JOURNAL OF BACTERIOLOGY, Mar. 1998, p. 1053–1062 Vol. 180, No. 5
0021-9193/98/$04.00+0

Control of *ftsZ* Expression, Cell Division, and Glutamine Metabolism in Luria-Bertani Medium by the Alarmone ppGpp in *Escherichia coli*

BRADFORD S. POWELL,† AND DONALD L. COURT*

Molecular Control and Genetics, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

Received 23 October 1997/Accepted 2 January 1998

Inactivation of transcription factor σ^{54}, encoded by *rpoN* (*glnF*), restores high-temperature growth in Luria-Bertani (LB) medium to strains containing the heat-sensitive cell division mutation *ftsZ84*. Mutational defects in three other genes involved in general nitrogen control (*glnD*, *glnG*, and *glnL*) also suppress lethal filamentation. Since addition of glutamine to LB medium fully blocks suppression by each mutation, the underlying cause of suppression likely derives from a stringent response to the limitation of glutamine. This model is supported by several observations. The *glnL* mutation requires RelA-directed synthesis of the nutrient alarmone ppGpp to suppress filamentation. Artificially elevated levels of ppGpp suppress *ftsZ84*, as do RNA polymerase mutations that reproduce global effects of the ppGpp-induced state. Both the *glnF* null mutation and an elevated copy number of the *relA* gene similarly affect transcription from the upstream (pQ) promoters of the *ftsQAZ* operon, and both of these genetic conditions increase the steady-state level of the FtsZ84 protein. Physiological suppression of *ftsZ84* by a high salt concentration was also shown to involve RelA. Additionally, we found that the growth of a *glnF* or *glnD* strain on LB medium depends on RelA or supplemental glutamine in the absence of RelA function. These data expand the roles for ppGpp in the regulation of glutamine metabolism and the expression of FtsZ during cell division.

Septation is the bacterial equivalent of cytokinesis and involves the regulated formation of a specialized cell wall at the midpoint of the dividing cell during vegetative growth. In *Escherichia coli*, loss of septation causes the formation of multinucleate cell filaments and eventual death. FtsZ is a GTPase and is the most abundant of several cell division proteins whose activity is essential for septation (for reviews, see references 29 and 51). FtsZ appears to guide septal invagination. Its subcellular distribution cycles with growth, coalescing at the division site during cytokinesis and then dispersing into the cytosol afterward (33, 40). This bimodal property could reflect a cyclic progression of FtsZ through active and inactive states. Much evidence supports the idea that the cellular activity of FtsZ depends on a dynamic self-polymerization reaction involving changes in localized concentration and a GTPase cycle akin to the tubulin proteins of eukaryotic cells (4, 6, 11, 35, 44, 60, 65). The thermosensitive allele *ftsZ84* has been the subject of numerous investigations into the nature of septation since its initial description more than 2 decades ago (47). Genetic, biochemical, and microscopic studies of *ftsZ84* strains have helped to generate a model that assigns a structural, and perhaps kinetic, role to FtsZ throughout cytokinesis, as well as a regulatory role for the commencement of septation. The process of discovery and characterization of additional factors affecting FtsZ continue to refine our understanding of its function in the cell cycle.

Purified FtsZ84 protein has reduced GTPase activity at elevated temperature, and cell filamentation is the consequence of this defect in vivo. This filamentation is relieved by growth media of high osmotic strength, a phenomenon originally called "salt repair" (47). The mechanism of salt repair for *ftsZ84* is not understood. Conditional lethality imparted by *ftsZ84* is genetically suppressed by increased dosages of some genes. Most notably, when present in multiple copies, the *ftsZ84* allele suppresses itself, verifying that lethality owes to its reduced activity (39). Three extragenic loci have been characterized as suppressors of *ftsZ84* when present in high dosages: the gene encoding the transcription factor SdiA (66) and two genes for regulators of capsular polysaccharide synthesis, *rcsB* and *rcsF* (20, 21). The connection between capsule gene regulation and septation is not clear. Too much FtsZ activity also disrupts normal septation (13, 67). Interestingly, this condition of FtsZ excess suppresses filamentation caused by abnormally high expression of another cell division gene, *ftsA*, which suggests the necessity of a dosage equilibrium between FtsA and FtsZ for their proper functioning (10, 12, 13).

The *ftsZ* and *ftsA* genes and another cell division gene, *ftsQ*, form a complex operon which comprises the distal end of a cluster of 16 aligned and overlapping genes concerning cell division and cell wall metabolism (3). The *ftsQAZ* operon contains several promoters, and there is genetic evidence for the influence of factors at some of these promoters. The majority of transcripts containing *ftsZ* appear to originate from promoters located 5′ of the operon (19, 69). One of these promoters (Qp2) is up-regulated by SdiA (66), and another (Qp1) is positively regulated by the stationary growth phase sigma factor σ^s (2, 56). Also contributing to FtsZ expression are four internal promoters (Ap, Zp2, Zp3, and Zp4) located within the *ftsQ* and *ftsA* coding regions (30) and antisense RNAs that are complementary to the 5′ region of *ftsZ* (14, 50, 58). The activities of some of these promoters vary inversely with the growth rate (2), and the levels of *ftsZ* transcripts appear to oscillate with the cell cycle (19). The short-lived nucleotide guanosine

* Corresponding author. Mailing address: Molecular Control and Genetics, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702. Phone: (301) 846-5940. Fax: (301) 846-6988. E-mail: court@ncifcrf.gov.

† Present address: Laboratory of Biochemical Genetics, National Institute of Mental Health, Bethesda, MD 20892.

tetraphosphate has recently received attention as a possible effector of cell division (61–63). Guanosine tetraphosphate and the related compound guanosine pentaphosphate, together referred to herein simply as ppGpp for brevity, function as an alarmone system and are believed to integrate cellular responses to various forms of nutrient stress (8). Natural synthesis of ppGpp in *Escherichia coli* occurs exclusively from the RelA and SpoT proteins, and the principal contribution is made by RelA. SpoT normally degrades ppGpp but has synthetic activity under certain conditions (27, 68). In the classic stringent response to the presence of uncharged tRNA, ppGpp increases the fidelity of protein translation (36). ppGpp also globally regulates gene expression by affecting initiation or pausing of RNA transcription and can have a positive or negative effect, depending on the targeted promoter. The identification of mutant σ^{70}, β, and β′ subunits of RNA polymerase that mimic the ppGpp-induced state supports the idea that this signal exerts an important effect at the level of transcription (8, 26).

Until now, no mutation has been reported that suppresses the high-temperature lethality of *ftsZ84* on Luria-Bertani (LB) medium. Several chromosomal mutations are identified here that restore septation and high-temperature growth on LB medium to strains having the *ftsZ84* mutation. Four of these suppressors are loss-of-function alleles for different genes that regulate general nitrogen assimilation and glutamine metabolism during nitrogen-restricted growth. The data presented here indicate that the likely mechanism of suppression by these mutations involves RelA-dependent synthesis of the nutrient starvation signal ppGpp in response to glutamine limitation. This introduces evidence associating ppGpp with glutamine regulation on LB medium.

MATERIALS AND METHODS

Strains, plasmids, bacteriophages, and growth media. The bacterial strains, plasmids, and bacteriophages used in this study are listed in Table 1. The *ftsZ84* strains used in this study have the genetic background of W3110 Δ(*lac*)*U169* (Court laboratory strain WJW45), except as specifically noted otherwise. Parental strain BSP610 and its derivatives were constructed by P1*vir* transductions using standard procedures (34) in the following steps. To make BSP610, the *ftsZ84* allele of strain JFL100 was first linked to a *leu*::Tn*10* marker and then cotransduced into WJW45 by selection for tetracycline resistance. Heat-sensitive transductants were then converted back to Leu^+ prototrophy by using a P1 lysate of WJW45 as the donor and selection on minimal-salts medium plus glucose medium, with subsequent confirmation of heat sensitivity and tetracycline sensitivity. Mutations of *glnA*, *glnD*, *glnE*, *glnF*, *glnG*, and *gltB* were introduced into BSP610 and congenic $FtsZ^+$ parental strain WJW45 by P1*vir* transductions from phage stocks prepared on donor strains containing the given mutation, employing selection for the drug resistance marker associated with each mutation. Unmarked mutations in *glnL*, *glnB*, and *gltBDF* were introduced, respectively, by transductions into the *glnA*, *glyA*, and *glnF* derivatives of BSP610 and WJW45 by selection for prototrophic conversion of these linked mutations.

Strains that lacked ppGpp, either partially (*relA251*::*kan*) or completely (*relA251*::*kan spoT207*::*cat*), were constructed by selection for the drug resistance marker associated with either disruption (68). The order of introduction of various mutations and discovery of certain medium requirements were determined empirically. The following restrictions were employed for construction of the final test strains. LB medium containing glutamine at 0.2% (LBQ) was used for the construction and propagation of *ftsZ84* and/or *relA*-derived strains having the Ntr^- phenotype, i.e., with a mutation of *glnF*, *glnD*, *glnG*, or *glnL*. Tetracycline was sometimes included in the medium during the construction of *ftsZ84 relA glnF* strains.

The phenotypes of glutamine prototrophy versus auxotrophy and the presence versus the lack of nitrogen control were tested, respectively, by plating at 32°C on M63 salts with glucose with or without glutamine (0.2% [wt/vol] each) and on ammonium-free W salts plus glucose medium containing either glutamine or arginine at 0.2% (wt/vol) as the sole source of nitrogen (57). Amino acid supplementation tests on *ftsZ84* suppressors used LB plates that individually contained each amino acid at a 0.05% (wt/vol) final concentration.

Thermosensitivity tests. While the thermosensitivity of *ftsZ84* is known to vary among laboratories, often requiring LB medium without added sodium chloride, we have found that our standard LB medium (10 g of Bacto Tryptone, 5 g of NaCl, 8 g of yeast extract; sterilized via autoclave) consistently produces filamentation and lethality at 42°C for *ftsZ84* strains in our W3110 genetic background. We observed that other *ftsZ84* strains of different genetic backgrounds were less sensitive on this LB medium, and so all heat sensitivity tests were performed on BSP610 and its derivatives. Heat sensitivities and filamentation were assayed by monitoring colony formation on LB plates and by microscopic inspection of suspended colonies or liquid LB cultures.

Assays for *ftsQAZ* operon expression. Reporter constructs containing *ftsQAZ* fusions to *lacZ* were introduced into tester strains either on low-copy-number plasmids (9) or in single copy on lambda prophages (Table 1). Phages λBBP133 and λBBP134 contain 342 nucleotides 5′ of the *ftsZ* start codon, which includes promoter Zp2, along with the first 11 codons of *ftsZ* joined to *lacZ* in protein and operon fusion configurations, respectively. To make these, DNA was amplified from the chromosome by using the oligonucleotide primers gttgacgaattcaagctcgccaacaaggggttaaacatcac and actttaggatccgcgtcattggtaagttccattggttcaaac and ULTma DNA polymerase (Perkin Elmer) and then digested with *Eco*RI and *Bam*HI and cloned into plasmids pRS414 or pRS415 (55), making plasmids pBP133 and pBP134, respectively. The absence of potential errors acquired during cloning was confirmed by direct sequencing of plasmids with the ABI Prism DNA Sequencing kit (Perkin Elmer) and a 373A DNA sequencer (Perkin Elmer). DNA sequences were assembled and analyzed by using Sequencher 3.0 (Gene Codes Corp.) and the Genetics Computer Group (Madison, Wis.) package, version 8.0. Each fusion was recombined from a plasmid onto λBDC531 to make phages λBBP133 and λBBP134 and used to infect BSP610, and prophage lysogens were screened for unit copy number as previously described (42). These strains were used as parental strains for all related strain constructions. β-Galactosidase activities were assayed by method B of Miller (34) with the following modification. Cultures were harvested by twofold dilution into ice-cold stop solution comprised of Z buffer plus 60-μg/ml chloramphenicol and 0.04% sodium azide.

Quantitation of FtsZ protein. The steady-state level of FtsZ84 protein in various strains was measured by Western blot assay done by standard procedures (23) and the protocols specified by the manufacturers of the electrophoretic and immunologic reagents. Overnight LB cultures were refreshed by 200-fold dilution and grown at 30°C to an optical density at 600 nm of 0.2 and then shifted to 42°C and grown for 2.5 h. Aliquots were collected at equivalent points in their respective growth curves, and volumes were normalized by optical density (5 ml at an optical density at 600 nm of 0.2). Cells were collected by centrifugation, washed in stop solution (described above), frozen immediately on dry ice, and then stored frozen until later use. To prepare extracts, cell pellets were thawed in 0.5 ml of buffer (50 mM sodium phosphate [pH 7.4], 10 μl of 10-mg/ml phenylmethylsulfonyl fluoride [Boehringer Mannheim]), mixed gently, passed through another freeze-thaw cycle, and then disrupted by sonication. Total protein was estimated via protein assay (Bio-Rad), and then cell extracts were normalized among each other by total protein concentration. Extracts were serially diluted into sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer and boiled briefly. Several dilutions of extract from each strain were tested. Proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose (Schleicher & Schuell Inc.) or Immobilon (Millipore) for immunoblot analysis. FtsZ was detected with anti-FtsZ serum and compared to purified FtsZ (gifts from the Rothfield laboratory) by using a peroxide-labeled secondary antibody and a LumiGlo chemiluminescent substrate kit (Kierkegaard & Perry). Band densities on X-Omat AR autoradiograph films (Kodak) were quantified with an LKB Ultroscan XL enhancer laser densitometer (Pharmacia LKB Biotechnologies). To check for consistency of measurements, total proteins were compared to estimates made with bicinchoninic acid and Coomassie Plus Protein Assay reagents (Pierce), and antigen detection was compared to immunoblots analyzed with a Storm 820 Phosphorimage Analyzer and the Vistra Systems Western Blot kit (Molecular Dynamics). Units representing the percent maximum signal of the desired protein band were compiled for FtsZ and an internal standard, NusA, detected with anti-NusA serum (59). FtsZ values from several measurements were normalized against their respective NusA values, adjusted for their respective loading volumes and exposure times, and finally summed to calculate an average FtsZ value for each strain.

RESULTS

Suppression correlates with the Ntr^- phenotype. While exploring the functions of newly identified genes of the *rpoN* operon (41), we discovered that mutations which disrupt the *rpoN* gene (41) allowed *ftsZ84* strains to grow on LB agar at 42°C. Examination of these suppressor mutations by complementation tests showed that plasmid or lambda clones that replenish only the wild-type *rpoN* gene could abolish suppression (Table 2). Thus, loss of RNA polymerase sigma factor σ^{54} appeared to be the causative defect for suppression. From this information, the mechanism of *ftsZ84* suppression could be direct or indirect; attributable either directly to an inability to induce promoters under σ^{54} control or indirectly to the phys-

TABLE 1. Strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Genotype and markers	Derivation, source, or reference
Strains		
W3110	*E. coli* K-12	Laboratory stock
WJW45	W3110 Δ(*lac*)*U169*	Laboratory stock
BSP396	WJW45 *glnF208*(*rpoN*)::Tn*10*	41
BSP440	WJW45 *rpoN*::ΔTn*10-kan*	41
BSP546	WJW45 Δ(*rpoN* operon)::*kan*	41
BSP609	WJW45 *leu*::Tn*10*	WJW45 × P1/SG13082
BSP610	WJW45 *ftZ84*	BSP609 × P1/JFL100
BSP621	BSP610 *rpoN*::ΔTn*10-kan*	BSP610 × P1/BSP440
BSP623	BSP610 *glnF208*(*rpoN*)::Tn*10*	BSP610 × P1/BSP396
BSP624	BSP610 Δ(*rpoN-npr*)::*kan*	BSP610 × P1/BSP546
BSP640	BSP610 *glnA*::Tn*5*	BSP610 × P1/TH16
BSP641	BSP610 *glnD99*::Tn*10*	BSP610 × P1/RB9040
BSP642	BSP610 *glnG10*::Tn*5*	BSP610 × P1/YMC12
BSP656	BSP610 *relA251*::*kan*	BSP610 × P1/CF1693
BSP666	BSP610 Δ*glnL2001*	BSP640 × P1/RB9132
BSP672	BSP610 *glnE*::Tn*5-131* (*tetR*)	BSP610 × P1/EB2853
BSP677	BSP610 *glyA*::Tn*5*	BSP610 × P1/NC124
BSP685	BSP610 *glnL2302*	BSP640 × P1/YMC15
BSP686	BSP610 Δ*glnB2307*	BSP677 × P1/RB9011
BSP690	BSP666 Δ*rpoS*::*kan*	BSP666 × P1/CF5006
BSP701	BSP610 *gltBDF500*	BSP624 × P1/EB2904
BSP718	BSP666 *relA251*::*kan*	BSP666 × P1/CF1693
BSP732	W3110 *glnA*::Tn*5*	W3110 × P1/TH16
BSP742	BSP610 *gltF226*Ω*kan*	BSP610 × P1/MX3003
BSP743	W3110 Δ*glnL2001*	BSP732 × P1/RB9132
BSP747	BSP610 *rpoD504 zgh-3075*::Tn*10-specR*	BSP610 × P1/BSP760
BSP748	BSP610 *rpoD506 zgh-3075*::Tn*10-specR*	BSP610 × P1/BSP761
BSP760	CF3744 Tn*10-specR*	CF3744 × λTSK
BSP761	CF3746 Tn*10-specR*	CF3746 × λTSK
BSP762	WJW45 *relA251*::*kan*	WJW45 × P1/CF1693
BSP785	BSP701 *glnG10*::Tn*5*	BSP701 × P1/YMC12
BSP808	WJW45 *glnD99*::Tn*10*	WJW45 × P1/RB9040
BSP809	BSP747 *relA251*::*kan*	BSP747 × P1/CF1693
BSP810	BSP762 *glnD99*::Tn*10*	BSP762 × P1/RB9040
BSP813	BSP809 *spoT207*::*cat*	BSP809 × P1/CF1693
BSP814	BSP762 *spoT207*::*cat*	BSP762 × P1/CF1693
BSP815	BSP813 *rpoS*::Tn*10*	BSP813 × P1/CF5025
BSP816	BSP762 *glnF208*(*rpoN*)::Tn*10*	BSP762 × P1/BSP396
BSP818	BSP814 *rpoD504 zgh-3075*::Tn*10-specR*	BSP814 × P1/BSP760
BSP821	BSP814 *glnF208*(*rpoN*)::Tn*10*	BSP814 × P1/BSP396
BSP822	BSP814 *glnD99*::Tn*10*	BSP814 × P1/RB9040
BSP823	BSP821 *glnF208*(*rpoN*)::Tn*10*	BSP821 × P1/BSP396
BSP824	BSP822 *glnD99*::Tn*10*	BSP822 × P1/RB9040
BSP828	BSP743 *relA251*::*kan*	BSP743 × P1/CF1693
BSP838	BSP640 *glnF208*(*rpoN*)::Tn*10*	BSP640 × P1/BSP396
BSP845	BSP640 *glnD99*::Tn*10*	BSP640 × P1/RB9040
BSP846	W3110 *relA251*::*kan*	W3110 × P1/CF1693
CF1693	*relA251*::*kan spoT207*::*cat*	M. Cashel
CF5006	Δ*rpoS*::*kan*	D. Gentry
CF3744	*rpoD504 zgh-3075*::Tn*10*	J. Hernandez
CF3746	*rpoD506 zgh-3075*::Tn*10*	J. Hernandez
CF5025	*rpoS*::Tn*10*	D. Gentry
EB2853	*glnE*::Tn*5-131* (*tetR*)	B. Bender
EB2904	*gltBDF500*	B. Bender
JFL100	*ftsZ84 ilv his thyA deo ara*(Am) *lac125*(Am) *galU42*(Am) *tyrT supF*(Ts)*A81*	J. Lutkenhaus
MX3003	*gltF226*Ω*kan*	F. Valle
NC124	*glyA*::Tn*5*	N. Costantino
RB9040	*glnD99*::Tn*10*	L. Reitzer
RB9132	Δ*glnL2001*	L. Reitzer
RB9011	Δ*glnB2307*	L. Reitzer
SG13082	*leu*::Tn*10*	S. Gottesman
TH16	*glnA*::Tn*5*	L. Reitzer
YMC12	*glnG10*::Tn*5*	L. Reitzer
YMC15	*glnL2302*	L. Reitzer
Plasmids		
pRS414	Protein fusion vector, Ap^r	R. Simons
pRS415	Operon fusion vector, Ap^r	R. Simons
pCX	pZ-*lacZ*	L. Rothfield
pCX32	pQ-*lacZ*	L. Rothfield
pCX39	Qp2-*lacZ*	L. Rothfield

Continued on following page

TABLE 1—*Continued*

Strain, plasmid, or phage	Genotype and markers	Derivation, source, or reference
pCX40	Qp1-*lacZ*	L. Rothfield
pCX38	pQ-pZ-*lacZ*	L. Rothfield
pGB2	pSC101 *aadA*	Laboratory stock
pHM675	pGB2(*tacp relA′*)	M. Cashel
pALS10	pBR322(*tacp relA*)	M. Cashel
pALS14	pBR322(*tacp relA″*)	M. Cashel
pBP133	pRS414(Zp2 *ftsZ′-lacZ*)PF	This study
pBP134	pRS415(Zp2 *ftsZ′-lacZ*)OF	This study
pBP124	pRS415(*orf-241 rpoN orf-95 ptsN orf-284 npr*)	41
pBP130	pRS415(*orf-241 rpoN orf-95 ptsN′*)	41
pBP131	pRS415(*orf-241* Δ*rpoN orf-95 ptsN orf-284 npr*)	41
Phages		
λTSK	λ cI857 Tn*10 tetA′*Ω(*kanR specR*)	Laboratory stock
λGL100	*plac ftsZ*	J. Garcia-Lara
λBDC531	λ *lacYZ′ supF 'bla att imm*^{21} Ap^{s}	Laboratory stock
λBP100	λBDC531 *lacYZ*$^{+}$ *bla*$^{+}$	41
λBP124	λBDC531 *bla*$^{+}$ (*orf-241 rpoN orf-95 ptsN orf-284 npr-lacZY*)	41
λBP124.1	λBDC531 *bla*$^{+}$ (*orf-241 rpoN orf-95 ptsN*::*kan orf-284 npr-lacZY*)	41
λBP124.2	λBDC531 *bla*$^{+}$ (*orf-241 rpoN*::*kan orf-95 ptsN orf-284 npr-lacZY*)	41
λBBP133	λBDC531 *bla*$^{+}$(Zp2 *ftsZ′-lacZ*)PF	λBDC531 × pBP133
λBBP134	λBDC531 *bla*$^{+}$(Zp2 *ftsZ′-lacZ*)OF	λBDC531 × pBP134

iological state of diminished glutamine metabolism, since the loss of σ^{54} activity creates both deficiencies. The relevance of each effect for suppression was tested as described next.

Mutations of several other genes affecting the regulation of glutamine metabolism were tested singly or in combination for the ability to suppress *ftsZ84*. This enabled us to distinguish between components causing a general defect in nitrogen regulation, called Ntr^{-}, and those causing glutamine auxotrophy, called Gln^{-} (for reviews of nitrogen regulation see references 28, 31, 32, and 45). Table 3 lists the regulatory genes and their protein products that are commonly known to be important for general nitrogen control and ammonium assimilation in *E. coli*. The Gln and Ntr phenotypes of strains having mutations in these genes are tabulated alongside findings of whether or not they suppressed *ftsZ84*. As shown, four single mutations, *glnD*, *glnF*, *glnG*, and *glnL*, allowed high-temperature growth with significant reductions in cell filamentation. Examination of data from all of these strains revealed that suppression generally correlated with the Ntr^{-} phenotype and not with the Gln^{-} phenotype (Table 3). The only exceptions to this correlation were strains containing *gltBDF* operon mutations. We reasoned that this apparent inconsistency may reflect the unusual physiology of *gltBDF* operon mutants. Such mutants possess substantially greater-than-normal levels of free glutamine due to the absence of glutamate synthase activity, which consumes glutamine in making glutamate (45). By combining either of the two different *gltBDF* operon mutations with the *glnG* null allele, we observed that suppression of *ftsZ84* by *glnG* was reversed genetically (Table 3, footnote *e*; compare BSP742 with BSP785). Since such strains are still defective for Ntr regulation, these findings indicated that suppression of filamentation likely relates to glutamine limitation as a consequence of the loss of general nitrogen control. This disfavored the possibility of a more direct effect on FtsZ transcription by σ^{54}. Interestingly, loss of glutamine synthetase (*glnA*), which is the only biosynthetic enzyme for glutamine in *E. coli*, does not suppress, and is itself unimportant for suppression by the *glnF* or *glnD* mutation (Table 3). It is not well understood how cells lacking both glutamine synthetase and σ^{54}-dependent activation of the Ntr pathway survive on autoclaved LB medium, which contains no detectable free glutamine. The absence of free glutamine in our LB medium was verified by physiological high-pressure liquid chromatography (HPLC) analysis (data not shown). The survival of this mutant and certain other Ntr^{-} mutants on LB medium, however, was found to require active RelA, suggesting a role for RelA in glutamine metabolism, as discussed below.

TABLE 2. Effects of extrachromosomal genes of the *rpoN* operon on suppression of *ftsZ84*

Strain	Relevant genotype	Growth at 42°C[b]			
		λBP100[a]	λBP124	λBP124.1	λBP124.2
WJW45		+	+	+	+
BSP610	*ftsZ84*	−	−	−	−
BSP621	*ftsZ84 rpoN*::ΔTn*10-kan*	+	−	−	+

[a] Clones contain various extents of the *rpoN* operon as diagrammed in Fig. 1 of Powell et al. (41): λBP100, vector; λBP124, *rpoN* operon; λBP124.1, same as λBP124 but with a transposon in the *ptsN* gene downstream of *rpoN*; λBP124.2, same as λBP124 but with a transposon in the *rpoN* gene. Strains containing a different *rpoN* mutation (BSP623) or a large deletion of the *rpoN* operon (BSP624) behaved similarly to BSP621 in these tests. Plasmids containing wild-type *rpoN* (e.g., pBP130) also reversed suppression, while those containing *rpoN* mutations (e.g., pBP131) did not.

[b] LB medium was used as described in Materials and Methods. Average colony size was scored as follows after 24 h of growth at 32 or 42°C: +, good growth at both temperatures; −, growth only at the lower temperature.

Suppression of *ftsZ84* by Ntr^{-} mutants is blocked by externally added glutamine. It is known that LB medium contains no free glutamine due to deamidation that occurs during autoclaving (49). We confirmed this absence by physiological HPLC (16a). *glnA* mutants presumably acquire glutamine from glutamine-containing oligopeptides in the medium. The hypothesis of glutamine limitation for the Ntr^{-} mutants was first tested by enriching LB medium separately with each of the 20 standard amino acids. The added presence of glutamine reversed suppression by all four mutations, as exemplified for the *glnF* mutation in Table 4. These mutants were not identically sensitive, however, since the *glnF* strain required an amount of glutamine (0.25% [wt/vol]) five times as great as that required by the other mutants to completely restore the high-temperature lethality of *ftsZ84*. No other amino acid fully blocked

TABLE 3. Effect on *ftsZ84* exerted by inactivating genes for regulation of glutamine metabolism

ftsZ84 strain	Mutation(s)	Protein(s) lost	Description or function	Phenotype[a]		Suppression of *ftsZ84*[b]
				Gln	Ntr	
BSP640	*glnA*::Tn*5*	GS	Glutamine synthetase	−	+	−
BSP686	Δ*glnB2307*	P_{II}	Activates NR_{II} phosphatase	+	+	−
BSP641	*glnD99*::Tn*10*	UT/URase	Regulates P_{II} by uridylation	−	−	+
BSP672	*glnE*::Tn*5-131*	AT/ARase	Regulates GS by adenylation	+	+	−
BSP623	*glnF208*::Tn*10*	σ^{54}	RNA polymerase sigma factor	−	−	+
BSP642	*glnG10*::Tn*5*	NR_I	Enhancer protein for σ^{54}	+	−	+
BSP666	Δ*glnL2001*[c]	NR_{II}	Kinase/phosphatase of NR_I	+	−	+
BSP838	*glnF208*::Tn*10*, *glnA*::Tn*5*[d]	σ^{54}, GS	As described above	−	−	+
BSP701	*gltBDF500*[e]	GOGAT, GltF	Glutamate synthase, regulator	++	−	−
BSP785	*gltBDF*, *glnG10*::Tn*5*	GOGAT, GltF, NR_I	As described above	++	−	−

[a] Glutamine prototrophy (Gln) and nitrogen gene regulation (Ntr) phenotypes are as follows: Gln^+, glutamine prototroph; Gln^-, glutamine auxotroph; Gln^{++}, glutamine prototroph with very high constitutive levels of glutamine; Ntr^+, able to use poor nitrogen sources; Ntr^-, unable to use poor nitrogen sources.
[b] Suppression of *ftsZ84* was measured and scored as for Table 2.
[c] The *glnL2302* mutant (BSP685) encoding NR_{II}* with constitutive NR_I kinase activity (Gln^+ Ntr^+) was also tested and did not affect *ftsZ84* temperature sensitivity.
[d] An *ftsZ84* mutant containing *glnA* plus *glnD* (BSP845) behaved similarly to BSP838.
[e] A different *gltB* operon mutant (BSP742 with *gltF226*Ω-*kan*) was also tested and behaved identically to *gltBDF500* whether alone or with *glnG10*::Tn*5*.

suppression, although leucine, glycine, and methionine produced mild effects (Table 4). Since a return to glutamine sufficiency reversed suppression in all of the suppressor mutants, the physiological state created by glutamine limitation seemed to be important for their suppression of *ftsZ84* heat sensitivity.

Dependence of ppGpp for suppression of *ftsZ84*. Because ppGpp is a global regulator induced by nutrient restriction, we tested whether suppression is affected by changes in the level of ppGpp. This association was first explored by artificially increasing ppGpp with plasmid pALS10. The RelA protein produced from this plasmid is unregulated and gratuitously expresses ppGpp in direct relation to its transcription from a *tac* promoter (53). Induction of RelA from this plasmid allowed high-temperature growth of all of the *ftsZ84* strains tested (Table 5). This indicated that all types of suppression may resemble or be identical to effects caused by the induction of ppGpp.

Next, we tested whether suppression by the Ntr mutants requires RelA activity. For this, we wished to remove natural sources of ppGpp in the tester strains; however, this proved to be difficult (see Materials and Methods). FtsZ84 mutant strains combining *relA* with either *glnF* or *glnD* required glutamine enrichment to survive on LB medium (i.e., LBQ medium), even at low temperature, and therefore were not useful for suppression tests. The same effect was found for congenic *ftsZ*$^+$ strains. In contrast to the *glnD relA* and *glnF relA* strains (which are both phenotypically Gln^- and Ntr^-), the *glnL relA* strain (Gln^+ Ntr^-) did grow on plain LB at all of the temperatures tested. We reasoned that this was because the *glnL* mutant has slightly higher levels of internal glutamine (17, 46). The *glnL* mutation was therefore used to test the importance of *relA*. While the *ftsZ84 glnL* strain grew at 42°C, the congenic *relA* derivative did not (Table 5). We interpret this to mean that suppression of *ftsZ84* by *glnL* depends on *relA* activity, as predicted. This conclusion presumably extends to the other Ntr^- mutants, although this was not tested due to difficulties in constructing the desired multiply defective strains. These data show that increased ppGpp suppresses *ftsZ84* and concur with the idea that RelA-directed ppGpp synthesis is of primary importance for *ftsZ84* suppression by mutations conferring the Ntr^- phenotype.

Finally, we employed mutant forms of sigma factor σ^{70}, *rpoD** (Table 4; see Table 7), that constitutively convert RNA

TABLE 4. Effect of added amino acids on suppression of *ftsZ84* temperature sensitivity

Strain	Relevant genotype	Growth at 42°C[a]	
		LB	LBQ[b]
WJW45	W3110 (parental strain)	+	+
BSP610	*ftsZ84*	−	−
BSP623	*ftsZ84 glnF*[c]	+	−

[a] Suppression of *ftsZ84* was measured and scored as for Table 2.
[b] LB plates were used without additional supplements or prepared separately with each of 20 amino acids as described in Materials and Methods. Shown here are data for LBQ plates. Leucine affected suppression similarly to glutamine but to a much lesser degree. Glycine and methionine caused a much weaker effect than leucine. No other single amino acid affected suppression of *ftsZ84*.
[c] Shown here are data for *glnF* suppression. Suppression of an isogenic strain with a substitution of a *glnD* (BSP641), *glnG* (BSP642), or *glnL* (BSP666) mutation for *glnF* was also fully blocked by glutamine.

TABLE 5. Importance of *relA* or a ppGpp-like effect for genetic suppression of *ftsZ84*

Strain/plasmid	Relevant genotype[a]	LB[b]	
		32°C	42°C
WJW45	Wild type	+	+
BSP610/pALS14	*ftsZ84*/vector	+	−
BSP610/pALS10	*ftsZ84*/RelA	+	+
BSP610	*ftsZ84*	+	−
BSP743	*glnL*	+	+
BSP666	*ftsZ84 glnL*	+	+
BSP846	*relA*	+	+
BSP828	*glnL relA*	(+)	(+)
BSP718	*ftsZ84 glnL relA*	(+)	−
BSP747	*ftsZ84 rpoD**	+	+
BSP813	*ftsZ84 relA spoT rpoD**	+	+

[a] Plasmid pALS10 expresses functional RelA′ from a *tacp* promoter, and pALS14 expresses an inactive RelA″ fragment and served as a vector control. These plasmids were used without isopropyl-β-D-thiogalactopyranoside induction since the multicopy nature of the plasmids is sufficient to produce elevated ppGpp levels. The test medium contained 15-μg/ml ampicillin for plasmid maintenance. Two *rpoD** mutations, *rpoD504* (shown) and *rpoD506*, were tested, and both suppressed *ftsZ84*, although *rpoD504* did so better. See Table 1 for complete genotypes.
[b] Colony growth: +, large colonies; (+), tiny colonies; −, no colonies.

TABLE 6. Importance of *relA* for salt suppression of *ftsZ84*

Strain	Relevant genotype	LB		LB + salt[a]	
		32°C	42°C	32°C	42°C
BSP610	*ftsZ84*	+	−	+	+
BSP656	*ftsZ84 relA*	+	−	+	−

[a] Colony formation was scored as for Table 5. Salt was added to LB medium at 0.15 to 0.3% (wt/vol). The following salts suppressed *ftsZ84* temperature sensitivity: NaCl, NH_4Cl, $(NH_4)_2SO_4$, and $Na(NH_4)HPO_4$. Na_2HPO_4 and NaH_2PO_4 did not suppress temperature sensitivity.

polymerase into a form that behaves as though it had been modified by ppGpp (26). These mutations also suppressed *ftsZ84* and could do so in the complete absence of cellular ppGpp, i.e., in *relA spoT* double null mutants (Table 5). This indicated that suppression is caused by an effect generated through ppGpp on RNA polymerase and not by ppGpp itself.

Suppression of *ftsZ84* by a high salt concentration also depends on RelA function. The *ftsZ84 relA* strain was used to explore the necessity of ppGpp for a different but familiar kind of suppression, i.e., that of salt repair (47). The presence of additional NaCl in the LB medium suppressed the filamentation of our parental *ftsZ84* strain but not that of its derivative which lacks RelA activity (Table 6). Furthermore, a requirement for RelA activity was also observed for suppression by $(NH_4)_2SO_4$ and NH_4Cl salts, indicating that the connection between suppression and *relA* is not limited to sodium salts (52). It is known that high sodium chloride levels are associated with accumulation of ppGpp (24).

Suppression does not involve some other known effectors of *ftsZ*. To explore the possible involvement of well-known effectors of the FtsZ protein, *ftsZ84* strains were constructed that combined either *glnL*, *glnD*, or *glnF* with a mutation of *sdiA*, *sulA*, *rcsB*, *rcsF*, or *rpoS*. Perhaps of most relevance is our finding that suppression by *glnL* is unaffected by a null mutation of *rpoS* since strain BSP690 (*ftsZ84 glnL rpoS*) grew at 42°C as well as parental strain BSP666 (*ftsZ84 glnL*). Importantly, loss of the σ^s transcription factor did not alter suppression by a σ^{70} (*rpoD**) mutation (strain BSP815) or by a high salt concentration (data not shown). The added disruption of either *sdiA*, *sulA*, *rcsB*, or *rcsF* had no effect on the suppression of *ftsZ84* by *glnF*. A potential involvement of the leucine regulatory protein Lrp was tested because we had seen a slight effect of added leucine on suppression (Table 4). Since an *lrp glnF ftsZ84* strain grew well at all temperatures and an *lrp ftsZ84* strain was still heat sensitive, Lrp appears to be unimportant for this kind of suppression by ppGpp on LB medium. Surprisingly, however, the *ftsZ84 lrp* strain acquired a heat-sensitive phenotype on M63 minimal salts plus glucose medium where none exists for the parental *ftsZ84* (Lrp^+) strain.

With the elimination of participation by the proteins tested as described above, the following model for suppression of *ftsZ84* emerged: growth on LB medium without Ntr-dependent regulation causes a limitation of glutamine and increased levels of ppGpp. This leads to increased functional activity of FtsZ, which then restores septation. To test this hypothesis, it was sensible to determine whether ppGpp affects FtsZ expression.

Suppression causes changes in transcription of the *ftsZ* operon. Since filamentation caused by the *ftsZ84* allele is known to be suppressed by its own overexpression, we tested whether the *glnF* mutation or artificially high RelA activity could alter patterns of transcription within the *ftsQAZ* operon. Transcription of two sets of *ftsQAZ* operon promoters was measured by using reporter plasmids developed and tested

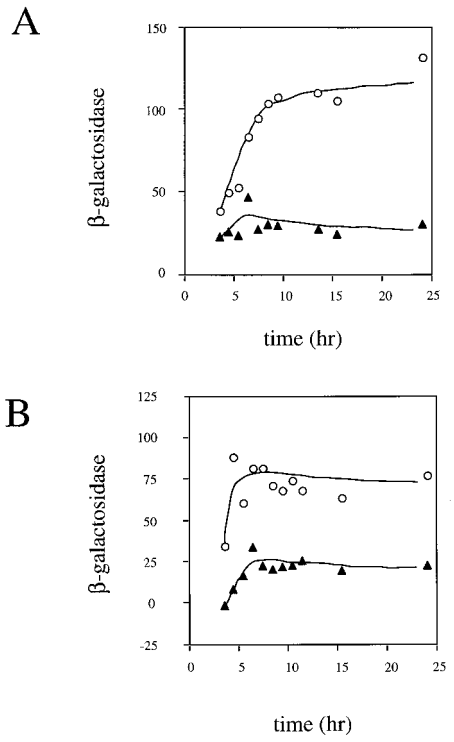

FIG. 1. Activity of the Qp1 and Qp2 promoters in *ftsZ84* heat-sensitive and heat-resistant strains. Transcription as measured by β-galactosidase activity of plasmid pCX32 (66) is plotted as a function of growth at 32°C, beginning from a culture density of 10^7 cells/ml and growth with selection for plasmids. (A) Suppression by *glnF*. Symbols: ○, BSP610 (*ftsZ84*); ▲, BSP623 (*ftsZ84 glnF*). (B) Suppression by multicopy *relA′*. Symbols: ○, BSP610/pGB2 (*ftsZ84*/vector); ▲, BSP610/pHM675 (*ftsZ84*/*relA′*). We noted that the strains containing plasmid pHM675 grew slowly, and this may reflect the toxic effect of high RelA′ concentrations observed by others (53).

elsewhere (66). These low-copy-number plasmids separately place *lacZ* under the transcriptional control of *ftsZ* promoters Zp2, Zp3, and Zp4 (herein called the pZ promoters) or *ftsQ* promoters Qp1 and Qp2 (herein called the pQ promoters). Contrary to what we expected, the activity of the pQ set of promoters was two- to threefold lower in the *glnF* strain than in the wild-type strain (Fig. 1A). *glnD*, *glnG*, and *glnL* mutants produced similar effects (data not shown). The effects on *ftsZ* operon transcription caused by artificially elevated levels of ppGpp were measured by using a compatible plasmid that overproduces RelA′ as the genetic condition of suppression. As was seen for the Ntr^- mutations, constitutively high RelA activity also reduced transcription from the pQ promoters about twofold (Fig. 1B). Plasmids that separately contained *lacZ* fusions to either Qp1 or Qp2 (pCX39 and pCX40, respectively) were tested, and both had decreased β-galactosidase activity in the *glnF* mutant compared to the wild type (data not shown). In contrast, no consistent change in activity of the pZ set of promoters was observed that was common to both conditions of suppression (data not shown). The activities of protein and operon fusions to the Zp2 promoter carried on λBBP133 and λBBP134, respectively, did not differ between the wild-type and *glnF* genetic backgrounds (data not shown). This suggested that any change in FtsZ activity caused by suppression is not due to alterations at the level of *ftsZ* translation and is not observable by using an isolated pZ promoter. Thus, induction of RelA activity correlates with a two- or

TABLE 7. Levels of FtsZ84 and NusA proteins in temperature-sensitive and temperature-resistant strains

Strain/plasmid	Genotype/plasmid	Normalized relative[a] amt of FtsZ84	
		Avg (SD)	Ratio
BSP610	*ftsZ84*	0.23 (0.02)	1
BSP623	*ftsZ84 glnF*	0.92 (0.17)	3.9
BSP610/pGB2	*ftsZ84*/vector	0.35 (0.02)	1.5
BSP610/pHM675	*ftsZ84*/*relA′*	0.78 (0.16)	3.4

[a] Levels of the FtsZ84 and NusA proteins relative to the total protein level were estimated by quantitative immunoblot analysis as described in the text. Raw FtsZ84 values were normalized by division by the respective NusA values and averaged among four appropriately weighted measurements per strain. The average relative amount of NusA among 16 measurements was 0.23, and the standard deviation was 0.03.

threefold decrease in the activity of the Qp1 and Qp2 promoters.

Suppression correlates with increased levels of FtsZ84 protein. To estimate possible changes in the amount of FtsZ protein that may accompany suppression, the steady-state level of FtsZ84 protein in these strains was measured by immunoblot analysis of whole-cell extracts. The amount of FtsZ84 protein was measured relative to that of an internal standard, NusA. A weighted average among several different loadings and measurements was calculated so as to minimize variations due to sample handling and antigen detection and finally compared between strains. The amount of FtsZ84 relative to the total amount of protein was greater in both of the suppressed strains than in their respective parental strains (Table 7). The *glnF* mutant strain had 3.9 times more FtsZ84 than the heat-sensitive parent, and the multicopy *relA′* strain had 3.4 times more FtsZ84 protein. It appears, then, that the operative effect of suppression by ppGpp is an increase in the steady-state amount of FtsZ84 protein.

***E. coli* requires ppGpp to grow on LB medium in the absence of a functional Ntr system.** While investigating the role of RelA in the suppression of filamentation, we found that our *ftsZ84* strains that lack ppGpp and genes for nitrogen regulation (Ntr^-) do not grow in LB medium. To further explore this effect, we constructed Ntr^- $ftsZ^+$ derivatives of strains having different levels of ppGpp and tested them for growth on various media. The growth of a *relA glnF* double mutant strain (BSP816) on LB medium, was severely affected compared to that of its $relA^+$ parental strain (BSP396), while a *relA glnD* strain (BSP810) was completely nonviable on LB medium (Table 8). Furthermore, the $ppGpp^0$ state rendered both Ntr^- strains (BSP821 and BSP822) fully nonviable on LB medium. The lethality of this LB effect was relieved genetically by the *rpoD** mutations (Table 8) or physiologically by addition of glutamine to the medium (Table 8). Therefore, ppGpp appears to be critical for fulfillment of the glutamine requirement of Ntr^- cells grown on LB medium. This introduces the possibility of a ppGpp-dependent survival mechanism for making glutamine available from LB medium that functions independently of σ^{54}-dependent nitrogen regulation. Interestingly, these *relA* Ntr^- ($ftsZ^+$) strains filamented soon after being transferred from LBQ medium to plain LB medium. However, neither septation nor survival was restored by artificially increasing *ftsZ* expression (data not shown). This suggests that this effect is not identical to the ppGpp effect on *ftsZ* under investigation or may involve an earlier stage of septation. In contrast to the *glnF* and *glnD* derivatives, a *glnA relA* strain, which is simply defective for glutamine synthesis (Gln^- Ntr^+), grew well on LB medium. Since the *glnA* strain is not stressed on LB medium, as surmised from its inability to suppress *ftsZ84*, while Ntr^- mutants are, it stands to reason that the Ntr system functions in LB medium to provide glutamine in spite of the conventional understanding that the Ntr system is not active in this regard during growth in LB medium (46). From our data, we propose that both the Ntr regulon and an undefined ppGpp-dependent pathway facilitate the supplying of glutamine from a source(s) in LB medium other than de novo synthesis or the transport of free glutamine, which is absent from standard LB medium.

DISCUSSION

This study demonstrates that an aspect of septation directed by FtsZ is influenced positively by nutritional stress and that the mechanism of this effect likely depends on the synthesis of ppGpp by RelA. We propose that ppGpp may effect suppression of FtsZ84 by increasing the total activity of the protein through an altered pattern of transcription, and we offer the following arguments in support of this hypothesis.

Suppression of heat sensitivity on LB medium by loss of nitrogen control, i.e., the Ntr^- phenotypic condition, corre-

TABLE 8. Effects of *relA*, *spoT*, and *rpoD* mutations on the growth of a *glnF* or *glnD* strain on LB

Strain	Allele present[a]					Avg colony size[b] on:	
	glnF	*glnD*	*relA*	*spoT*	*rpoD*	LB	LBQ
Parental	WT	WT	WT	WT	WT	++	++
BSP396	208	WT	WT	WT	WT	++	++
BSP808	WT	99	WT	WT	WT	++	++
BSP762	WT	WT	251	WT	WT	++	++
BSP816	208	WT	251	WT	WT	(+)	++
BSP810	WT	99	251	WT	WT	−	++
BSP814	WT	WT	251	207	WT	+	+
BSP821	208	WT	251	207	WT	−	+
BSP822	WT	99	251	207	WT	−	+
BSP818	WT	WT	251	207	504	+	+
BSP823	208	WT	251	207	504	+	+
BSP824	WT	99	251	207	504	(+)	+

[a] Strains contain mutations in the listed genes, as indicated by allele number, or have the wild-type (WT) locus. The parental strain is WJW45. Full strain genotypes are listed in Table 1.

[b] LB medium was used with or without glutamine (Q) at 6.8 mM. Average colony sizes were scored after 24 h of incubation at 32°C as follows: ++, large to medium (0.5- to 0.2-mm diameter); +, small (0.1-mm diameter); (+), tiny (<0.05-mm diameter); −, no growth or no individual colonies.

lates with an increased steady-state concentration of the FtsZ84 protein (Table 7). This concurs with other findings that the cellular amount of FtsZ is normally rate limiting (38, 67) and that an increase of as little as a 1.5-fold can suppress heat-sensitive filamentation of *ftsZ84* (66). It is worth noting here that suppression of filamentation by ppGpp appears to be allele specific since the Ntr^- mutants did not abate heat sensitivity caused by the *ftsZ26* allele, which cannot suppress itself by increased copy number (5) or by the *ftsA12* mutation (data not shown). We postulate that the increase in FtsZ protein that accompanies loss of nitrogen control probably involves an elevated basal level of the nutrient stress signal ppGpp for the following reasons. The *glnL* mutant requires RelA to suppress *ftsZ84* (Table 5), an abundance of glutamine annuls suppression by the nitrogen control mutations (Tables 2 and 4), and genetic conditions that overproduce RelA or mimic the ppGpp-induced state (*rpoD**) suppress *ftsZ84* (Table 5). The requirement of RelA for salt repair shows separately that the relief of *ftsZ84* heat sensitivity by ppGpp operates there as well (Table 6).

A reasonable mechanism for RelA-dependent suppression would invoke changes in the transcription of *ftsZ* due to the known effect of ppGpp on RNA polymerase (8, 26). Indeed, the restoration of high-temperature growth of *ftsZ84* strains by SdiA correlates with altered transcription and increased FtsZ protein levels (66). However, the mechanism of SdiA suppression is not similar to that described here since ppGpp decreases expression at both promoters Qp1 and Qp2 (Fig. 1), while SdiA activates the Qp2 promoter. Furthermore, SdiA is not needed for suppression by ppGpp. We note that there is no measured effect of ppGpp on transcription from the downstream pZ promoters or on translation of FtsZ.

We offer two possible scenarios of suppression that might accommodate the decrease in promoter activity upstream of *ftsZ84*. If the pZ promoters adjacent to *ftsZ* function independently of the pQ promoters upstream, then suppression might be the consequence of an increase in the ratio of FtsZ to FtsA and/or FtsQ. This scenario alone, however, does not explain why the absolute amount of FtsZ84 protein increases upon suppression. Alternatively, the phenomenon of promoter occlusion may account for the effect of decreased promoter activity, as well as that of increased FtsZ84 protein levels. Promoter occlusion is the inhibitory effect that elongating RNA polymerase molecules have upon transcription initiation at relatively weaker promoters downstream. This was originally suggested with respect to the activities of multiple *trp* operon promoters (25) and substantiated with respect to the lambda p_L promoter and nearby pGal promoters (1). Promoter occlusion is a well-known phenomenon often called transcription interference at eukaryotic promoters (18, 43). By comparison, then, the predominant transcription arising early in the *ftsQAZ* operon may inhibit the activity of promoters nearer to *ftsZ*. Thus, downstream promoters may be activated by reducing promoter activity upstream. A corollary of this model is that translation is more efficient from mRNA originating at the downstream promoter (1). The predicted activation of the reporter-linked promoters on prophages λBBP133 and λBBP134 would not be detectable in these experiments since they do not contain the upstream promoters in *cis*. Plasmids carrying reporter fusions that do contain both sets of promoters in *cis* (68) are not stable under the genetic conditions tested here. Perhaps such constructs adversely affect the cell by introducing extra copies of *ftsQ* and *ftsA*. If promoter occlusion explains *ftsZ84* suppression by decreased activity of promoters Qp1 and Qp2, then how can it agree with SdiA suppression by increased activity of promoter Qp2? We predict that although activation of Qp2 would further occlude pZ, the increase in total *ftsZ* translation from an increased amount of the longer transcripts must allow sufficient FtsZ expression. These models do not necessarily exclude one another and, in fact, there are probably many ways to activate FtsZ. For example, a *cis*-dependent model for regulation of the activities of *ftsQAZ* operon promoters based on DNA-looping and putative multiple operator sites has also been proposed (15). Early on, we had considered the possibility of a direct effect by ppGpp on the GTPase activity of FtsZ, but this idea was dismissed by the finding that the *rpoD** mutation suppresses *ftsZ84* in the complete absence of ppGpp (Table 5). Thus, it appears that the essence of suppression by ppGpp may be an increase in the total activity of FtsZ84 protein through transcriptional regulation. These data, however, do not distinguish between a direct and an indirect effect of ppGpp on *ftsZ* transcription.

Connections between ppGpp and septation have been suggested previously. Studies on the first $ppGpp^0$ strains, which filament upon nutritional downshift (68), provided the first direct indication that ppGpp may normally stimulate septation. Gervais et al. mentioned unpublished data showing that multiple copies of *relA* suppress *ftsZ84* (21). ppGpp has been shown to help confer resistance to the cell wall inhibitor mecillinam via several different mutations (62–64). In these examples, it is proposed that FtsZ levels could be especially limiting due to the abnormally wide diameter of these cells, and ppGpp may exert a suppressive effect by increasing the total amount of FtsZ. A similar explanation is given for suppression by ppGpp of heat-sensitive filamentation caused by a mutant of the β subunit of RNA polymerase (61). Perhaps this *rpoB* mutation affects the same step in transcription as the *rpoD** mutants used here, except that it creates an RNA polymerase that is less responsive to ppGpp. Another *rpoB* mutant appears to resemble *rpoD** and increases expression of *ftsZ* (7). The work reported here concurs with these findings and is the first direct evidence that RelA-mediated synthesis of ppGpp suppresses the *ftsZ84* mutant on LB medium. Other indications of a role for ppGpp have come from its effect on growth rate control. It has been known for some time that *E. coli* cell size decreases under poorer growth conditions. In concordance with this, FtsZ expression has been shown to vary inversely with growth (2, 16, 48) and, by inference, with ppGpp, although a direct connection has not been shown until now. Thus, increased ppGpp correlates with increased FtsZ protein levels in *E. coli* cultures that enter stationary growth (2, 19, 56) or are otherwise under nutritional stress (54).

Altogether, the data presented here may elucidate some previously unexplained phenomena. One example is the suppression of *ftsZ84* by multiple copies of the capsule regulators *rcsB* and *rcsF* (20, 21). Since suppression involved high-copy plasmids containing both an intact *rcs* regulator gene and its σ^{54} promoter, it is plausible that the plasmids may titrate a finite and constitutive supply of σ^{54} (41), thus leading to glutamine insufficiency, elevated ppGpp, and, hence suppression. The physiological suppression of *ftsZ84* by high-osmotic-strength medium (Table 6) probably operates partly through a similar mechanism, since osmotic stress induces ppGpp (24). Interestingly, salt and ppGpp act oppositely on the isomerization reaction that converts the RNA polymerase holoenzyme into the elongating conformation at the *rrn* promoters of *E. coli* (22, 37). In this regard, it would be informative to measure the activities of *ftsQAZ* promoters as a function of medium osmolarity.

During this study, we found evidence that Ntr regulation is important for supplying glutamine in cells grown in LB medium since the absence of Ntr activity requires the nutrient

stress factor ppGpp or enrichment of the LB medium with glutamine. This also reveals that ppGpp-dependent gene induction comprises an auxiliary path for supplying glutamine on LB medium. Peptide-bound glutamine can be less susceptible than free glutamine to nonenzymatic deamidation under harsh conditions (e.g., autoclaving) (49), and so the source of glutamine in LB made accessible by the ppGpp pathway may derive from the abundant supply of oligopeptides. Specific mutants which bypass the ppGpp requirement of this peptide transport and degradation pathway might be found by using combinations of Ntr$^-$ *glnA relA* strains and selecting for growth on LB medium.

These findings interrelate nitrogen control and cell division by a global signal of nutrient stress, ppGpp, and begin to explain some of the previously observed difficulty and variability encountered in working with Ntr$^-$ strains, as well as with *ftsZ84* strains. While *glnF*, *glnG*, *glnD*, *glnL*, and *rpoD** are the first mutations reported to suppress *ftsZ84* on LB medium, the detailed mechanism of the ppGpp effect on FtsZ is not readily apparent and the promoter occlusion model proposed here awaits more direct experimental examination.

ACKNOWLEDGMENTS

We are thankful to the sources of strains, lambda phages, antisera, and plasmids listed in Table 1. We are particularly indebted to the following people for kindly responding to numerous requests and for helpful discussions: L. Reitzer, J. Garcia-Lara, L. Rothfield, J. Hernandez, D. Gentry, D. Vinella, and M. Cashel. We sincerely thank R. Dinterman for generously performing the amino acid analysis of LB medium by physiological HPLC.

This research was sponsored by the National Cancer Institute under contract with ABL.

REFERENCES

1. **Adhya, S., and M. Gottesman.** 1982. Promoter occlusion: transcription through a promoter may inhibit its activity. Cell **29:**939–944.
2. **Aldea, M., T. Garrido, J. Pla, and M. Vicente.** 1990. Division genes in Escherichia coli are expressed coordinately to cell septum requirements by gearbox promoters. EMBO J. **9:**3787–3794.
3. **Ayala, J. A., T. Garrido, M. A. de Pedro, and M. Vicente.** 1994. Molecular biology of bacterial septation, p. 73–101. *In* J. M. Ghuysen and R. Hackenbeck (ed.), Bacterial cell wall. Elsevier Science, Amsterdam, The Netherlands.
4. **Bi, E., and J. Lutkenhaus.** 1990. FtsZ regulates frequency of cell division in *Escherichia coli*. J. Bacteriol. **172:**2765–2768.
5. **Bi, E., and J. Lutkenhaus.** 1992. Isolation and characterization of *ftsZ* alleles that affect septal morphology. J. Bacteriol. **174:**5414–5423.
6. **Bi, E. F., and J. Lutkenhaus.** 1991. FtsZ ring structure associated with division in Escherichia coli [see comments]. Nature **354:**161–164.
7. **Cam, K., A. Cuzange, and J. P. Bouche.** 1995. Sigma S-dependent overexpression of ftsZ in an Escherichia coli K-12 rpoB mutant that is resistant to the division inhibitors DicB and DicF RNA. Mol. Gen. Genet. **248:**190–194.
8. **Cashel, M., D. R. Gentry, J. Hernandez, and D. Vinella.** 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
9. **Cortay, J. C., and A. J. Cozzone.** 1983. A study of bacterial response to polypeptide antibiotics. FEBS Lett. **157:**307–310.
10. **Dai, K., and J. Lutkenhaus.** 1992. The proper ratio of FtsZ to FtsA is required for cell division to occur in *Escherichia coli*. J. Bacteriol. **174:**6145–6151.
11. **de Boer, P., R. Crossley, and L. Rothfield.** 1992. The essential bacterial cell-division protein FtsZ is a GTPase. Nature **359:**254–256.
12. **Descoteaux, A., and G. R. Drapeau.** 1987. Regulation of cell division in *Escherichia coli* K-12: probable interactions among proteins FtsQ, FtsA, and FtsZ. J. Bacteriol. **169:**1938–1942.
13. **Dewar, S. J., K. J. Begg, and W. D. Donachie.** 1992. Inhibition of cell division initiation by an imbalance in the ratio of FtsA to FtsZ. J. Bacteriol. **174:**6314–6316.
14. **Dewar, S. J., and W. D. Donachie.** 1993. Antisense transcription of the *ftsZ-ftsA* gene junction inhibits cell division in *Escherichia coli*. J. Bacteriol. **175:**7097–7101.
15. **Dewar, S. J., and W. D. Donachie.** 1990. Regulation of expression of the *ftsA* cell division gene by sequences in upstream genes. J. Bacteriol. **172:**6611–6614.
16. **Dewar, S. J., V. Kagan-Zur, K. J. Begg, and W. D. Donachie.** 1989. Transcriptional regulation of cell division genes in Escherichia coli. Mol. Microbiol. **3:**1371–1377.

16a. **Dinterman, R., and B. Powell.** Unpublished data.

17. **Feng, J., M. R. Atkinson, W. McCleary, J. B. Stock, B. L. Wanner, and A. J. Ninfa.** 1992. Role of phosphorylated metabolic intermediates in the regulation of glutamine synthetase synthesis in *Escherichia coli*. J. Bacteriol. **174:**6061–6070.
18. **Friesen, P. D., and L. K. Miller.** 1985. Temporal regulation of baculovirus RNA: overlapping early and late transcripts. J. Virol. **54:**392–400.
19. **Garrido, T., M. Sanchez, P. Palacios, M. Aldea, and M. Vicente.** 1993. Transcription of ftsZ oscillates during the cell cycle of Escherichia coli. EMBO J. **12:**3957–3965.
20. **Gervais, F. G., and G. R. Drapeau.** 1992. Identification, cloning, and characterization of *rcsF*, a new regulator gene for exopolysaccharide synthesis that suppresses the division mutation *ftsZ84* in *Escherichia coli* K-12. J. Bacteriol. **174:**8016–8022.
21. **Gervais, F. G., P. Phoenix, and G. R. Drapeau.** 1992. The *rcsB* gene, a positive regulator of colanic acid biosynthesis in *Escherichia coli*, is also an activator of *ftsZ* expression. J. Bacteriol. **174:**3964–3971.
22. **Hamming, J., G. Ab, and M. Gruber.** 1980. E. coli RNA polymerase-rRNA promoter interaction and the effect of ppGpp. Nucleic Acids Res. **8:**3947–3963.
23. **Harlow, E., and D. Lane (ed.).** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Harshman, R. B., and H. Yamazaki.** 1972. MSI accumulation induced by sodium chloride. Biochemistry **11:**615–618.
25. **Hausler, B., and R. L. Somerville.** 1979. Interaction in vivo between strong closely spaced constitutive promoters. J. Mol. Biol. **127:**353–356.
26. **Hernandez, V. J., and M. Cashel.** 1995. Changes in conserved region 3 of Escherichia coli sigma 70 mediate ppGpp-dependent functions in vivo. J. Mol. Biol. **252:**536–549.
27. **Johnson, G. S., C. R. Adler, J. J. Collins, and D. Court.** 1979. Role of the spoT gene product and manganese ion in the metabolism of guanosine 5′-diphosphate 3′-diphosphate in Escherichia coli. J. Biol. Chem. **254:**5483–5487.
28. **Kustu, S., E. Santero, J. Keener, D. Popham, and D. Weiss.** 1989. Expression of sigma 54 (ntrA)-dependent genes is probably united by a common mechanism. Microbiol. Rev. **53:**367–376.
29. **Lutkenhaus, J., and A. Mukherjee.** 1996. Cell division, p. 1615–1626. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
30. **Lutkenhaus, J. F., and H. C. Wu.** 1980. Determination of transcriptional units and gene products from the *ftsA* region of *Escherichia coli*. J. Bacteriol. **143:**1281–1288.
31. **Magasanik, B.** 1982. Genetic control of nitrogen assimilation in bacteria. Annu. Rev. Genet. **16:**135–168.
32. **Magasanik, B.** 1993. The regulation of nitrogen utilization in enteric bacteria. J. Cell Biochem. **51:**34–40.
33. **Magasanik, B.** 1989. Regulation of transcription of the *glnALG* operon of *Escherichia coli* by protein phosphorylation. Biochimie **71:**1005–1012.
34. **Miller, J. (ed.).** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Mukherjee, A., and J. Lutkenhaus.** 1994. Guanine nucleotide-dependent assembly of FtsZ into filaments. J. Bacteriol. **176:**2754–2758.
36. **Nystom, T.** 1994. Role of guanosine tetraphosphate in gene expression and the survival of glucose or seryl-tRNA starved cells of Escherichia coli K12. Mol. Gen. Genet. **245:**355–362.
37. **Ohlsen, K. L., and J. D. Gralla.** 1992. Interrelated effects of DNA supercoiling, ppGpp, and low salt on melting within the Escherichia coli ribosomal RNA rrnB P1 promoter. Mol. Microbiol. **6:**2243–2251.
38. **Palacios, P., M. Vicente, and M. Sanchez.** 1996. Dependency of Escherichia coli cell-division size, and independency of nucleoid segregation on the mode and level of ftsZ expression. Mol. Microbiol. **20:**1093–1098.
39. **Phoenix, P., and G. R. Drapeau.** 1988. Cell division control in *Escherichia coli* K-12: some properties of the *ftsZ84* mutation and suppression of this mutation by the product of a newly identified gene. J. Bacteriol. **170:**4338–4342.
40. **Pla, J., M. Sanchez, P. Palacios, M. Vicente, and M. Aldea.** 1991. Preferential cytoplasmic location of FtsZ, a protein essential for Escherichia coli septation. Mol. Microbiol. **5:**1681–1686.
41. **Powell, B. S., D. L. Court, T. Inada, Y. Nakamura, V. Michotey, X. Cui, A. Reizer, M. H. Saier, Jr., and J. Reizer.** 1995. Novel proteins of the phosphotransferase system encoded within the rpoN operon of Escherichia coli. Enzyme IIANtr affects growth on organic nitrogen and the conditional lethality of an erats mutant. J. Biol. Chem. **270:**4822–4839.
42. **Powell, B. S., M. P. Rivas, D. L. Court, Y. Nakamura, M. P. Rivas, and C. L. Turnbough, Jr.** 1994. Rapid confirmation of single copy lambda prophage integration by PCR. Nucleic Acids Res. **22:**5765–5766. (Erratum, **11:**1278, 1995.)
43. **Proudfoot, N. J.** 1986. Transcriptional interference and termination between

duplicated alpha-globin gene constructs suggests a novel mechanism for gene regulation. Nature **322:**562–565.
44. **RayChaudhuri, D., and J. T. Park.** 1992. Escherichia coli cell-division gene ftsZ encodes a novel GTP-binding protein. Nature **359:**251–254.
45. **Reitzer, L. J.** 1996. Ammonium assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine, and D-alanine, p. 391–433. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
46. **Reitzer, L. J.** 1996. Sources of nitrogen and their utilization, p. 380–390. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, D.C.
47. **Ricard, M., and Y. Hirota.** 1973. Process of cellular division in *Escherichia coli*: physiological study on thermosensitive mutants in cell division. J. Bacteriol. **116:**314–322.
48. **Robin, A., D. Joseleau-Petit, and R. D'Ari.** 1990. Transcription of the *ftsZ* gene and cell division in *Escherichia coli*. J. Bacteriol. **172:**1392–1399.
49. **Robinson, A. B., and C. J. Rudd.** 1974. Deamidation of glutaminyl and asparaginyl residues in peptides and proteins. Curr. Top. Cell Regul. **8:**247–295.
50. **Robinson, A. C., D. J. Kenan, G. F. Hatfull, N. F. Sullivan, R. Spiegelberg, and W. D. Donachie.** 1984. DNA sequence and transcriptional organization of essential cell division genes *ftsQ* and *ftsA* of *Escherichia coli*: evidence for overlapping transcriptional units. J. Bacteriol. **160:**546–555.
51. **Rothfield, L. I., W. R. Cook, and P. A. de Boer.** 1991. Biogenesis of the Escherichia coli cell division system. Cold Spring Harbor Symp. Quant. Biol. **56:**751–756.
52. **Rowbury, R. J., M. Goodson, Z. Lazim, and T. J. Humphrey.** 1996. Sensitization to acid induced by sodium ions in Escherichia coli: dependence of (p)ppGpp and cAMP and suppression of the relA-associated defect by mutations in envZ. Microbios **85:**161–177.
53. **Schreiber, G., S. Metzger, E. Aizenman, S. Roza, M. Cashel, and G. Glaser.** 1991. Overexpression of the relA gene in Escherichia coli. J. Biol. Chem. **266:**3760–3767.
54. **Schreiber, G., E. Z. Ron, and G. Glaser.** 1995. ppGpp-mediated regulation of DNA replication and cell division in Escherichia coli. Curr. Microbiol. **30:** 27–32.
55. **Simons, R., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy lac-based cloning vectors for protein and operon fusions. Gene **53:** 85–96.
56. **Sitnikov, D. M., J. B. Schineller, and T. O. Baldwin.** 1996. Control of cell division in Escherichia coli: regulation of transcription of ftsQA involves both rpoS and SdiA-mediated autoinduction. Proc. Natl. Acad. Sci. USA **93:**336–341.
57. **Smith, G. R., Y. S. Halpern, and B. Magasanik.** 1971. Genetic and metabolic control of enzymes responsible for histidine degradation in Salmonella typhimurium 4-imidazolone-5-propionate amidohydrolase and *N*-formimino-L-glutamate formiminohydrolase. J. Biol. Chem. **246:**3320–3329.
58. **Tetart, F., and J. P. Bouche.** 1992. Regulation of the expression of the cell-cycle gene ftsZ by DicF antisense RNA. Division does not require a fixed number of FtsZ molecules. Mol. Microbiol. **6:**615–620.
59. **Tsugawa, A., M. Saito, D. L. Court, and Y. Nakamura.** 1988. *nusA* amber mutation that causes temperature-sensitive growth of *Escherichia coli*. J. Bacteriol. **170:**908–915.
60. **van Heeswijk, W. C., M. Rabenberg, H. V. Westerhoff, and D. Kahn.** 1993. The genes of the glutamine synthetase adenylylation cascade are not regulated by nitrogen in Escherichia coli. Mol. Microbiol. **9:**443–457.
61. **Vinella, D., and R. D'Ari.** 1994. Thermoinducible filamentation in *Escherichia coli* due to an altered RNA polymerase beta subunit is suppressed by high levels of ppGpp. J. Bacteriol. **176:**966–972.
62. **Vinella, D., R. D'Ari, and A. Jaff.** 1992. Penicillin binding protein 2 is dispensable in Escherichia coli when ppGpp synthesis is induced. EMBO J. **11:**1493–1501. (Erratum, **11:**5137, 1992.)
63. **Vinella, D., B. Gagny, D. Joseleau-Petit, R. D'Ari, and M. Cashel.** 1996. Mecillinam resistance in *Escherichia coli* is conferred by loss of a second activity of the AroK protein. J. Bacteriol. **178:**3818–3828.
64. **Vinella, D., D. Joseleau-Petit, D. Thevenet, P. Bouloc, and R. D'Ari.** 1993. Penicillin-binding protein 2 inactivation in *Escherichia coli* results in cell division inhibition, which is relieved by FtsZ overexpression. J. Bacteriol. **175:**6704–6710.
65. **Wang, X., and J. Lutkenhaus.** 1993. The FtsZ protein of Bacillus subtilis is localized at the division site and has GTPase activity that is dependent upon FtsZ concentration. Mol. Microbiol. **9:**435–442.
66. **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.
67. **Ward, J. E., Jr., and J. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicell formation in E. coli. Cell **42:**941–949.
68. **Xiao, H., M. Kalman, K. Ikehara, S. Zemel, G. Glaser, and M. Cashel.** 1991. Residual guanosine 3′,5′-bispyrophosphate synthetic activity of relA null mutants can be eliminated by spoT null mutations. J. Biol. Chem. **266:**5980–5990.
69. **Zhou, P., and C. E. Helmstetter.** 1994. Relationship between *ftsZ* gene expression and chromosome replication in *Escherichia coli*. J. Bacteriol. **176:**6100–6106.