickness to the division mutants to different extents.

Transcriptional regulation of *sufI*. To examine whether the expression of *sufI* is regulated by conditions of stress, the promoter of *sufI* was cloned into the single-copy promoter-probe vector pMU2385 (45), and its expression was measured by β-galactosidase reporter gene assays. The basal level of β-galactosidase activity of the vector was in the range of 3 to 5 Miller units, whereas plasmid pMN59 (with the *sufI* promoter) showed activity of 25 to 30 Miller units, indicating that the promoter is weak. None of the perturbations tested, including altered temperature, osmolarity, stationary phase, oxidative stress, DNA damage, or mutations in either the stationary-phase sigma factor gene *rpoS* or *soxR*, encoding an activator of the superoxide regulon, affected the promoter strength of the *sufI* promoter.

DISCUSSION

In this study, we have made an attempt to understand the function of SufI by analyzing mutant phenotypes and multicopy effects. The results implicate a role for SufI in the stabilization of divisomal components since it is shown to be required for the viability of *E. coli* under several conditions in which division appears to be compromised. *sufI* deletion mutants exhibited apparently pleiotropic phenotypes, i.e., (i) cell filamentation, (ii) sensitivity to superoxide stress, (iii) sensitivity to DNA damage, and (iv) temperature sensitivity on low-osmotic-strength media during aerobic growth (Fig. 1). However, the fact that all of these phenotypes were simultaneously suppressed by high levels of the division proteins FtsQAZ and FtsN or the presence of the *ftsA** allele indicates that they have a common mechanistic basis (Fig. 2).

Role of SufI in cell division. The *sulA*-independent filamentation phenotype of the *sufI* deletion mutant at 42°C indicates that some step of cell septation is blocked at high temperatures (Fig. 1C). The filamentation pattern of the *sufI* mutant is interesting in that cells in the early exponential phase exhibit cell elongation but gradually regain their normal cell shape at later stages of growth (Fig. 1C), suggesting the existence of an adaptation mechanism that operates in stationary phase. Since the stationary-phase sigma factor RpoS regulates a morphogene, *bolA*, that controls cell shape in stationary phase (27), an *rpoS* deletion was introduced but was shown not to alter the viability or filamentation pattern of MR22 (data not shown). One other possible reason for this growth phase-dependent filamentation of the *sufI* mutant could be the generation of partially anaerobic conditions in the medium with increasing culture density; however, cells grown with high or low aeration did not show significant variation in filamentation (data not shown).

The suppression of *sufI* mutant filamentation by factors that stabilize the FtsZ ring assembly strongly argues for the functional involvement of *sufI* in the division process. Most of the phenotypes of the *sufI* mutant (Fig. 1) were suppressed by multiple copies of FtsQAZ, showing that a coordinated increase of all these divisomal proteins removes the defect of *sufI* deletion (Fig. 2). Likewise, multicopy FtsN also abolished all the defects of the *sufI* mutant. Furthermore, the presence of the gain-of-function *ftsA** allele, which codes for an altered FtsA (R286W) protein, alleviated the phenotypes of the *sufI* mutant very efficiently (Fig. 2). This allele emerged as a nonspecific suppressor of most of the division mutants and, interestingly, was able to eliminate the requirement for several essential division proteins, such as ZipA (15), FtsK (14), FtsN (4), and FtsEX (Reddy, unpublished observations), most likely by stabilizing the FtsZ ring assembly (16). In accordance with all of the above observations, a SufI-green fluorescent protein fusion protein has been shown to localize to the division septum (David Weiss, personal communication).

SufI is required for growth under conditions of stress. Most of the phenotypes of the *sufI* mutant were more striking at 37 or 42°C, reflecting the need for SufI at high temperatures (Fig. 1A) and/or implicating high temperature as a sensitizing factor for the assembly of the divisome (38). It appears that SufI is rendered essential for the growth of *E. coli* whenever the division process is compromised by various conditions, as described below.

(i) Decreased FtsZ function through high levels of SulA, generated either by treatment with the DNA-damaging agent MMC or by a mutation in Lon protease, is detrimental to the *sufI* mutant (Fig. 1A). This premise is also strengthened by the extreme sickness of an *ftsZ84 sufI* double mutant at 42°C (on LBON plates that contained 0.7% NaCl, on which both the single mutants grew reasonably well) and also by its filamentation phenotype in LB at 30°C, where the single mutants showed normal cell morphology (data not shown). Hence, it seems that the MMC^s phenotype of the *sufI* mutant is due to two overlapping mechanisms, i.e., compromised division in the absence of SufI coupled to the lowered activity of FtsZ.

(ii) SufI is also required for the growth and viability of the various division-defective mutants carrying the *ftsQ1* (Ts), *ftsK44* (Ts), *ftsI23* (Ts), or Δ*ftsEX* allele. The extent of dependence on SufI may vary with the severity of the division-defective mutation, and this could be the most likely basis for the absolute requirement of SufI for the viability of either the Δ*ftsEX* or *ftsI23* mutant (38; this study).

(iii) Unlike the MMC^s phenotype, both the PQ^s and LBON-Ts phenotypes of the *sufI* mutant are not suppressed by a mutation in *sulA*, showing that these are not the result of a DNA damage response (Fig. 1A). This observation permitted us to speculate that oxidative damage may cause stress to the divisomal assembly, and the fact that most of the division mutants are sensitive to superoxide radicals (Fig. 4) supports the idea that the division process could be intrinsically sensitive to oxidative stress.

However, the effect of oxidative stress on the division assembly could be indirect, as growth at high temperatures coupled with increased oxidative stress or low osmotic strength may require a higher activity of chaperones and therefore may affect the proper folding of division proteins. It was recently shown that the chaperonin GroE is involved in folding of the division protein FtsE, and the filamentation phenotype of a GroE depletion mutant is due to the improper folding of FtsE (13). It has been shown that the rate of superoxide formation in the periplasm is fairly high (~3 μM/s, when normalized to the estimated periplasmic volume, whereas the rate in the cytosol is ~5 μM/s), and it also contains a Cu, Zn superoxide dismutase (SodC) for scavenging the periplasmic superoxides

(19, 26). Yet *sodC* mutants are not sensitive to superoxide-generating agents, indicating that there probably should be some hitherto unidentified factor(s) that protects the periplasm from superoxide stress (19). However, the preliminary observations show that a *sufI sodC* double mutant is viable and just as PQ sensitive as the *sufI* single mutant. Likewise, mutations in *sodAB* which abolish the cytoplasmic superoxide dismutase activity (10) also did not alter the viability or filamentation pattern of the *sufI* mutant (data not shown).

SufI stabilizes the divisomal assembly. In an earlier study (38), we speculated that both SufI and FtsEX may have redundant functions, based on the findings that (i) the presence of *sufI* is essential for the viability of an *ftsEX* deletion mutant and (ii) the multicopy SufI protein is able to substitute for the functions of FtsEX. However, in this study, it was observed that the absence of SufI is detrimental to the growth of several division-defective mutants. Hence, it is possible that the function of SufI may not be particularly redundant or overlapping with that of FtsEX alone but may have a wide-ranging role in protecting the divisomal assembly or its components. In support of this, multicopy *sufI* suppressed the growth defects of division mutants carrying the *ftsA12*, *ftsEX*, *ftsK44*, *ftsQ1*, or *ftsI23* allele (25, 38; this study). In addition to suppressing the thermosensitivity of the division mutants (Fig. 3A), multicopy *sufI* also abolished the PQ and MMC sensitivity of *ftsI23* and *ftsEX* mutants (Fig. 4), validating the idea that SufI protects the divisomal components against damage caused by various stress conditions. Preliminary observations from this laboratory showed that increased FtsQAZ or FtsN also suppresses the PQ and MMC sensitivity of *ftsI23* and *ftsEX* mutants.

The restoration of the growth defects of the division mutants by multicopy *sufI* is reminiscent of the suppression shown by multicopy *ftsN* (11). Both *sufI* and *ftsN* in multiple copies are able to confer growth to all of the above mutants, except the *ftsZ84* mutant. The role of FtsN in division is not clear; in vitro, it binds murein sacculus and is presumed to be involved in peptidoglycan assembly or stabilization of the divisome components (42). It is possible that SufI may also function in an analogous way. As proposed earlier (14), division proteins may perform two main functions: one is to stabilize the interactions between divisome components, and the second is to generate the division septum. SufI may fall into the former class of divisome proteins.

However, the precise biochemical function of SufI does not emerge from these studies. A homology search with SufI has shown that it belongs to the multicopper oxidase family. It closely resembles the blue copper oxidase (CueO) of *E. coli* and, to a lesser extent, the spore coat protein (CotA) of *Bacillus subtilis*. Both CueO and CotA are copper-dependent oxidoreductases. CueO is a TatABC substrate that confers copper tolerance to *E. coli* by oxidizing the toxic cuprous ions into cupric ions in the periplasm (20), whereas CotA is a thermostable laccase that protects the spores of *B. subtilis* against UV irradiation and oxidative stress (23). Unlike its homologs, SufI is not known to bind any cofactor (3). SufI does not appear to be a ubiquitous protein. In this context, it is interesting that both FtsN and SufI are present only in organisms belonging to the orders *Enterobacteriales* and *Pasteurellales* of the *Gammaproteobacteria*.

Our results, along with the localization data of David Weiss, strongly argue for a role of *sufI* in the cell division of *E. coli*. Since *sufI* fulfils the requirements of an *fts* gene (as defined in reference 9), we propose that it be redesignated *ftsP*. In addition, this will avoid confusion with other known *suf* genes (the *sufABCDSE* operon located at 37.9 min) that are involved in the biogenesis of iron-*sulf*ur (Fe-S) clusters in *E. coli* (37).

ACKNOWLEDGMENTS

We thank Jon Beckwith, Donald Court, James Imlay, William Margolin, and Miroslav Radman for various strains and plasmids, R. N. Amrutha for construction of strain MR32, and J. Gowrishankar for advice and suggestions on the manuscript. We are grateful to David Weiss for sharing results prior to publication and to Mary Berlyn (CGSC) for advice on renaming *sufI*. We thank the anonymous reviewers for useful suggestions, in particular the reviewer who suggested the use of the DNA-damaging agent MMC.

This work was supported in part by funds from the Department of Biotechnology (DBT), Government of India. H.S. is a DBT postdoctoral fellow.

REFERENCES

1. **Aarsman, M. E. G., A. Piette, C. Fraipont, T. M. F. Vinkenvleugel, M. Nguyen-Disteche, and T. den Blaauwen.** 2005. Maturation of the *Escherichia coli* divisome occurs in two steps. Mol. Microbiol. **55:**1631–1645.
2. **Alami, M., I. Luke, S. Deitermann, G. Eisner, H.-G. Koch, J. Brunner, and M. Muller.** 2003. Differential interactions between a twin-arginine signal peptide and its translocase in *Escherichia coli*. Mol. Cell **12:**937–946.
3. **Berks, B. C., T. Palmer, and F. Sargent.** 2005. Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. Curr. Opin. Microbiol. **8:**174–181.
4. **Bernard, C. S., M. Sadasivam, D. Shiomi, and W. Margolin.** 2007. An altered FtsA can compensate for the loss of essential cell division protein FtsN in *Escherichia coli*. Mol. Microbiol. **64:**1289–1305.
5. **Bernhardt, T. G., and P. A. de Boer.** 2003. The *Escherichia coli* amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. Mol. Microbiol. **48:**1171–1182.
6. **Bernhardt, T. G., and P. A. de Boer.** 2004. Screening for synthetic lethal mutants in *Escherichia coli* and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. Mol. Microbiol. **52:** 1255–1269.
7. **Bouvier, J., C. Richaud, W. Higgins, O. Bogler, and P. Stragier.** 1992. Cloning, characterization, and expression of the *dapE* gene of *Escherichia coli*. J. Bacteriol. **174:**5265–5271.
8. **Broder, D. H., and C. G. Miller.** 2003. DapE can function as an aspartyl peptidase in the presence of Mn^{2+}. J. Bacteriol. **185:**4748–4754.
9. **Buddelmeijer, N., and J. Beckwith.** 2002. Assembly of cell division proteins at the *E. coli* cell center. Curr. Opin. Microbiol. **5:**553–557.
10. **Carloz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5:**623–630.
11. **Dai, K., Y. Xu, and J. Lutkenhaus.** 1993. Cloning and characterization of *ftsN*, an essential cell division gene in *Escherichia coli* isolated as a multicopy suppressor of *ftsA12* (Ts). J. Bacteriol. **175:**3790–3797.
12. **Errington, J., R. A. Daniel, and D. J. Scheffers.** 2003. Cytokinesis in bacteria. Microbiol. Mol. Biol. Rev. **67:**52–65.
13. **Fujiwara, K., and H. Taguchi.** 2007. Filamentous morphology in GroE-depleted *Escherichia coli* induced by impaired folding of FtsE. J. Bacteriol. **189:**5860–5866.
14. **Geissler, B., and W. Margolin.** 2005. Evidence for functional overlap among multiple bacterial cell division proteins: compensating for the loss of FtsK. Mol. Microbiol. **58:**596–612.
15. **Geissler, B., D. Elraheb, and W. Margolin.** 2003. A gain-of-function mutation in *ftsA* bypasses the requirement for the essential cell division gene *zipA* in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **100:**4197–4202.
16. **Geissler, B., D. Shiomi, and W. Margolin.** 2007. The *ftsA** gain-of-function allele of *Escherichia coli* and its effects on the stability and dynamics of the Z ring. Microbiology **153:**814–825.
17. **Gerding, M. A., Y. Ogata, N. D. Pecora, H. Niki, and P. A. de Boer.** 2007. The *trans*-envelope Tol-Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in *E. coli*. Mol. Microbiol. **63:**1008–1025.
18. **Goehring, N. W., and J. Beckwith.** 2005. Diverse paths to midcell: assembly of the bacterial cell division machinery. Curr. Biol. **15:**514–526.
19. **Gort, A. S., D. M. Ferber, and J. A. Imlay.** 1999. The regulation and role of the periplasmic copper, zinc superoxide dismutase of *Escherichia coli*. Mol. Microbiol. **32:**179–191.
20. **Grass, G., and C. Rensing.** 2001. CueO is a multi-copper oxidase that confers

copper tolerance in *Escherichia coli*. Biochem. Biophys. Res. Commun. **286:** 902–908.
21. **Guthrie, B., and W. Wickner.** 1990. Trigger factor depletion or overproduction causes defective cell division but does not block protein export. J. Bacteriol. **172:**5555–5562.
22. **Guzman, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
23. **Hullo, M.-F., I. Moszer, A. Danchin, and I. Martin-Verstraete.** 2001. CotA of *Bacillus subtilis* is a copper-dependent laccase. J. Bacteriol. **183:**5426–5430.
24. **Ize, B., N. R. Stanley, G. Buchanan, and T. Palmer.** 2003. Role of the *Escherichia coli* Tat pathway in outer membrane integrity. Mol. Microbiol. **48:**1183–1193.
25. **Kato, J., Y. Nishimura, M. Yamada, H. Suzuki, and Y. Hirota.** 1988. Gene organization in the region containing a new gene involved in chromosome partition in *Escherichia coli*. J. Bacteriol. **170:**3967–3977.
26. **Korshunov, S., and J. A. Imlay.** 2006. Detection and quantification of superoxide formed within the periplasm of *Escherichia coli*. J. Bacteriol. **188:** 6326–6334.
27. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor sigma S. J. Bacteriol. **173:**4474–4481.
28. **Lee, P. A., D. Tullman-Ercek, and G. Georgiou.** 2006. The bacterial twin-arginine translocation pathway. Annu. Rev. Microbiol. **60:**373–395.
29. **Lerner, C. G., and M. Inouye.** 1990. Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. Nucleic Acids Res. **18:**4631.
30. **Liu, G., G. C. Draper, and W. D. Donachie.** 1998. FtsK is a bifunctional protein involved in cell division and chromosome localization in *Escherichia coli*. Mol. Microbiol. **29:**893–903.
31. **Lutkenhaus, J., and S. G. Addinall.** 1997. Bacterial cell division and the Z-ring. Annu. Rev. Biochem. **66:**93–116.
32. **McCarty, J. S., and G. C. Walker.** 1994. DnaK mutants defective in ATPase activity are defective in negative regulation of the heat shock response: expression of mutant DnaK proteins results in filamentation. J. Bacteriol. **176:**764–780.
33. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and other bacteria. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
34. **Mukherjee, A., C. Cao, and J. Lutkenhaus.** 1998. Inhibition of FtsZ polymerization by SulA, an inhibitor of cell septation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **95:**2885–2890.
35. **Nanninga, N.** 1998. Morphogenesis of *Escherichia coli*. Microbiol. Mol. Biol. Rev. **62:**110–129.
36. **Ogino, H., M. Wachi, A. Ishii, N. Iwai, T. Nishida, S. Yamada, K. Nagai, and M. Sugai.** 2004. FtsZ-dependent localization of GroEL protein at possible division sites. Genes Cells **9:**765–771.
37. **Patzer, S. I., and K. Hantke.** 1999. SufS is a NifS-like protein, and SufD is necessary for stability of the [2Fe-2S] FhuF protein in *Escherichia coli*. J. Bacteriol. **181:**3307–3309.
38. **Reddy, M.** 2007. Role of FtsEX in cell division of *Escherichia coli*: viability of *ftsEX* mutants is dependent on functional SufI or high osmotic strength. J. Bacteriol. **189:**98–108.
39. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
40. **Schmidt, K. L., N. D. Peterson, R. J. Kustusch, M. C. Wissel, B. Graham, G. J. Phillips, and D. S. Weiss.** 2004. A predicted ABC transporter, FtsEX, is needed for cell division in *Escherichia coli*. J. Bacteriol. **186:**785–793.
41. **Uehara, T., H. Matsuzawa, and A. Nishimura.** 2001. HscA is involved in the dynamics of FtsZ-ring formation in *Escherichia coli* K12. Genes Cells **6:**803–814.
42. **Ursinus, A., F. van den Ent, S. Brechtel, M. de Pedro, J. V. Holtje, J. Lowe, and W. Vollmer.** 2004. Murein (peptidoglycan) binding property of the essential cell division protein FtsN from *Escherichia coli*. J. Bacteriol. **186:** 6728–6737.
43. **Vicente, M., and A. I. Rico.** 2006. The order of the ring: assembly of *Escherichia coli* cell division components. Mol. Microbiol. **61:**5–8.
44. **Walker, G. C.** 1996. The SOS response of *Escherichia coli*, p. 1400–1416. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, DC.
45. **Wang, P., J. Yang, and A. J. Pittard.** 1997. Promoters and transcripts associated with the *aroP* gene of *Escherichia coli*. J. Bacteriol. **179:**4206–4212.
46. **Wang, X. D., P. A. de Boer, and L. I. Rothfield.** 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. EMBO J. **10:**3363–3372.
47. **Weiss, D. S.** 2004. Bacterial cell division and the septal ring. Mol. Microbiol. **54:**588–597.
48. **Wu, B., C. Georgopoulos, and D. Ang.** 1992. The essential *Escherichia coli msgB* gene, a multicopy suppressor of a temperature-sensitive allele of the heat shock gene *grpE*, is identical to *dapE*. J. Bacteriol. **174:**5258–5264.
49. **Yahr, T. L., and W. T. Wickner.** 2001. Functional reconstitution of bacterial Tat translocation *in vitro*. EMBO J. **20:**2472–2479.
50. **Yu, D., H. M. Ellis, E. C. Lee, N. A. Jenkins, N. G. Copeland, and D. L. Court.** 2000. An efficient recombination system for chromosome engineering in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**5978–5983.