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Control of cell division in *Escherichia coli*: Regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction

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ABSTRACT **The conditioning of culture medium by the production of growth-regulatory substances is a well-established phenomenon with eukaryotic cells. It has recently been shown that many prokaryotes are also capable of modulating growth, and in some cases sensing cell density, by production of extracellular signaling molecules, thereby allowing single celled prokaryotes to function in some respects as multicellular organisms. As *Escherichia coli* shifts from exponential growth to stationary growth, many changes occur, including cell division leading to formation of short minicells and expression of numerous genes not expressed in exponential phase. An understanding of the coordination between the morphological changes associated with cell division and the physiological and metabolic changes is of fundamental importance to understanding regulation of the prokaryotic cell cycle. The *ftsQA* genes, which encode functions required for cell division in *E. coli*, are regulated by promoters P_1 and P_2, located upstream of the *ftsQ* gene. The P_1 promoter is *rpoS*-stimulated and the second, P_2, is regulated by a member of the LuxR subfamily of transcriptional activators, SdiA, exhibiting features characteristic of an autoinduction (quorum sensing) mechanism. The activity of SdiA is potentiated by *N*-acyl-homoserine lactones, which are the autoinducers of luciferase synthesis in luminous marine bacteria as well as of pathogenesis functions in several pathogenic bacteria. A compound(s) produced by *E. coli* itself during growth in Luria Broth stimulates transcription from P_2 in an SdiA-dependent process. Another substance(s) enhances transcription of *rpoS* and (perhaps indirectly) of *ftsQA* via promoter P_1. It appears that this bimodal control mechanism may comprise a fail-safe system, such that transcription of the *ftsQA* genes may be properly regulated under a variety of different environmental and physiological conditions.**

The intricate autoregulatory mechanism employed by luminous marine bacteria in the cell density-dependent regulation of bioluminescence is also employed by other Gram-negative bacteria to control a wide variety of different biochemical functions, including pathogenicity (1–5). The paradigm of this phenomenon, the luminous marine bacteria (6, 7), has been studied for over three decades. When luminous marine bacteria are free-living in the ocean, they do not express bioluminescence; rather they must attain a critical cell density in order to stimulate transcription of the *lux* gene cluster. The first systems to be studied in molecular detail were those of *Vibrio harveyi* (7) and *Vibrio fischeri* (8, 9). The onset of bioluminescence results from increased transcriptional levels of the luminescence functions (7). Eberhard *et al.* (8, 9) discovered that these bacteria produce a molecular signal which can be extracted from the growth medium. Accumulation of this molecule, termed autoinducer, results in the increased transcription of the bioluminescence functions.

There is a long and growing list of bacteria which apparently employ an autoinduction mechanism (10, 11). The autoinduction mechanism in *V. fischeri* is mediated through a transcriptional activator protein, LuxR, and LuxI, which produces autoinducer from cytoplasmic components (6). There are at least eight other proteins that show extensive similarity to LuxR: SdiA from *Escherichia coli* (12, 13), LasR and RhlR from *Pseudomonas aeruginosa* (1, 14), the nopaline- and octopine-type TraR proteins from *Agrobacterium tumefaciens* (4, 15), RhiR from *Rhizobium leguminosarum* (16), ExpR from *Erwinia carotovora* (5), and PhzR from *Pseudomonas aureofaciens* (3). The structures of seven autoinducers have been determined: *V. fischeri* autoinducer, *N*-(3-oxohexanoyl)-L-homoserine lactone (9); *V. harveyi* autoinducer, *N*-(3-hydroxybutanoyl)-L-homoserine lactone (17); *A. tumefaciens* autoinducer, *N*-(3-oxooctanoyl)-L-homoserine lactone (18); *P. aeruginosa* autoinducer, *N*-(3-oxododecanoyl)-L-homoserine lactone (19); *P. aeruginosa* factor 2, *N*-(3-butyryl)-L-homoserine lactone (20); and two other autoinducers produced by *V. fischeri*—the *luxI*-independent compound *N*-octanoyl-L-homoserine lactone (AI-2), and *N*-hexanoyl-L-homoserine lactone (AI-3) which is dependent on *luxI* for its synthesis (21, 22). These autoinducers differ in the acyl chain attached to the homoserine lactone. The LasR protein has been shown to be stimulated by *V. fischeri* autoinducer (23), and TraR by *V. fischeri* autoinducer and other *N*-acyl-L-homoserine lactones (18). The production of autoinducer-like molecules by many other bacteria has been reported, suggesting that *N*-acyl-L-homoserine lactones may be signals used widely in prokaryotes to effect cell density-dependent gene regulation (10, 11).

Most known autoinduction systems share three main characteristics: (*i*) transcription of genes is activated by a LuxR homolog showing cell density dependence; (*ii*) addition of conditioned medium to the culture results in an earlier induction of the LuxR homolog-dependent transcription, a consequence of the diffusible character of the autoinducer; and (*iii*) different LuxR homologs can crossreact with different autoinducers.

The SdiA protein from *E. coli* has an amino acid sequence similar to that of LuxR and has been proposed to activate transcription of the *ftsQAZ* gene cluster, which is required for cell division (13). The homology between LuxR and SdiA suggested to us the possibility that SdiA might function by a mechanism similar to that of LuxR. The *ftsQAZ* gene cluster is regulated by multiple promoters, with the P_1 and P_2 promoters being upstream of the *ftsQA* genes (24, 25). It has been shown that only the P_2 promoter is affected by SdiA overexpression (13). Here we show that transcription from the SdiA-regulated P_2 promoter satisfies all three requirements of an autoinduction system, suggesting that an autoinduction mechanism is involved in regulation of these genes. Further-

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Table 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Relevant characteristics	Source or ref.
E. coli strains		
HB101	*recA*	28
TB1	Δ*lac*	29
LE392	*recA*$^+$	30, 31
NM522	Δ*lac*	*
UT481	Δ*lac*	†
WX2	UT481, *sdiA*::Km^r	13
ZK126	Δ*lac*	32, ‡
ZK1000	ZK126, *rpoS*::Km^r	33, ‡
Plasmids		
pVFR901	pTZ18R, P_{lac}–*luxR*	34
pJHD600	*luxR*$^-$, *lux* reporter	34, 35
pCX16	*sdiA*$^+$	13
pFZY	Copy number 1–2	13
pCX32	pFZY, *fts*(P_1+P_2)–*lacZ*	13
pCX39	pFZY, *fts*(P_2)–*lacZ*	13
pCX40	pFZY, *fts*(P_1)–*lacZ*	13
pRSkatF5	P_{rpoS}(bp −500 to +65)–*lacZ*	36
pFP53	P_{rpoS}(bp −350 to +526)–*lacZ*	37
Phages		
λRSkatF5	λRS45 × pRSkatF5	37
λFP53	λRS45 × pFP53	37

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more, we demonstrate that transcription from the second *ftsQA* promoter is dependent on a functional *rpoS* gene, the product of which is a σ factor involved in regulation of genes expressed in the stationary phase (26).

MATERIALS AND METHODS

All chemicals used for synthesis of autoinducers and analogs were purchased from Aldrich. *V. fischeri* autoinducer and *V. harveyi* autoinducer were synthesized as described by Eberhard *et al.* (9) and by Cao and Meighen (17), respectively. L-Homoserine lactone was obtained from Sigma. *N*-decanoyl-DL-homoserine lactone was synthesized by the method of Eberhard *et al.* (27) and purified by recrystallization (methanol/water). *V. fischeri* and *V. harveyi* autoinducers were purified by silica gel column chromatography (chloroform/methanol) to 95% purity as determined by HPLC. Structures were confirmed by NMR spectroscopy and fast-atom bombardment mass spectroscopy. The bacterial strains, plasmids, and phages used are listed in Table 1. pVFR901 is a pTZ18R derivative with *luxR* from *V. fischeri* ATCC-7744 under control of a wild-type *lac* promoter (34).

Growth of Cultures and Measurement of Transcription with Bioluminescence and β-Galactosidase Activity *in Vivo*. All experiments were performed in Luria–Bertani (LB) medium at 28°C. Transcription from the *lux* promoter was determined by monitoring luminescence *in vivo* (38). For determination of β-galactosidase activity *in vivo*, overnight cultures were diluted 100-fold into LB medium containing the appropriate antibiotics, grown to mid-logarithmic phase, and diluted back to $OD_{600} < 0.05$. Conditioned medium was prepared by centrifugation of the *E. coli* culture at 10,000 × *g* for 10 min at 4°C. Sterility of the conditioned medium prepared by this method was confirmed by the lack of cell growth upon continued incubation. Prior to use, conditioned media were supplemented with 1% Casamino Acids and 0.4% glycerol, as well as the required antibiotic. Addition of 1% Casamino Acids and 0.4% glycerol to LB medium had no effect on β-galactosidase or bioluminescence activities compared with those in unsupplemented LB medium. β-Galactosidase activity was assayed as described by Miller (39).

RESULTS

Stimulation of the SdiA-Dependent Transcription of *ftsQA* by *V. fischeri* and *V. harveyi* Autoinducers and *N*-Decanoyl-DL-homoserine Lactone. To determine whether the SdiA-mediated *ftsQA* transcription was affected by different autoinducers, we used reporters with a *lacZ* transcriptional fusion to the SdiA-regulated P_2 promoter (pCX39), the SdiA-independent P_1 promoter (pCX40), or both *ftsQA* promoters (pCX32). The data (Table 2) confirm the previous report that overexpression of SdiA increases transcription from the P_2 promoter but not from the P_1 promoter (13) and, furthermore, demonstrate that addition of *V. fischeri* autoinducer, *V. harveyi* autoinducer, or *N*-decanoyl-DL-homoserine lactone stimulated SdiA-mediated *ftsQA* transcription from the P_2 promoter but had no effect on transcription from the P_1 promoter. This effect was dependent on the presence of the *sdiA* gene. The concentration range over which *V. fischeri* autoinducer enhanced transcription from the SdiA-dependent promoter was the same as for the LuxR-dependent promoter (Fig. 1). L-Homoserine lactone (up to 1 mM) had no effect on transcription from either the P_1 or the P_2 promoter (Table 2 and data not shown).

The *ftsQA* P_1 Promoter Is *rpoS*-Stimulated. The *ftsQA* P_1 promoter belongs to a family of "gearbox" promoters, transcription from which is growth-rate dependent (25). This promoter contains sequences similar to *rpoS*-regulated promoters (40); to determine whether P_1 was *rpoS*-stimulated, *rpoS*$^-$ and *rpoS*$^+$ strains of *E. coli* were used (Fig. 2). Transcription from P_1 was decreased in the *rpoS*$^-$ strain (Fig. 2*C*); *rpoS* had no effect on transcription from P_2 (Fig. 2*B*). In the

Table 2. Effect of *V. fischeri* and *V. harveyi* autoinducers, *N*-decanoyl-DL-homoserine lactone, and L-homoserine lactone on *ftsQA* transcription

sdiA phenotype and *ftsQ* promoter(s)	β-Galactosidase activity, Miller units				
	No addition	*V. fischeri* AI	*V. harveyi* AI	DHSL	HSL
sdiA, *ftsQ*(P_1+P_2)	14.6 ± 0.9	11.5 ± 0.6	11.3 ± 0.9	10.9 ± 0.3	10.3 ± 0.9
sdiA$^+$, *ftsQ*(P_1+P_2)	38 ± 2	67 ± 4	62 ± 2	59 ± 3	37 ± 1
sdiA, *ftsQ* (P_2)	11.6 ± 0.7	10.6 ± 1.5	10.3 ± 0.3	10.0 ± 0.7	11.0 ± 0.7
sdiA$^+$, *ftsQ* (P_2)	49 ± 3	79 ± 2	73 ± 4	70 ± 4	42 ± 2
sdiA, *ftsQ* (P_1)	6.5 ± 0.2	4.7 ± 0.3	4.7 ± 0.5	3.8 ± 1.1	5.2 ± 0.3
sdiA$^+$, *ftsQ* (P_1)	3.9 ± 0.3	3.3 ± 0.2	3.9 ± 0.4	2.9 ± 0.2	3.3 ± 0.2

E. coli WX2 (*sdiA*$^-$) was transformed with either pGB2 (*sdiA*$^-$) or pCX16 (*sdiA*$^+$) and one of the *ftsQA* reporter plasmids (pCX32, pCX39, or pCX40). Growth conditions were as described in *Materials and Methods*. The *V. fischeri* or *V. harveyi* autoinducers (AI), *N*-decanoyl-DL-homoserine lactone (DHSL), or L-homoserine lactone (HSL) was added to a final concentration of 1 μM. Values are the average of two independent cultures measured at an OD_{600} of 0.65 ± 0.12. β-Galactosidase activities were determined as described in the legend to Fig. 1.

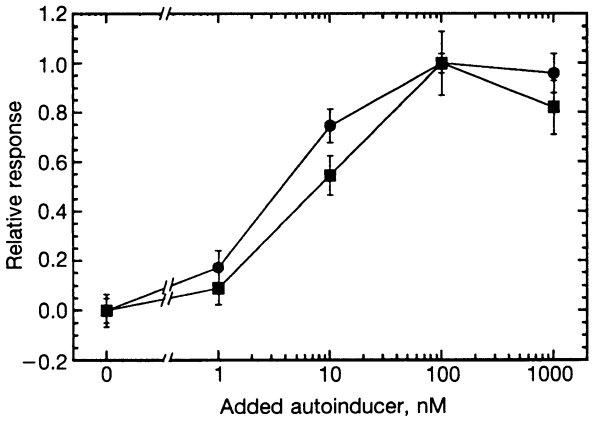

FIG. 1. Effect of different concentrations of *V. fischeri* autoinducer on *V. fischeri lux* and *E. coli fts* transcription. ■, *E. coli* WX2 transformed with pCX16 (*sdiA*$^+$) and pCX39 [*ftsQA* (P_2)]. β-Galactosidase activity was determined by the method of Miller (39). A 0.1-ml aliquot of culture of known OD_{600} was mixed with 0.9 ml of Z buffer (100 mM sodium phosphate/10 mM KCl/1 mM $MgSO_4$/50 mM 2-mercaptoethanol, pH 7.0), 2 drops of chloroform, and 1 drop of 0.1% SDS; mixed vigorously for 10 sec; and equilibrated at 28°C. The substrate (0.2 ml of a 4 mg/ml solution of *o*-nitrophenyl β-D-galactopyranoside) was added and allowed to react until yellow color developed. The reaction was stopped by addition of 0.5 ml of 1 M Na_2CO_3, and the time was recorded. The OD_{550} and the OD_{420} were determined, and the β-galactosidase activity was calculated in Miller units as $1000 \cdot [OD_{420} - (1.75 \cdot OD_{550})]/(\text{time in minutes}) \cdot 0.1 \cdot OD_{600}$. ●, *E. coli* WX2 transformed with pVFR901 (*luxR*$^+$) and pJHD600 (*lux* promoter). The methods used for determination of bioluminescence have been described in detail (38).

rpoS$^-$ background, deletion of the P1 promoter had a stimulatory effect on *ftsQA* transcription, suggesting other regulatory mechanisms in the absence of *rpoS* (Fig. 2 *A* and *B*). The *rpoS*$^-$ mutation had no effect on *lux* expression in *E. coli* (data not shown). The *rpoS*-mediated activation of transcription from P_1 showed cell density dependence (Fig. 2 *A* and *C*), occurring in mid-logarithmic phase rather than in stationary phase (26). Activation of transcription from the SdiA-dependent P_2 promoter also showed cell density dependence (Fig. 2*B* and Fig. 3).

Transcription from *lux*, *ftsQA*, and *rpoS* Promoters Is Stimulated by *E. coli* Conditioned Medium. Even though in the absence of autoinducer the level of luminescence is low, we have consistently observed a cell density-dependent increase of *lux* transcription in *E. coli* (38, 41). This increase in transcription in early stationary phase is *luxR*-dependent, *sdiA*-independent, and *rpoS*-independent (data not shown). By analogy with the *V. fischeri* autoinduction mechanism, it would be reasonable to propose that *E. coli* itself excretes a compound into the medium, and when a certain threshold concentration of the compound is attained, LuxR responds by stimulating *lux* transcription in *E. coli*. This hypothesis predicts that addition of *E. coli* conditioned medium to *E. coli* transformed with *lux* plasmids should induce *lux* transcription at lower cell density. To test this hypothesis, *E. coli* HB101 transformed with the *lux* plasmids pVFR901 and pJHD600 was grown in medium conditioned by *E. coli* strains HB101, LE392, or TB1. In all cases, the *E. coli* conditioned medium stimulated luminescence in the culture (Table 3).

In a similar series of experiments, the effect of *E. coli* conditioned medium on transcription from the *ftsQA* promoters was tested (Table 3 and Fig. 3). Transcription from both P_1 and P_2 promoters was enhanced in the presence of conditioned medium; the effect appeared to be greater for P_2 than for P_1 (Table 3). As discussed above (Fig. 2), transcription from both P_1 and P_2 shows a lag at low cell densities. In the presence of

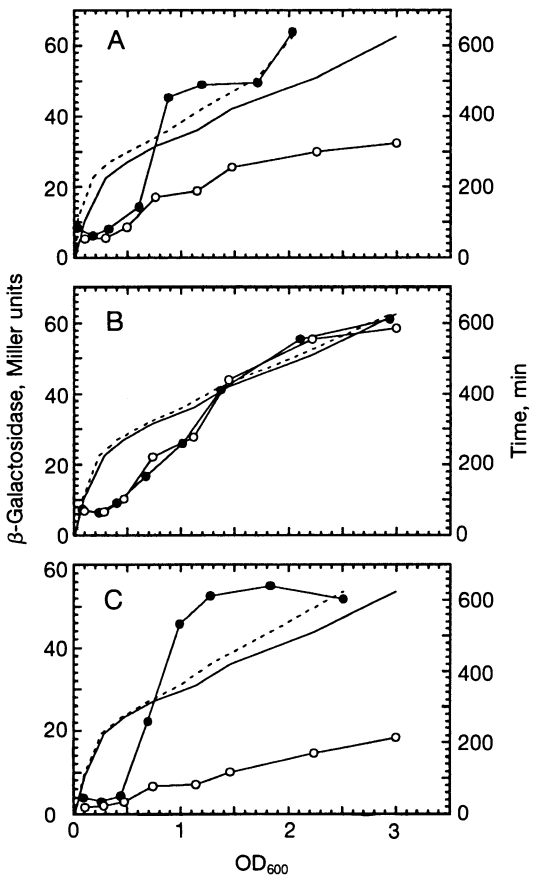

FIG. 2. Effect of *rpoS* mutation on *ftsQA* transcription. β-Galactosidase activity was determined as described in the legend to Fig. 1. Filled symbols, *E. coli* ZK126 (*rpoS*$^+$); open symbols, *E. coli* ZK1000 (*rpoS*$^-$). Dashed and solid lines represent time courses of growth (OD_{600} is plotted against time in minutes) of *E. coli* ZK126 (*rpoS*$^+$) and *E. coli* ZK1000 (*rpoS*$^-$), respectively. The kinetics of cell growth were included to show that the *rpoS* background did not significantly alter the growth rate and to show that induction of *ftsQA* transcription occurred prior to stationary phase. (*A*) Transcription from *ftsQA* (P_1 and P_2) on pCX32. (*B*) Transcription from *ftsQA* (P_2) on pCX39. (*C*) Transcription from *ftsQA* (P_1) on pCX40. These data demonstrate that transcription from P1 is *rpoS*-stimulated. Deletion of P2 resulted in reduced transcription in the *rpoS*$^-$ background (*C* relative to *A*), whereas deletion of P1 resulted in increased transcription (*B* relative to *A*).

conditioned medium, transcription from the SdiA-mediated P_2 promoter occurred at lower cell densities with a correspondingly reduced lag phase.

It has been reported that *E. coli* conditioned medium activates *rpoS* transcription (36). Using different experimental conditions, we have observed similar effects (Table 3). With two different P_{rpoS}–*lacZ* transcriptional fusions, conditioned medium resulted in a 1.5- to 2-fold stimulation of transcription in mid- to late-logarithmic-phase cells.

We also employed an *E. coli rpoS*$^-$ strain to determine the effect of *E. coli* conditioned medium on transcription from the *ftsQA* P_1 promoter in the *rpoS*$^-$ background (Table 4). Under these conditions, the stimulatory effect of conditioned medium on transcription from the P_1 promoter was greatly reduced (11-fold in *rpoS*$^+$ vs. 4-fold in *rpoS*$^-$), while the effect of conditioned medium on transcription from the SdiA-dependent P_2 promoter was not changed by the status of *rpoS*.

Neither *V. fischeri* Autoinducer nor *V. harveyi* Autoinducer Has an Effect on *rpoS* Expression. Even though *V. fischeri* autoinducer at the concentrations employed had no effect on the *ftsQA* P_1 promoter, we asked whether the autoinducer

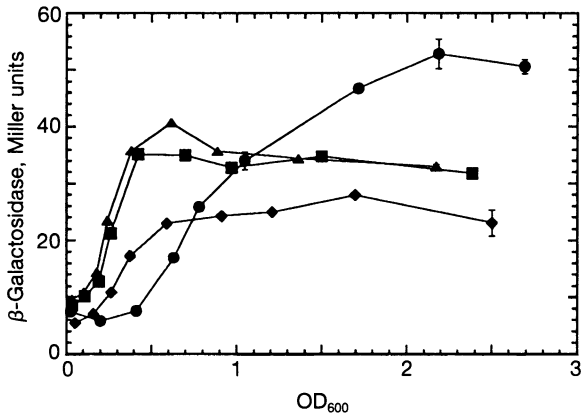

FIG. 3. Effect of conditioned medium on transcription from the SdiA-regulated *ftsQA* P_2 promoter. Conditions were the same as described in Table 3. ●, UT481(pCX39) in LB medium; ◆, UT481 (pCX39) in TB1 conditioned medium; ■, UT481(pCX39) in HB101 conditioned medium; ▲, UT481(pCX39) in LE392 conditioned medium. β-Galactosidase activity was determined as described in the legend to Fig. 1.

would have any effect on *rpoS* expression, thereby mimicking the effect of the conditioned medium (36). To address this question, two P_{rpoS}–*lacZ* transcriptional fusions were used, both as a plasmid and as a single chromosomal copy (pRSkatF5, pFP53, λRSkatF5, and λFP53). Neither *V. fischeri* autoinducer (up to 2 μM) nor *V. harveyi* autoinducer (up to 1 mM) had any effect on *rpoS* transcription (data not shown). L-Homoserine lactone had no effect on *rpoS* transcription at concentrations up to 1 mM (data not shown).

DISCUSSION

SdiA from *E. coli* has been shown to be involved in a complex transcriptional regulation of genes required for cell division (13). Since SdiA shows extensive homology to LuxR, it was reasonable to assume that *E. coli* employs an autoinduction mechanism to regulate these functions. Autoinduction utilizes a signal molecule, autoinducer, that is freely diffusible (42); thus, addition of conditioned medium can stimulate transcription at lower cell densities. Our results show that (*i*) *E. coli* conditioned medium activates transcription from the SdiA-dependent promoter, (*ii*) SdiA-mediated *ftsQA* expression shows cell density dependence, and (*iii*) SdiA-regulated transcription can be stimulated by different autoinducers and an autoinducer analog, strongly suggesting that *E. coli* uses an autoinduction mechanism to regulate transcription of the *ftsQA* genes.

Table 4. Effect of *E. coli* conditioned medium on *ftsQA* expression in *rpoS*$^+$ and *rpoS*$^-$ *E. coli*

Promoter and *rpoS* phenotype	β-Galactosidase,* Miller units		Fold stimulation
	LB	LE392†	
ftsQA(P_1+P_2), *rpoS*$^+$	5.6 ± 0.2	40 ± 1	7.0
ftsQA(P_1+P_2), *rpoS*$^-$	5.2 ± 0.2	21 ± 1	4.0
ftsQA(P_2), *rpoS*$^+$	6.1 ± 0.2	31 ± 1	5.0
ftsQA(P_2), *rpoS*$^-$	6.1 ± 0.2	30 ± 1	5.0
ftsQA(P_1), *rpoS*$^+$	2.2 ± 0.1	24 ± 1	11.0
ftsQA(P_1), *rpoS*$^-$	1.6 ± 0.1	6.5 ± 0.2	4.0

E. coli ZK126 (*rpoS*$^+$) and ZK1000 (*rpoS*$^-$) were transformed with pCX32 [*ftsQA*(P_1+P_2)], pCX39 [*ftsQA*(P_2)], or pCX40 [*ftsQA*(P_1)]. Data are the average of two independent cultures.
*Measured at an OD_{600} of 0.42 ± 0.02.
†Conditioned medium from *E. coli* LE392 at an OD_{600} of 5.4.

At the concentrations of the autoinducers used, there were large effects on transcription of the *lux* genes, but relatively small effects on SdiA-mediated transcription (about 1.5- to 2.0-fold); *E. coli* conditioned medium effected a 5-fold stimulation of transcription from the SdiA-dependent promoter. This effect is low compared with other autoinduction systems, where autoinducer-dependent stimulation of transcription can exceed 100-fold. However, if we assume that *E. coli* produces its own autoinducer to which SdiA responds, it is reasonable to suggest that the basal level of transcription from the SdiA-dependent promoter would be lower in the absence of the endogenous signal molecule.

Very little is known about the proposed autoinduction system in *E. coli*. No corresponding LuxI homolog in *E. coli* has been identified. Several facts indicate that the SdiA-mediated transcription might show different features and requirements compared with other autoinduction systems. When the *sdiA* gene is deleted, transcription from the SdiA-dependent promoter is not changed greatly and cells divide normally (13). It is unlikely that another copy of *sdiA* exists, since addition of autoinducers did not have an effect on the *ftsQA* P_2 promoter in the *sdiA*$^-$ strain (Table 2). This suggests that other regulatory mechanism(s) compensate for the absence of *sdiA*. The amount of FtsQ is about 25 molecules per cell (43) and FtsA is present at about 150 molecules per cell (44), compared with 5×10^4–2×10^5 molecules of FtsZ per cell (45). For comparison, the amount of luciferase is about 5×10^3–5×10^4 molecules per cell. Since small amounts of the *ftsQA* gene products are required by the cell, the level of activation by SdiA

Table 3. Effect of *E. coli* conditioned medium on *ftsQA*, *lux*, and *rpoS* expression

Promoter	β-Galactosidase or luciferase activity*			
	LB	HB101	LE392	TB1
lux	2.6	7.7 (3.0)	7.8 (3.0)	6.7 (2.6)
ftsQA(P_1+P_2)	10	38 (3.8)	33 (3.3)	26 (2.6)
ftsQA(P_2)	7.4	34 (4.6)	36 (4.9)	18 (2.4)
ftsQA(P_1)	3.7	9.9 (2.7)	10.2 (2.8)	10.4 (2.8)
rpoS†	27 ± 1	42 ± 1 (1.6)	45 ± 5 (1.7)	50 ± 2 (1.9)
rpoS‡	30 ± 1	44 ± 3 (1.4)	47 ± 1 (1.6)	48 ± 6 (1.6)

For *ftsQA* (UT481 transformed with pCX32, pCX39, or pCX40) and *lux* (HB101 carrying pVFR901 and pJHD600) transcription, complete growth curves were performed and β-galactosidase (Miller units) and luciferase (light units/ml) activities are shown for bacteria at an OD_{600} of 0.4. The OD_{600} of HB101, LE392, and TB1 at which the conditioned media were prepared for the *ftsQA* and *lux* experiments was 5.4, 5.4, and 2.5, and for the *rpoS* experiments was 6.6, 6.5, and 6.2, respectively. The methods for determination of β-galactosidase and luciferase activities are given in the legend to Fig. 1. The error in all cases was ±10%.
*Numbers in parentheses indicate the fold increase in the conditioned medium.
†NM522(λRSkatF5), three independent experiments.
‡NM522(λFP53), three independent experiments.

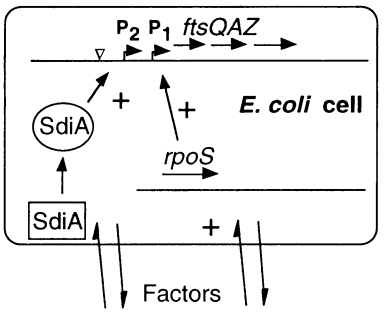

FIG. 4. Model for regulation of *ftsQA*. See text for details. Positions of the *ftsQA* P_1 and P_2 promoters are shown; ▽ indicates the location of the inverted repeat 5′-AGCAGAAA△TTTCTGCT-3′ discussed in the text.

may also be relatively low compared with activation of *lux* transcription by LuxR.

The *ftsQA* P_1 promoter belongs to a family of "gearbox" promoters that are regulated by growth rate (25). Some of the gearbox promoters are *rpoS*-stimulated (40). Sequence similarities with other *rpoS*-regulated promoters led to the proposal that the *ftsQA* P_1 promoter is *rpoS*-dependent (40). Our data show that the *ftsQA* P_1 promoter is rpoS-stimulated. This promoter is induced in mid-logarithmic phase and there is no increase of transcription in stationary phase, whereas most other *rpoS*-dependent promoters are activated in the stationary phase. There are other regulatory mechanisms which are mediated through the P_1 promoter, since deletion of this promoter or addition of conditioned medium to *E. coli* carrying an *ftsQA* P_1 reporter plasmid resulted in stimulation of transcription in an *rpoS*$^-$ background (Fig. 2 *A* and *B*; Table 4).

E. coli conditioned medium stimulates transcription from both the P_1 and P_2 promoters of *ftsQA*. It has been demonstrated that *E. coli* conditioned medium induces *rpoS* transcription (36). In this report, we confirm this observation and show that *E. coli* conditioned medium activates transcription from the *ftsQA* P_1 promoter. Even though *E. coli* conditioned medium stimulated both *rpoS*-stimulated and SdiA-dependent *ftsQA* transcription, addition of the *V. fischeri* and *V. harveyi* autoinducers increased transcription only from *ftsQA* P_2, not from the P_1 promoter. These results suggest that different factors may be responsible for activation of SdiA- and *rpoS*-stimulated *ftsQA* promoters. We propose that the stimulation of transcription from P_1 by conditioned medium is due to increased transcription of the *rpoS* gene or, alternatively, that σ^S itself may respond to a factor excreted into the medium.

We propose the following model for regulation of *ftsQA* transcription in *E. coli* (Fig. 4). At low cell densities there is a basal level of transcription from the *ftsQA* P_1 and P_2 promoters. Upon reaching a quorum (46), when the concentration of the proposed freely diffusible factors inside the cell reaches some threshold, SdiA and σ^S respond by induction of *ftsQA* transcription from the P_1 and P_2 promoters. This induction could be either the result of direct interactions of SdiA and σ^S with the *ftsQA* promoters or mediated through activation of other stimulatory factors. Although there is no obvious consensus σ^S-regulated promoter, the sequence similarity between the *ftsQA* P_1 promoter and other *rpoS*-dependent promoters suggests that activation of σ^S-mediated *ftsQA* transcription could occur through direct interactions with the P_1 promoter. Inverted DNA repeats are found upstream of the LuxR homolog-regulated promoters and are proposed to be the activator–DNA binding sites (46, 47). There is an inverted repeat upstream of the P_2 promoter, suggesting that this repeat could be a binding site for SdiA (see Fig. 4). An alternative mechanism(s) for *ftsQA* regulation must exist, since regulation is observed in the absence of *sdiA* (13). The nature of the factor(s) responsible for the induction of the *ftsQA* transcription is not known. A homoserine lactone derivative has been proposed to be the factor responsible for the regulation of the *rpoS* expression, and high concentrations of homoserine lactone (>0.5 mM) resulted in an increase in σ^S (48). Our results, obtained under different conditions, show no effect of L-homoserine lactone (up to 1 mM), either on *rpoS* transcription or on the *rpoS*-stimulated *ftsQA* P_1 promoter, suggesting that homoserine lactone itself is not responsible for the regulation of *rpoS* transcription.

FtsQ and FtsA are required for cell division, but little is known of the details of their function in the process of septation, how their activities are regulated, or whether they may be involved in processes other than cell division. It is not obvious that stimulation of transcription of *ftsQA* results directly in cell division. Overexpression of FtsQ has no effect on cell division in Luria broth (43), whereas FtsZ overexpression leads to minicell formation (49). It is possible that unknown processes require transcription of *ftsQA* in mid-logarithmic phase. Alternatively, involvement of both σ^S and SdiA is suggestive of a bimodal mechanism of *ftsQA* regulation. Under dilution conditions in a nutrient-poor environment, *rpoS*-mediated transcription could supply the *ftsQA* gene products, while in a rich medium at high cell density, the SdiA system may control transcription of the critical *ftsQA* functions.

In summary, regulation of cell division is a complex process involving multiple transcriptional regulatory elements. We propose that one of these elements is an autoinduction mechanism employed by *E. coli* to regulate transcription of the *ftsQA* genes. It is possible that the *ftsQA* genes are not the only target of SdiA and that other genes might be regulated by this transcriptional activator.

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