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An Extracellular Factor Regulates Expression of *sdiA*, a Transcriptional Activator of Cell Division Genes in *Escherichia coli*

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The *sdiA* gene codes for a protein that regulates expression of the *ftsQAZ* cluster of essential cell division genes of *Escherichia coli*. SdiA up-regulates the *ftsQ2p* promoter that initiates transcription into the *ftsQAZ* cluster. In this paper, we report that expression of *sdiA* is itself regulated by a factor that is released into the growth medium by *E. coli*. When medium that had previously supported growth of *E. coli* (conditioned medium) was used to support growth of an indicator *E. coli* strain that contained an *sdiA-lacZ* transcriptional reporter, there was a 50 to 80% decrease in *sdiA* expression as monitored by β-galactosidase activity. The down-regulation of P_{sdiA} was associated with a decrease in expression of the SdiA target promoter *ftsQ2p*, as monitored by expression of an *ftsQ2p-lacZ* transcriptional fusion. An effect of conditioned medium on *ftsQ2p* expression was not seen when the wild-type *sdiA* gene was disrupted by insertional mutagenesis, indicating that the effect on *ftsQ2p* expression was secondary to the down-regulation of P_{sdiA}. Conditioned medium had no effect on expression of P_{lac}, P_{rpoS}, or several other promoters associated with the *ftsQAZ* gene cluster (*ftsQ1p* and *ftsZ1-4p*). This suggests that the response is specific for P_{sdiA} and for promoters that are regulated by the *sdiA* gene product and that cell-to-cell signalling may play a role in regulating expression of this group of genes.

Several extracellular factors have recently been shown to regulate differentiation and behavior in bacteria by altering the transcription of specific target genes. The most extensively studied group of such extracellular regulatory factors are *N*-acyl derivatives of homoserine lactone (HSL) that are released by bacteria and accumulate in the medium (reviewed in references 13 and 36). The effects of these molecules are mediated by transcriptional regulatory proteins (R proteins) within the target cell. For example, in *Vibrio fischeri*, the extracellular factor *N*-(3-oxohexanoyl)-HSL (OHHL) and the corresponding R protein (LuxR) are both required for induction of the *lux* operon that is responsible for production of bioluminescence (12, 20, 34).

Genes homologous to *luxR* have also been identified in other gram-negative bacteria that use HSL derivatives as extracellular signals. The combination of the R proteins and their cognate extracellular factors is responsible for regulating a variety of cellular processes, including production of exoproteins in *Erwinia carotovora* (3, 30), conjugal transfer in *Agrobacterium tumefaciens* (29, 42), and virulence in *Pseudomonas aeruginosa* (14, 25, 26) and *Yersinia enterocolitica* (38). The extracellular HSL derivatives in these systems differ in their acyl moieties, which are therefore thought to be responsible for specific recognition of the cognate transcriptional regulatory proteins. LuxR homologs have also been identified in several bacterial strains (*Rhizobium leguminosarum*, *Enterobacter agglomerans*, and *P. aureofaciens*) in which diffusible factors have not been identified (6, 28, 37).

SdiA, a protein involved in the regulation of expression of the *ftsQAZ* cluster of cell division genes in *Escherichia coli* (39), shows sequence similarity to the R proteins that are thought to mediate the transcriptional effects of HSL-related signal molecules that are released into the medium by other species (17, 28). In this paper, we report that *E. coli* cells release a factor into the medium that down-regulates transcription of the *sdiA* gene, leading to a decrease in *ftsQ2p* expression. This finding raises the possibility that cell-to-cell signalling plays a role in modulating cell division events in *E. coli* and other bacterial species.

MATERIALS AND METHODS

Strains and growth conditions. The bacterial strains used in this study are described in Table 1. Cells were grown in the dark in culture tubes (15 by 1.5 cm) containing 5 ml of Luria-Bertani broth (LB) (23) at 37°C with vigorous shaking, unless otherwise indicated. Ampicillin or kanamycin was present at 50 μg/ml when required for plasmid maintenance.

Where indicated, HSL (Sigma) was added to LB from a 1 M solution prepared in 0.1 M potassium phosphate buffer (pH 7.0). OHHL was a gift from T. Baldwin of Texas A&M University. Where indicated, OHHL was added to the culture tubes from a 9.4 mM solution in ethyl acetate, dried at 37°C under nitrogen, and then resuspended by shaking in fresh LB at 37°C.

Plasmids. pFZY1 is a mini-F derivative (average copy number, 1 to 2 per cell) with a polycloning site upstream of a promoterless *galK′-lacZYA* reporter segment (21). pSP3 (Fig. 1) is a pFZY1 derivative that contains P_{sdiA} immediately upstream of *galK′-lacZYA* and was constructed by inserting the *Bam*HI-*Kpn*I fragment from pCX2 (39) into *Bam*HI-*Kpn*I-digested pFZY1. pGL101 (Km^r Amp^s) is a pUC19 derivative in which the 1,894-bp *Eco*RI-*Xmn*I fragment has been replaced by the 1,712-bp *Bam*HI-*Bam*HI fragment from pNK862 (40) that includes a kanamycin resistance element. pGL133 (Km^r Amp^s *rspA rspB*) was constructed by inserting the 4,400-bp *Sph*I-*Kpn*I fragment from pSPER1B ($rspA^+$ $rspB^+$) (18), containing *rspA* and *rspB*, into *Sph*I-*Kpn*I-digested pGL101. pSPER1B was provided by R. Kolter of Harvard Medical School. pGL136 (Km^r Amp^s) is identical to pGL133 except for the absence of *rspA* and *rspB*. pGL140 (P_{lac}-*lacZYA*) was constructed by placing P_{lac} from pMLB1115 upstream of *galK′-lacZYA* in pFZY1. pCX25, pCX39, and pCX40 (Fig. 1) are pFZY1 derivatives that have been described elsewhere (39).

Preparation and extraction of conditioned medium. Actively growing cultures of *E. coli* DH5α were diluted in LB to an optical density at 600 nm (OD_{600}) of 0.01 and grown for 24 h at 37°C to a final OD_{600} of 3.7 to 4.3. Cells were removed by centrifugation at 7,000 rpm (Sorvall RC2-B centrifuge, Sorvall SS-34 rotor) for 15 min at 4°C. The supernatant was sterilized by filtration through a 0.2-μm-pore-size Nalgene Tissue Culture Filter and kept frozen at −70°C until use (conditioned culture medium). When conditioned medium was used to support growth, tryptone and yeast extract were added to conditioned culture medium to the levels present in LB (10 and 5 g/liter, respectively) unless otherwise noted (conditioned medium). This medium supported growth with a generation time equal to that of cells grown in fresh LB (Fig. 2). There was no difference between growth rates of cultures grown in LB and cultures grown in LB containing HSL or OHHL at the concentrations indicated in the figures and tables (data not shown).

To prepare ethyl acetate extracts, 250 ml of conditioned culture medium or LB

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TABLE 1. *E. coli* strains used in this study

Strain	Genotype	Source or reference
UT481	Δ*lac-pro met pro zzz*::Tn*10 thy supD* r_K^- m_K^-	G. Carmichael
WX2[a]	Same as UT481 + *sdiA*::Kan^r	39
DH5α	Δ*lacU169 recA1 endA1 gyrA96 relA1 hsdR17 thi-1 supE44*	31
HS143[b]	Δ*lacU169* φ(*katF-lacZ*$^+$)*143*	32

[a] In strain WX2, a kanamycin resistance element replaces bp 196 to 693 of the 723-bp coding sequence of the chromosomal *sdiA* gene (39).
[b] Strain provided by R. Kolter of Harvard Medical School.

was extracted twice by shaking for 5 min with 2 volumes of high-pressure liquid chromatography-grade ethyl acetate. The combined organic phases were taken to dryness, redissolved in 30 ml of ethyl acetate, and back extracted by shaking with 15 ml of water. The final organic phase was taken to dryness and dissolved in 2.5 ml of ethyl acetate. When the extracts were to be tested on growing cells, aliquots were added to empty tubes, dried at 37°C under nitrogen, and then resuspended by shaking in fresh LB at 37°C.

Assays for effects on expression of *lacZ* transcriptional fusion plasmids. Overnight cultures in LB were used to inoculate 5 ml of LB to an OD_{600} of approximately 0.01. The cultures were then grown at 37°C until mid-exponential phase, and cells were collected by centrifugation, washed in M9 medium (23), and resuspended in fresh M9. This cell suspension was used to inoculate 5 ml of either LB or conditioned medium to an OD_{600} of approximately 0.01. The cultures were then incubated at 37°C with vigorous shaking in culture tubes (15 by 1.5 cm). Samples were removed at intervals for determination of OD_{600} and β-galactosidase activity. Cells were collected by centrifugation and resuspended in fresh LB before determination of β-galactosidase activity (23). Specific activity of β-galactosidase is expressed as Miller units (23, 24).

Characterization of extracellular factor. To determine heat stability, conditioned medium or fresh LB was incubated at 37 or 100°C for 20 min and then allowed to cool to 37°C. To determine alkali stability, conditioned medium or fresh LB was adjusted to pH 12 by addition of NaOH and then allowed to stand for 1 h at room temperature before neutralization by addition of HCl. The heated or alkali-treated samples were then used to support growth of indicator strains in the standard assay described above.

DNA sequence analysis. Comparison of DNA sequences was performed by using the Bestfit program from the Sequence Analysis Software Package GCG 6.0 (University of Wisconsin, 1989).

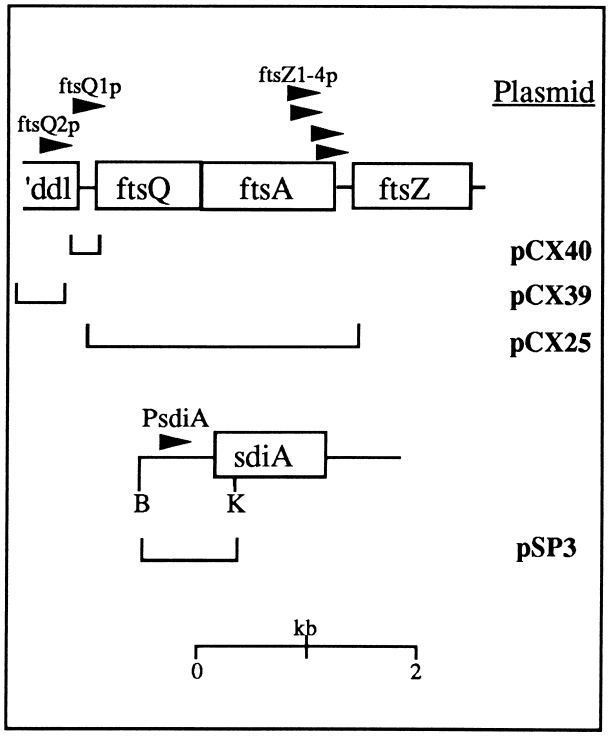

FIG. 1. Plasmids used in this study. The top drawing shows the transcriptional organization of the *ftsQAZ* gene cluster, and the bottom drawing shows the *sdiA* gene. Boxes indicate the open reading frames, and arrowheads indicate the positions of promoters (1, 34a) of the *ftsQAZ* gene cluster (top diagram) and the *sdiA* gene (bottom diagram). Under each diagram are shown the fragments transcriptionally fused to the *lacZ* gene in the indicated plasmids. B, *Bam*HI; K, *Kpn*I.

RESULTS

Effect of conditioned medium on expression of *ftsQ2p*. The sequence similarity between SdiA of *E. coli* and R proteins of other species prompted us to ask whether *E. coli* might produce an extracellular factor that acts via SdiA to modulate the expression of the SdiA target promoter *ftsQ2p*. To determine whether *E. coli* releases a factor that regulates *ftsQ2p* expression, *E. coli* UT481 containing an *ftsQ2p-lacZ* transcriptional reporter plasmid (pCX39; Fig. 1) was grown in LB medium and in conditioned medium prepared from LB that had previously supported growth of *E. coli* DH5α; β-galactosidase activity was used to monitor transcription from *ftsQ2p*. This revealed a moderate but reproducible decrease in *ftsQ2p* activity in cells grown in conditioned medium compared with cells grown in LB (Fig. 3a). The down-regulation of *ftsQ2p* expression ranged from 30 to 35% in mid- and late-exponential growth phase cells. For comparison, replacement of the chromosomal *sdiA* gene with an *sdiA* gene disrupted by insertional mutagenesis leads to a 40 to 60% decrease in *ftsQ2p* expression (39).

The effect on *ftsQ2p* did not reflect a global down-regulation of *E. coli* gene expression, as shown by the failure of conditioned medium to affect the expression of P_{lac} or P_{rpoS} or of

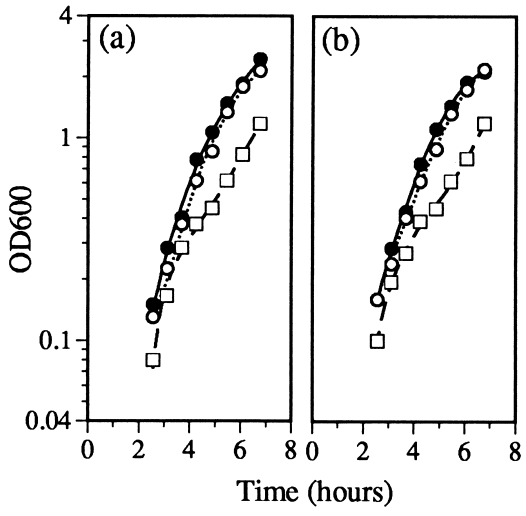

FIG. 2. Growth curves of *E. coli* UT481/pSP3 and UT481/pCX39 in LB and conditioned medium. *E. coli* UT481/pSP3 (a) and UT481/pCX39 (b) were grown in LB (●) or conditioned culture medium supplemented with either 1% tryptone and 0.5% yeast extract (○) or 0.4% glycerol and 1% Casamino Acids (□).

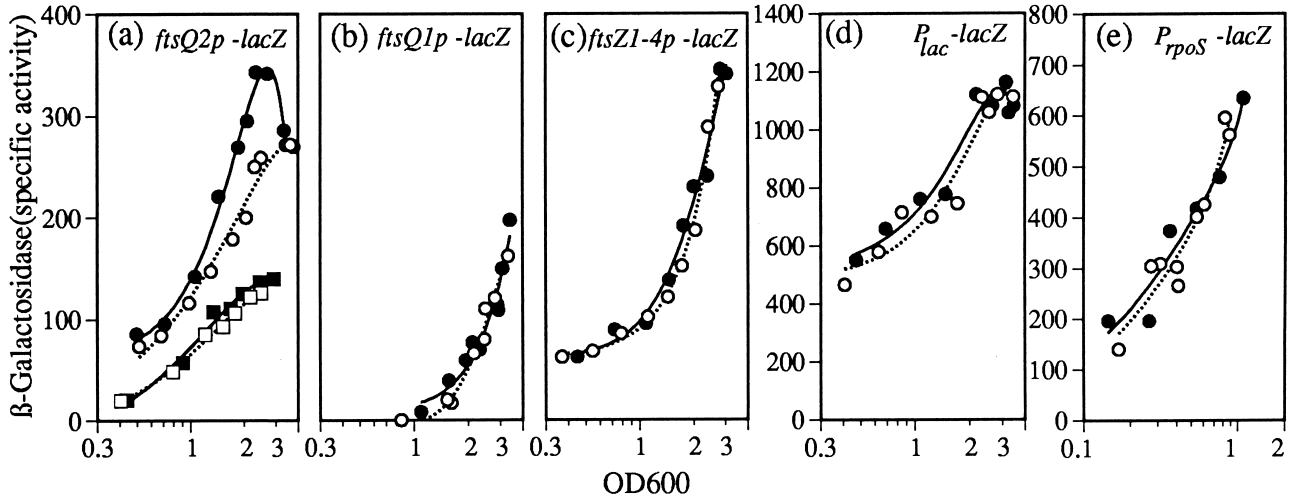

FIG. 3. Effect of conditioned medium on the expression of *ftsQ2p*, *ftsQ1p*, *ftsZ1-4p*, P_{lac}, and P_{rpoS}. β-Galactosidase activity was determined in the following strains: panel a, UT481/pCX39 (*sdiA*$^+$ *ftsQ2p-lacZ*) (●, ○) and WX2/pCX39 (*sdiA*::Kan^r *ftsQ2p-lacZ*) (■, □); panel b, UT481/pCX40 (*sdiA*$^+$ *ftsQ1p-lacZ*); panel c, UT481/pCX25 (*sdiA*$^+$ *ftsZ1-4p-lacZ*); panel d, UT481/pGL140 (*sdiA*$^+$ P_{lac}-*lacZ*); panel e, HS143 (P_{rpoS}-*lacZ*). Cells were grown in either LB (solid lines) or conditioned medium (dotted lines).

several other promoters located within the *ftsQAZ* gene cluster (*ftsQ1p* and *ftsZ1-4p*) (Fig. 3b to e).

In other systems, R proteins act as mediators of the effect of extracellular factors on target genes. This suggested that SdiA might play a similar role in mediating the effect of conditioned medium on *ftsQ2p* expression. We therefore asked whether conditioned medium was still capable of regulating the expression of *ftsQ2p* in cells in which the *sdiA* gene was inactivated by insertional mutagenesis in strain WX2/pCX39 (*sdiA*::Kan^r *ftsQ2p-lacZ*). This revealed that *ftsQ2p* expression in *sdiA*::Kan^r cells was not affected by growth in conditioned medium (Fig. 3a). Thus, the conditioned medium effect required an intact *sdiA* gene, consistent with the view that the effect of the extracellular factor on *ftsQ2p* was mediated by SdiA.

Regulation of *sdiA* expression by conditioned medium. In the *lux* system of *V. fischeri*, the extracellular factor OHHL leads to down-regulation of the *luxR* gene (9, 33). If a similar situation occurred in the *sdiA* system, the extracellular factor would be expected to down-regulate expression of P_{sdiA} and the resulting decrease in SdiA concentration might be the proximate cause of the observed decrease in *ftsQ2p* expression. To determine whether *sdiA* expression is modulated by the *E. coli* factor, β-galactosidase activity was used to monitor transcription from P_{sdiA}-*lacZ* in cells grown in LB and in conditioned medium.

Growth of strain UT481/pSP3 (P_{sdiA}-*lacZ*) in conditioned medium led to a reproducible 50 to 80% decrease in *sdiA* expression in mid- to late-exponential growth phase cells compared with cells grown in LB (Fig. 4a). Similar results were obtained with conditioned medium prepared from cultures grown at 30°C (data not shown).

In *V. fischeri*, the R protein LuxR is responsible for mediating the down-regulation of its own expression by the cognate extracellular factor OHHL (9, 33). To determine whether the down-regulation of P_{sdiA} by conditioned medium is mediated by SdiA in an analogous fashion, we examined the effect of conditioned medium on transcription of the P_{sdiA}-*lacZ* probe in *sdiA*$^+$ and *sdiA*::Kan^r cells. This showed that the extent of down-regulation of P_{sdiA} induced by conditioned medium was similar in the presence and absence of the *sdiA*$^+$ gene product (Fig. 4a and b). Thus, in contrast to the *V. fischeri* LuxR system, in which LuxR regulates its own expression in the presence of OHHL, the SdiA protein is not required for the effect of the *E. coli* extracellular factor on *sdiA* expression.

When cells were grown in conditioned medium, a tryptone-yeast extract supplement was routinely added to restore depleted nutrients and permit the medium to support growth (see Materials and Methods). Addition of the same supplement to fresh LB caused only a slight (10 to 15%) decrease in *sdiA* expression (Fig. 4c), compared with the 50 to 80% decrease observed in supplemented conditioned medium (Fig. 4a). Thus, the down-regulation of *sdiA* that was induced by growth in conditioned medium could not be explained by inhibitory compounds present in the supplement.

We conclude from these experiments that growth of *E. coli* leads to the appearance of an extracellular factor in the growth medium that specifically down-regulates expression of P_{sdiA}.

Characterization of the conditioned medium effect. The ex-

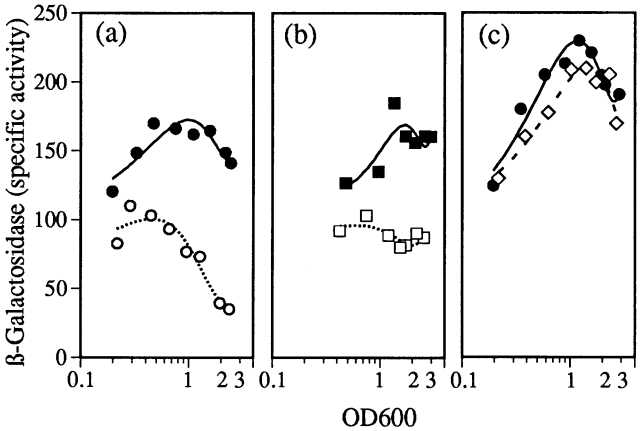

FIG. 4. Effect of conditioned medium on expression of P_{sdiA} in *sdiA*$^+$ and *sdiA* mutant cells. β-Galactosidase activity was determined in the following strains: panel a, UT481/pSP3 (*sdiA*$^+$ P_{sdiA}-*lacZ*); panel b, WX2/pSP3 (*sdiA*::Kan^r P_{sdiA}-*lacZ*); panel c, UT481/pSP3. Panels: a and b, cells grown in LB (●) or conditioned medium (○); c, cells grown in LB (●) or LB supplemented with tryptone (10 g/liter) and yeast extract (5 g/liter) (◇).

TABLE 2. Effect of extracellular factors from different strains on expression of P_{sdiA}

Addition to LB	P_{sdiA} expression[a]
OHHL[b] at:	
0	1.0
0.05	1.01
0.50	0.94
4.70	0.99
47.0	0.99
470.0	0.94
Ethyl acetate extract[c] of:	
LB	1.0
DH5α conditioned culture medium	0.72
DH5α/pSPER1B conditioned culture medium	0.64

[a] Expression of P_{sdiA} is expressed as the ratio of the specific activity of β-galactosidase in cells grown in supplemented LB to the specific activity of β-galactosidase in cells grown in LB.

[b] Strain UT481/pSP3 (P_{sdiA}-*lacZ*) was incubated in LB supplemented with the *V. fischeri* extracellular factor OHHL.

[c] Strain UT481/pSP3 (P_{sdiA}-*lacZ*) was incubated in LB supplemented with ethyl acetate extract of LB or ethyl acetate extract of conditioned culture medium from strain DH5α or DH5α/pSPER1B (*rspAB*). The ethyl acetate extracts were added to a concentration of 10 μl/ml of LB. Samples were removed for determination of β-galactosidase activity at an OD_{600} of 1.5.

tracellular factor that was responsible for the down-regulation of P_{sdiA} was soluble in ethyl acetate (see below), resistant to heating at 100°C for 20 min, and moderately sensitive to treatment at pH 12 at room temperature for 1 h. Strain UT481/pSP3 (P_{sdiA}-*lacZ*) was grown in LB and conditioned medium that had both been treated as indicated in Material and Methods. P_{sdiA} expression, expressed as the ratio of the specific activity of β-galactosidase in cells grown in treated conditioned medium to the specific activity of β-galactosidase in cells grown in treated LB, was determined on samples removed from cultures which had reached an OD_{600} of 1.5. P_{sdiA} expression was 0.52 and 0.55, when the medium had been treated at 37 and 100°C, respectively. P_{sdiA} expression was 0.71 and 0.41, when medium had been treated at pH 12.0 and 7.0, respectively. In these characteristics it resembled HSL derivatives that serve as extracellular signalling factors in other organisms (10).

Addition of the *V. fischeri* HSL-derived factor OHHL to LB did not affect the expression of the P_{sdiA}-*lacZ* transcriptional fusion (Table 2). Similarly, OHHL cannot substitute for the extracellular factor that induces the *lux* system of *V. harveyi* [*N*-(3-hydroxybutanoyl)-L-HSL] (16).

An ethyl acetate extract of conditioned medium retained the ability to down-regulate expression of the P_{sdiA}-*lacZ* transcriptional reporter (Fig. 5a). The effect of the extracted factor on P_{sdiA} expression was proportional to the amount of extract added to LB over a threefold concentration range. The maximum effect (a 58% decrease in activity) was obtained at a concentration of 30 μl of conditioned medium extract (obtained from 3 ml of conditioned medium) per ml of LB. Higher concentrations of conditioned medium extract (100 μl of conditioned medium extract per ml of LB) caused a greater decrease in P_{sdiA} expression but also resulted in decreased growth rates (data not shown). The conditioned medium extract had no effect on the expression of other *fts* promoters (*ftsZ1-4p*; Fig. 5b).

In contrast to the results obtained with the extract from conditioned medium, an ethyl acetate extract of fresh LB had only a slight effect on expression of *sdiA* (Fig. 5a). The failure of the LB extract to significantly affect *sdiA* expression confirms that the effects of conditioned medium on *sdiA* expression were not due to the LB components (tryptone-yeast extract) that were routinely added as a growth supplement (see Materials and Methods).

Effect of HSL. Because the factor in conditioned medium might be an HSL derivative (by analogy to other R-protein-mediated systems), we examined whether HSL could substitute for conditioned medium in down-regulating P_{sdiA} expression. Addition of HSL to LB at a 10 mM final concentration led to a decrease in expression of P_{sdiA} to approximately the same extent as that seen with conditioned medium (Fig. 6a and b). There was no effect of HSL at lower concentrations (≤1 mM). The effect of HSL upon P_{sdiA} was specific, since 10 mM HSL did not affect the expression of promoters *ftsZ1-4p* and P_{lac} (Fig. 6c and d).

The RpoS protein mediates important aspects of the cellular response to stationary-phase growth. Huisman and Kolter (18) have reported that the activity of RpoS-dependent genes was lower in a *lysC metL thrA* triple mutant than in wild-type cells. They also showed that the concentration of RpoS was increased when 0.1 to 1.0 mM HSL was added to the growth medium. On the basis of this evidence, they suggested that the triple mutant might be defective in HSL synthesis and that HSL (or metabolites derived from it) might play a role in modulating expression of the *rpoS* gene.

We therefore determined whether conditioned medium, which down-regulates the expression of P_{sdiA}, also affects *rpoS* expression. As noted above (Fig. 3e), expression of a chromosomal P_{rpoS}-*lacZ* transcriptional fusion was unaffected by growth in conditioned medium. Huisman and Kolter have made similar observations (personal communication).

It has also been reported that overexpression of the *E. coli* *rspA* and *rspB* genes partially suppresses the ability of HSL to correct the decrease in RpoS concentration that is seen in the *lysC metL thrA* triple mutant (18). In addition, overexpression of *rspA* and *rspB* prevented the response of the *V. fischeri lux*

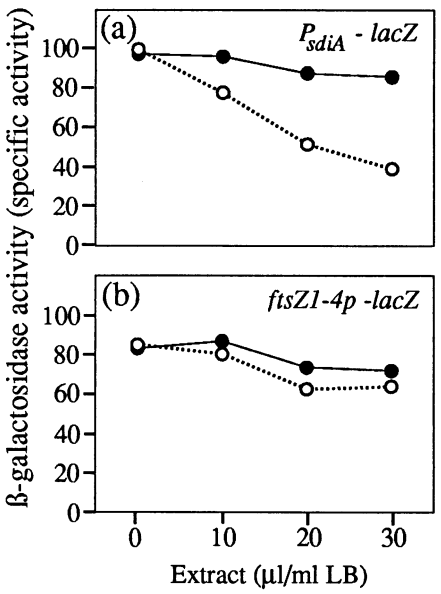

FIG. 5. Effect of ethyl acetate extracts of LB and conditioned medium on expression of P_{sdiA} and *ftsZ1-4p*. Strains UT481/pSP3 (*sdiA*$^+$ P_{sdiA}-*lacZ*) (a) and UT481/pCX25 (*sdiA*$^+$ *ftsQ1-4p-lacZ*) (b) were grown in LB containing the indicated amounts of ethyl acetate extracts of LB (●) or ethyl acetate extracts of conditioned culture medium (○). Samples were removed for β-galactosidase assays when the cultures reached an OD_{600} of 1.5. A 1-μl volume of extract represents material extracted from 1 ml of conditioned medium.

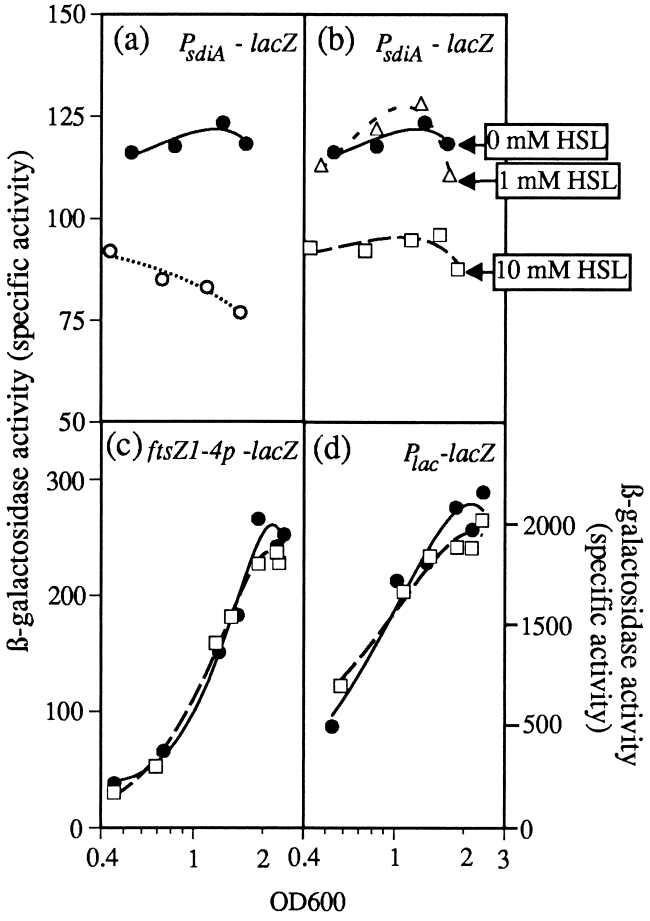

FIG. 6. Effect of HSL. Strain UT481 containing plasmid pSP3 (P_{sdiA}-*lacZ*) (a and b), pCX25 (*ftsZ1-4p*) (c), or pGL140 (P_{lac}-*lacZ*) (d) was grown in LB (●), conditioned medium (○), or LB containing HSL at a concentration of 1 mM (△) or 10 mM (□).

system to the cognate extracellular HSL-derived factor (18). In contrast, we observed that the extent of down-regulation of P_{sdiA} by conditioned medium was unaffected when *rspA* and *rspB* (from *rspAB*$^+$ plasmid pGL133) were overexpressed in the target cells (Table 3). Conditioned medium from cultures of cells that overexpressed *rspA* and *rspB* (DH5α/pSPER1B) was also indistinguishable from conditioned medium prepared from wild-type cells in the ability to down-regulate *sdiA* expression (Table 2).

It seems clear, therefore, that the mediator of the effect observed in the present study differs from that responsible for the HSL effect on *rpoS* expression.

DISCUSSION

This report demonstrates that *E. coli* can be added to the list of organisms in which extracellular factors cause specific transcriptional effects in target cells. In the present case, the factor negatively regulated the expression of *sdiA*. This, in turn, was associated with a decrease in expression of the SdiA-regulated *ftsQ2p* promoter, leading to a decreased level of transcription into the *ftsQAZ* cluster of cell division genes. The effect of the extracellular factor appeared to be specific for *sdiA* and its downstream target.

The present study showed that expression of P_{sdiA} and *ftsQ2p* is down-regulated by a factor in the culture medium of stationary-phase cells. If this observation is relevant to normal growth conditions, one would expect the cellular activity of the promoters to decrease as cell density increases in growing cultures. This was confirmed by the observation that expression of *lacZ* from the *ftsQ2p-lacZ* and P_{sdiA}-*lacZ* transcriptional probes in cells growing in LB began to decrease when cell densities exceeded 1.5×10^9 to 2.5×10^9 cells per ml (OD_{600}, 1.5 to 2.5) (Fig. 3a and 4). This was not true of promoters that did not respond to the conditioned medium factor (*ftsQ1p*, *ftsZ1-4p*, P_{lac}, and P_{rpoS}; Fig. 3b to e).

TABLE 3. Effect of *rspAB* overexpression on the activity of P_{sdiA}

Indicator strain[a]	β-Galactosidase sp act	
	LB	Conditioned medium
UT481/pSP3 (P_{sdiA}-*lacZ*)	129	76
UT481/pSP3/pGL136 (vector)	116	74
UT481/pSP3/pGL133 (*rspAB*)	119	79

[a] The indicator strains were grown at 30°C in LB or conditioned medium, and samples were removed at an OD_{600} of 1.5 for determination of β-galactosidase activity.

The down-regulation of *sdiA* expression did not require an intact *sdiA* gene product, implying that another *E. coli* protein exists that acts as a functional R-protein homolog to mediate the effect of the extracellular factor on *sdiA* expression. In this regard, the present system differs from the *V. fischeri* system, in which LuxR mediates the effect of the extracellular factor OHHL on expression of *luxR*, as well as on other target genes (8, 20, 22, 33).

The sequence similarity of SdiA to R proteins that respond to HSL-derived extracellular signals suggests that the factor responsible for down-regulating P_{sdiA} and *ftsQ2p* might also be an HSL derivative. Devine and co-workers (7) have shown that the binding site for LuxR may be located in a 20-bp palindrome centered 40 bp upstream of the start of transcription of *luxI*. Similar sites are located upstream of several other promoters that are regulated by HSL-derived extracellular factors (13, 15). A search of the region extending several hundred nucleotides upstream of P_{sdiA} and *ftsQ2p* failed to reveal any significant homology to the consensus sequence of these sites.

Among the R proteins identified so far, the PhzR protein of *P. aureofaciens* shows the highest homology to SdiA (28). Interestingly, conditioned medium from *E. coli* DH5α led to an increase in expression of PhzR target genes, although to a lesser extent that conditioned medium from *P. aureofaciens* (28).

High concentrations of HSL mimicked the effect of the *E. coli* extracellular factor on expression of P_{sdiA}. However, this required concentrations of HSL that were 10^4- to 10^6-fold higher than the effective concentrations of HSL-derived factors in other systems (2, 5, 11, 26, 27, 42). The *E. coli* extracellular factor is unlikely to be HSL itself. However, it is possible that HSL can act as a precursor of the *E. coli* factor or substitute for the *E. coli* factor when present at high concentrations. It may be relevant that in *A. tumefaciens*, heterologous HSL derivatives can mimic the activity of the *A. tumefaciens* extracellular factor but only when present at a concentration approximately 10^4-fold higher than that of the cognate factor (42).

The nature of the factor responsible for the effect on *sdiA* expression remains to be determined. Although the factor could be an HSL derivative, it should be noted that other types

of intercellular signalling molecules exist in bacterial systems. Examples of these include oligopeptides that act to transduce signals in *Bacillus subtilis*, *Myxococcus xanthus*, *Enterococcus faecalis*, and *Streptomyces coelicolor* (reviewed in references 13 and 19); weak acids that induce expression of *rpoS* (32); and aspartate, whose release in response to oxidative stress has been reported to induce cellular aggregation in *E. coli* (4).

The extracellular factor responsible for down-regulation of P_{sdiA} did not affect expression of *rpoS*. Therefore, although the factor was recovered from the medium of stationary-phase cells, it is unlikely to be responsible for the increase in *rpoS* expression that occurs when cells enter stationary phase.

Sitnikov et al. recently reported that expression of *ftsQ2p* increased at early times during growth in conditioned medium (35). The possible involvement of SdiA in this effect was not reported. In the present study, an increase in *ftsQ2p* expression during early exponential growth in conditioned medium was not observed. The discrepancy in results may reflect differences in experimental conditions in the two studies. We have observed that cells grown in conditioned medium containing the Casamino Acids-glycerol mixture used as a growth supplement by Sitnikov et al. grew at a significantly slower rate than cells grown in LB, whereas cells grown in conditioned medium containing the tryptone-yeast extract supplement used in the present study grew at the same rate as cells grown in LB (Fig. 2). The possible roles of this and other differences in experimental conditions remain to be defined.

SdiA resembles R proteins that mediate the transcriptional effects of extracellular factors in other systems. However, its own response to the *E. coli* conditioned medium factor is probably mediated by another protein, suggesting that more than one R protein is present in *E. coli*. This raises the possibility that the transcription of *sdiA* might be regulated by one extracellular signal whereas the activity of SdiA on target genes might be responsive to another. Other species have, in fact, been shown to produce more than one extracellular signalling factor (41).

The biological significance of having cells release an extracellular factor that down-regulates the expression of cell division genes remains to be established. The accumulation of the factor in the medium as cell density increases may play a role in mediating the down-regulation of the rate of cell division that occurs when cells slow their growth rate, for instance, at entrance into stationary phase. The possibility also should be considered that SdiA and/or the extracellular factor participate in the regulation of genes involved in other aspects of cell function. Further work is needed to distinguish between these and other possibilities.

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