Salmonella typhimurium Encodes an SdiA Homolog, a Putative Quorum Sensor of the LuxR Family, That Regulates Genes on the Virulence Plasmid

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Quorum sensing is a phenomenon in which bacteria sense and respond to their own population density by releasing and sensing pheromones. In gram-negative bacteria, quorum sensing is often performed by the LuxR family of transcriptional regulators, which affect phenotypes as diverse as conjugation, bioluminescence, and virulence gene expression. The gene encoding one LuxR family member, named sdiA (suppressor of cell division inhibition), is present in the Escherichia coli genome. In this report, we have cloned the Salmonella typhimurium homolog of SdiA and performed a systematic screen for sdiA-regulated genes. A 4.4-kb fragment encoding the S. typhimurium sdiA gene was sequenced and found to encode the 3′ end of YecC (homologous to amino acid transporters of the ABC family), all of SdiA and SizA (Salmonella invasion regulator), and the 5′ end of UvrC. This gene organization is conserved between E. coli and S. typhimurium. We determined that the S. typhimurium sdiA gene was able to weakly complement the E. coli sdiA gene for activation of fisQAZ at promoter 2 and for suppression of filamentation caused by fisZ(Ts) allele. To better understand the function of sdiA in S. typhimurium, we screened 10,000 random lacZY transcriptional fusions (MudI transposon mutations) for regulation by sdiA. Ten positively regulated fusions were isolated. Seven of the fusions were within an apparent operon containing ORF8, ORF9, rck (resistance to complement killing), and ORF11 of the S. typhimurium virulence plasmid. The three ORFs have now been named srgA, srgB, and srgC (for sdiA-regulated gene), respectively. The DNA sequence adjacent to the remaining three fusions shared no similarity with previously described genes.

Many species of bacteria sense and respond to their own population density by a phenomenon known as quorum sensing. Each bacterium in a population produces and releases a pheromone that can be detected by its neighbors. As the population density increases, so does the concentration of pheromone, allowing bacteria to gauge their own population density and possibly those of other species. Many of these sensory systems contain a positive feedback loop in which the synthesis of pheromone is increased as more pheromone is detected. The population density required to initiate the positive feedback loop defines a bacterial quorum. Many regulatory networks, with many of the genes being regulated to some degree by growth phase and/or nutrient limitation. For instance, SPI1 (Salmonella pathogenicity island 1) and the spv locus (Salmonella plasmid virulence) are two virulence gene

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clusters that are affected by growth phase (10, 17, 31). These observations led us to hypothesize that population density may be another regulatory input to virulence gene expression in S. typhimurium. To test this hypothesis, we determined that S. typhimurium encodes a LuxR homolog (SdiA) and then performed a screen for genes regulated by sdiA.

MATERIALS AND METHODS

Bacterial strains and media. Bacteria were grown in Luria-Bertani (LB) broth (Difco) unless otherwise indicated. M9 minimal medium contained (per liter) 11 g of Na2HPO4 · 7H2O, 3 g of KH2PO4, 0.5 g of NaCl, and 1 g of NH4Cl. The pH was adjusted to 7.4, the solution was autoclaved, and then MgSO4 was added to 2 mM. CaCl2 was added to 0.1 mM. 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was added to 40 μg/ml, and glucose or arabinose was added to 0.2%. Dulbecco modified Eagle medium (DMEM) was purchased from Gibco BRL, and when indicated, normal human serum (Sigma) was added to 10%. LB to 0.2%. Where indicated, Casamino Acids (Difco) were added to the M9 medium to 0.2%. Dulbecco modified Eagle medium (DMEM) was purchased from Gibco BRL, and when indicated, normal human serum (Sigma) was added to 10%. LB and M9 plates contained 1.5% agar (Difco).

Strain constructions and lineages are described in Table 1. Transductions were performed with phage P22HTint, followed by streaking to isolation in the presence of 10 mM EGTA and confirmation of smooth lipopolysaccharide (LPS) and lack of pseudolysogeny by cross-streaking transductants against P22vir on Evan’s Blue-Uranine plates (33). Plasmids were introduced by electroporation with a BTX electro cell manipulator 600. Antibiotics were used at the following concentrations: kanamycin, 60 μg/ml; tetracycline, 20 μg/ml; carbenicillin, 100 μg/ml (chromosomal) and 200 μg/ml (episomal); chloramphenicol, 30 μg/ml; streptomycin, 100 μg/ml; spectinomycin, 100 μg/ml; and naladixic acid, 50 μg/ml.

Molecular biology techniques. Plasmid DNA was isolated by using ion-exchange columns from Qiagen. Standard methods were used for restriction endonuclease digestion, ligation, and electroporation of plasmid DNA (2). All restriction endonucleases and T4 DNA ligase were purchased from Gibco BRL or Boehringer Mannheim. PCR was performed with Phi DNA polymerase (Stratagene) according to the instructions of the manufacturer. Southern hybridizations and probes were produced with the Genius nonradioactive nucleic acid detection kit and positively charged nylon membrane from Boehringer Mannheim. DNA probes were labeled with digoxigenin-dUTP (DIG) and detected with anti-DIG-alkaline phosphatase conjugate Fab fragments and the chemiluminescent substrate Lumi-Phos 530.

DNA sequencing was performed with a Pharmacia automated laser fluorescence and an Applied Biosystems 377 fluorescent DNA sequencer at our departmental core facility. Sequence analysis was performed with the following programs: AssemblyLign 1.0.7 (Eastman Kodak), MacVector 6.0 (Oxford Molecular Group), the BLAST programs from the National Center for Biotechnology Information (1), and the Genetics Computer Group (package version 9.0 from the University of Wisconsin).

β-Galactosidase assays. Expression of lacZ fusions was assessed by using β-galactosidase assays as described by Miller (37). Cloning of the S. typhimurium sda4 homolog. An E. coli luxR homolog (sda4) was identified by performing database searches with the Vibrio fischeri LuxR
amino acid sequence and the program TBlastN. sdiA-specific oligonucleotides (BA134 and BA135 [Table 2]) were synthesized, and the E. coli sdiA gene was amplified by PCR. The 654-bp PCR product was DIG labeled and used as a probe to clone the S. typhimurium homolog.

A genomic library of S. typhimurium 14028 prepared in the cosmid vector pLAFR2 has been previously described (32). Individual cosmids from this library were grouped into 30 pools of 30 cosmids each. The 30 pools were digested with HaeIII, separated by agarose gel electrophoresis, and transferred to Hybond-N\(^\text{+}\) (Amersham). The blot was probed with the DIG-labeled DNA that had been digested with Pfu DNA polymerase (Stratagene). Clones were sequenced on both strands with Universal-PCR and Reverse-PCR primers.

### TABLE 2. Oligonucleotides used

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal-PCR</td>
<td>GATAAACCGGCGCGGATGAGGCGGCC</td>
<td>Binds the KpnI side of the pCR-Script multiple cloning site (Stratagene)</td>
</tr>
<tr>
<td>Reverse-PCR</td>
<td>GGAACAAGCTATGACCATAGTATGAC</td>
<td>Binds the SacI side of the pCR-Script multiple cloning site (Stratagene)</td>
</tr>
<tr>
<td>Tn3-bla</td>
<td>GACGAGATCGTGAGATAGTGCCCT</td>
<td>Binds the end of mTn3 (oriented outward) within the (\beta)-lactamase gene</td>
</tr>
<tr>
<td>BA134</td>
<td>TCGGTTTTTCAGAAAGGAGGAGAG</td>
<td>Binds nucleotides 501 to 522 of E. coli sdiA (accession no. X00391)</td>
</tr>
<tr>
<td>BA135</td>
<td>AGGCAAACCTGGGCTTATTGT</td>
<td>Binds nucleotides 1154 to 1133 of E. coli sdiA (accession no. X00391)</td>
</tr>
<tr>
<td>BA174</td>
<td>CCAACAGAAAGCGTTGAGCTAAGGAG</td>
<td>Binds nucleotides 1970 to 1946 of the sequence reported here (accession no. U88651)</td>
</tr>
<tr>
<td>BA176</td>
<td>CGAGTGAAATACGCGTAATAAAC</td>
<td>Binds nucleotides 1678 to 1656 of the sequence reported here (accession no. U88651)</td>
</tr>
<tr>
<td>BA178</td>
<td>AATGATTATCAATATCTAAAAGCGGT</td>
<td>Binds nucleotides 437 to 461 of the sequence reported here (accession no. U88651)</td>
</tr>
<tr>
<td>MudOut</td>
<td>CCGAATATCCAAATCTGCCCTGCCGT</td>
<td>Binds nucleotides 54 to 30 of the Mu left end (accession no. M64097)</td>
</tr>
<tr>
<td>MudTaq</td>
<td>AGTGCCGATAAAATCGGCTGTCGTT</td>
<td>Binds nucleotides 701 to 725 of the Mu left end (accession no. M64097)</td>
</tr>
<tr>
<td>MudAlu</td>
<td>CGAAAGACACAAACAGACTGGGAATACATTTACATAC</td>
<td>Binds nucleotides 167 to 201 of the Mu left end (accession no. M64097)</td>
</tr>
</tbody>
</table>

Plasmid constructions and DNA sequencing. All plasmid constructions except pVR2 are described in Table 1. pVR2 is a cosmid vector that has been amplified with the high-fidelity DNA polymerase Pfu (Stratagene) and cloned into the Smal site of pBAD33. The PCR primers (BA176 and BA178 [Table 2]) were designed to amplify sdiA without its promoter, in order to allow control exclusively by the arabinose promoter of pBAD33 (18). Orientation of the insert was confirmed by restriction analysis, and the 3' and 5' junctions were confirmed by sequence analysis with oligonucleotides specific to pBAD33 (18).

The 4.4-kb insert of pBA301 was sequenced on both strands by several strategies. Subclones were sequenced with Universal-PCR and Reverse-PCR primers to sequence into the inserts of pBA301, pBA306, and a collection of Sau3AI subclones. Successive deletions into the insert of pBA301 were generated by exonuclease III and nuclelease S1 digestion followed by religation (2). The resulting plasmids were sequenced with Universal-PCR and Reverse-PCR primers. The DNAs adjacent to a collection of mTn3 insertions were sequenced with primer Tn3-bla. Finally, several oligonucleotides were synthesized to sequence gaps.

Transposon mutagenesis of pBA302 and construction of a chromosomal sdiA::mTn3. mTn3 mutagenesis was performed as described previously (52). Briefly, E. coli sdiA::mTn3 and plasmid pBlI01 encoding the Tn3 transposase) was transformed with pBA302. Transformants, now carrying cointegrates between pBA202 and the F-

Sequence analysis of the S. typhimurium sdiA region. The 4.4-kb insert of pBA301 was sequenced on both strands. Four open reading frames (ORFs) were identified, with the first and last being truncated (Fig. 1). The four ORFs share the same spacing and orientation as the homologous region in E. coli. The 3' end of the first ORF extends from nucleotides 1 to 257 and shares 91% amino acid identity with the homologous ORF in E. coli, which is named yecC. Database searches suggest that yecC encodes an amino acid transport protein of the ABC (ATP-binding cassette) family. YecC is similar to HisP, the ATP-binding component of the Salmonella histidine trans-
porter. Interestingly, YecC is also very similar to the opaline and nopaline transporters in A. tumefaciens (60% amino acid identity in certain regions). These transporters are located on the Ti plasmid, which is known to use quorum sensing to regulate conjugation (14, 23, 24).

The next open reading frame is sdiA, which extends from nucleotides 472 to 1191. SdiA is 69% identical at the amino acid level to its E. coli homolog, while it is 26% identical and 47% similar to LuxR of V. fischeri (Fig. 2). The ORF downstream of sdiA is known as sirA in S. typhimurium, uvrY or ORF2 in E. coli, and gacA in Pseudomonas species. The S. typhimurium sirA gene was recently identified genetically as a regulator of SPI1 gene expression (26). As with gacA of the Pseudomonas species and uvrY of E. coli, sirA of S. typhimurium is directly followed by the uvrC gene. uvrC encodes a subunit of the UvrABC DNA excision repair enzyme (39, 51). Assuming that S. typhimurium uvrC is similar in size to the E. coli uvrC gene, all but the last two amino acids of UvrC are encoded by pBA301. The E. coli and S. typhimurium coding regions share 94% amino acid identity.

Construction of an S. typhimurium sdiA mutation and an arabinose-inducible sdiA gene. To identify lacZY fusions that are regulated by sdiA, we constructed a strain in which sdiA was under control of the tightly regulated arabinose-inducible PBAD promoter (18). First, the chromosomal copy of sdiA was disrupted in S. typhimurium 14028 to create BA612. Disruption of sdiA was accomplished by mutagenizing pBA302 with mTn3.
as described in Materials and Methods (52). One mutant clone, pBA366, contained an mTn3 between nucleotides 716 and 717 of the sequence reported here, which corresponds to codon 82 of the sdiA ORF. This plasmid-encoded sdiA::mTn3 was used to disrupt the chromosomal sdiA of *S. typhimurium* by allelic exchange as described in Materials and Methods, resulting in strain BA612.

The second step was to create a system for controlling expression of sdiA. Oligonucleotides were designed to amplify the sdiA gene, including its putative ribosome binding site, but lacking any upstream promoter sequences. This PCR product was cloned into pBAD33, which placed the sdiA gene under control of the *F*<sub>BAD</sub> arabinose-inducible promoter (see Materials and Methods). The resulting plasmid, pJVR2, was then transformed into BA612 (14028 sdiA::mTn3), yielding an *S. typhimurium* strain in which sdiA expression was dependent on the presence of arabinose (18).

*S. typhimurium* sdiA weakly complements *E. coli* sdiA. In *E. coli*, when sdiA is expressed from multicopy plasmids, it suppresses filamentation of an *ftsZ*(Ts) mutant grown at the nonpermissive temperature (62). The mechanism for this suppression is increased expression from promoter 2 of the *ftsQAZ* operon by sdiA. Presumably, a fraction of wild-type FtsZ activity is present at the nonpermissive temperature; thus, increasing expression of the *ftsZ*(Ts) allele raises the concentration of active FtsZ to a level capable of supporting cell division (62). To determine if *S. typhimurium* sdiA can also activate the *E. coli* *ftsQAZ* operon, pJVR2 was transformed into *E. coli* WX2/pCX39 (sdiA mutant carrying a lacZ fusion to promoter 2 of *ftsQAZ*). Expression of β-galactosidase activities from cultures grown in various concentrations of glucose or arabinose were determined (Fig. 3). pJVR2, but not the vector pBAD33, activated promoter 2 of *ftsQAZ* in the presence of arabinose. This demonstrates that (i) *S. typhimurium* sdiA is expressed by pJVR2 in the presence of arabinose and (ii) *S. typhimurium* sdiA can at least partially complement *E. coli* sdiA for regulation of *ftsQAZ* promoter 2. Promoter 1 of *ftsQAZ* is known to be insensitive to *E. coli* sdiA, and in agreement with this, pJVR2 had no effect on this promoter (WX2/pCX40 [Fig. 3]).

To further assess the ability of *S. typhimurium* sdiA to complement the filamentation phenotype of an *E. coli* *ftsZ*(Ts) mutant, filamentation assays were performed. *E. coli* sdiA and *S. typhimurium* sdiA were both expressed from low-copy-number pSC101 replicons and compared directly for their abilities to complement the filamentation phenotype of an *E. coli* *ftsZ*(Ts) mutant grown at the nonpermissive temperature. Figure 4 demonstrates that *S. typhimurium* sdiA does indeed complement the filamentation phenotype, but the complementation is weak compared to that for *E. coli* sdiA.

**Isolation of sdiA-regulated genes.** To address the role of sdiA in *S. typhimurium*, we performed a systematic screen for genes regulated by sdiA using a strategy recently used to identify *rpoS*-regulated genes (11). BA612/pJVR2 (*S. typhimurium* containing sdiA under arabinose control) was mutagenized with MudJ as previously described (22) and plated on M9 minimal medium containing glucose as the carbon source (M9 glucose). A total of 10,000 random mutants from these plates were patched on M9 glucose versus M9 arabinose, with all plates containing the colorimetric β-galactosidase substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Mutants with altered β-galactosidase activities on glucose versus arabinose were streaked to isolation, and the differences in color were confirmed.

The next step was to transduce each MudJ fusion into four distinct genetic backgrounds with plasmid P22HTtim. These constructions and backgrounds are listed in Table 1. The first background was wild-type *S. typhimurium* 14028 (resulting in the BA1100 series of mutants). The second background was a simple retransduction into BA612/pJVR2 (the BA1200 series of mutants) to eliminate the possibility of secondary mutations contributing to the expression phenotype. The BA1300 series of mutants were the result of each MudJ fusion being transduced into the sdiA mutant (BA612) background. The fourth background, BA612/pBAD33 (the BA1400 series of mutants), was an isogenic vector control to show that these fusions were not regulated by arabinose or the pBAD33 vector.

As seen in Fig. 5, the 10 fusions identified in this study are expressed only in the presence of arabinose and only in the arabinose-conditioned sdiA background, not in the isogenic control background BA612/pBAD33. This demonstrates that expression of these fusions is sdiA specific and is not due to arabinose or vector sequences. Of course, fusions that failed these tests were isolated and discarded as arabinose-regulated genes.

Inverse PCR was used to clone and sequence a small portion of genome flanking each sdiA-regulated fusion as described in Materials and Methods. Database searches were performed, and the results are listed in Table 3. Seven of the MudJ mu-
tations were located within four ORFs on the S. typhimurium virulence plasmid: ORF8, ORF9, rck (resistance to complement killing), and ORF11 (Fig. 6). These ORFs are located at the 3' terminus of the previously identified pef region (5, 6, 12). We have renamed ORF8, ORF9, and ORF11 srgA, srgB, and srgC, respectively (sdiA regulated gene). Three other positively regulated fusions were also identified. These three fusions shared no similarity with current GenBank entries and are not present in the E. coli genome.

The expression of each fusion in the wild-type and sdiA mutant backgrounds was also assessed (BA1100 and BA1300 series mutants). None of the fusions demonstrated any significant activity in either background (data not shown). This experiment was performed with stationary-phase cultures grown in LB or M9 minimal medium with no activity in either case (always less than 10 U). Since 7 of 10 fusions are within a gene cluster containing rck (resistance to complement killing), we assayed the β-galactosidase activities of the rck fusion in both sdiA+ and sdiA mutant backgrounds (BA1105 and BA1305) in M9 glucose, M9 glucose plus Casamino Acids, LB, DMEM, and DMEM containing human serum. β-Galactosidase activities at time points throughout the growth curve for all cultures were assayed. In every case, less than 10 U of activity was obtained, and the residual activity was not dependent on sdiA (data not shown).

DISCUSSION

Pathogenic bacteria sense and respond to a wide variety of environmental signals during the transition from a free-living state to infection of a suitable host. Oxygen tension, osmolarity, iron availability, pH, nutrient limitation, temperature, and even specific bacterium-host physical interactions regulate expression of virulence genes (see reference 3 and references therein). Population density is emerging as yet another environmental factor that is sensed by bacterial pathogens. Most gram-negative bacteria, including pathogens, sense population density by using members of the LuxR-LuxI family of quorum-sensing components in which LuxR is the autoinducer sensor and transcriptional regulator and LuxI is the autoinducer synthase (for reviews, see references 13, 50, 54, and 58). A second type of autoinducer synthase, AinS, has also been identified (16). E. coli encodes a LuxR homolog named SdiA. In E. coli, SdiA weakly activates ftsQAZ in response to V. fischeri and Vibrio harveyi autoinducer and more dramatically in response to spent E. coli culture supernatant (53). These findings suggest that the native E. coli autoinducer is different in some way from the Vibrio autoinducers. Interestingly, upon completion of the E. coli genome sequencing project, we searched again for the quorum-sensing components LuxR, LuxI, and AinS. SdiA remains the only LuxR homolog, and there is no autoinducer synthase of any known family (LuxI or AinS). This leaves open the nature of the autoinducer (if any) produced by E. coli.

To determine whether S. typhimurium encodes a homolog of SdiA, we probed a cosmid library of the S. typhimurium genome with the E. coli sdiA gene. A 4.4-kb fragment encoding the S. typhimurium sdiA gene was cloned and sequenced. The order, spacing, and orientation of the genes adjacent to sdiA (yeeC, sirA, and uvrC) are conserved between E. coli and S. typhimurium. This gene organization may be conserved in Pseudomonas species as well, but to date only gacA (sirA) and uvrC have been sequenced (30, 47). Interestingly, the S. typhimurium yeeC, sirA, and uvrC genes all encode products that are
at least 90% identical at the amino acid level to the corresponding *E. coli* homologs. However, *sdiA* shares only 69% amino acid identity with its *E. coli* counterpart (Fig. 1 and 2). The function of *sdiA* may differ in the two organisms, resulting in rapid sequence divergence. This selective pressure could affect either autoinducer specificity or DNA recognition specificity and raised the possibility that the *S. typhimurium sdiA* gene and the *E. coli sdiA* gene are not completely interchangeable. To determine whether *S. typhimurium sdiA* could complement *E. coli sdiA*, we first tested the ability of *S. typhimurium sdiA* to activate promoter 2 of *E. coli ftsQAZ*. We found that *S. typhimurium sdiA* under control of an arabinose promoter was able to activate *E. coli ftsQAZ* by 2-fold (Fig. 3). Using a different expression system, Wang et al. found that *E. coli sdiA* activated *ftsQAZ* by 5- to 13-fold (62). Second, we tested the ability of *S. typhimurium sdiA* to suppress the filamentation phenotype of an *E. coli ftsZ(Ts)* strain grown at nonpermissive temperature. *S. typhimurium sdiA* was capable of partially suppressing filamentation of the *E. coli* strain (Fig. 4). Both assays taken together demonstrate that *S. typhimurium sdiA* can only partially complement *E. coli sdiA*.

To isolate genes regulated by *sdiA* in *S. typhimurium*, we screened 10,000 random lacZ fusions for regulation by *S. typhimurium sdiA*. Ten *sdiA*-regulated fusions were isolated. Three genes were not homologous to any sequences present in GenBank, including the recently completed *S. typhimurium* genome. The remaining seven fusions were located within a four-gene cluster on the *S. typhimurium* virulence plasmid. The four ORFs *orf8*, *orf9*, and *orf11* were adjacent to each other at the 3′ end of the previously identified *pef* region (12). We have named the genes *srgA*, *srgB*, and *srgC* to ORF8, ORF9, and ORF11, respectively. The clustering of the fusions suggests that these four genes were transcribed independently of the other genes in the *pef* region. Interestingly, *S. typhi*, the causative agent of human typhoid fever, lacks a virulence plasmid but has retained homologs of four plasmid-encoded genes in its chromosome: *pefI*, *orf7*, *orf8*, and *orf9* (48). The identification of genes not present in *E. coli* confirms the hypothesis that *sdiA* of *S. typhimurium* may not have precisely the same functions as *sdiA* of *E. coli*, possibly explaining the relatively large degree of sequence divergence.

The four-plasmid-encoded ORFs regulated by *sdiA* begin with *srgA* (ORF8), which encodes a *dsbA* homolog. DsbA is a disulfide bond isomerase involved with periplasmic protein folding (4). Several bacterial pathogens have fimbrae that require *dsbA* for proper fimbrial subunit folding and assembly (25, 55, 64). It would be reasonable to speculate that *srgA* plays some role in Pef assembly; however, mutations in *srgA* have no apparent effect on fimbrial biogenesis (12).

\[ \text{size of genomic DNA portion of inverse PCR product.} \]

### TABLE 3. *sdiA*-regulated lacZ fusions

<table>
<thead>
<tr>
<th>Strain</th>
<th>Homology/aa identity (%)</th>
<th>No. of nucleotides sequenced</th>
<th>Insertion site</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA1101</td>
<td>No homology</td>
<td>463</td>
<td>NA</td>
</tr>
<tr>
<td>BA1102</td>
<td><em>S. typhimurium</em> ORF9 (srgB)/100</td>
<td>112</td>
<td>10,603</td>
</tr>
<tr>
<td>BA1103</td>
<td><em>S. typhimurium</em> ORF8 (srg4)/100</td>
<td>111</td>
<td>10,114</td>
</tr>
<tr>
<td>BA1104</td>
<td><em>S. typhimurium</em> rck/100</td>
<td>252</td>
<td>11,412</td>
</tr>
<tr>
<td>BA1105</td>
<td><em>S. typhimurium</em> rck/100</td>
<td>234</td>
<td>11,430</td>
</tr>
<tr>
<td>BA1107</td>
<td><em>S. typhimurium</em> ORF9 (srgB)/100</td>
<td>195</td>
<td>10,519</td>
</tr>
<tr>
<td>BA1109</td>
<td><em>S. typhimurium</em> ORF11 (srgC)/100</td>
<td>136</td>
<td>12,604</td>
</tr>
<tr>
<td>BA1110</td>
<td>No homology</td>
<td>699</td>
<td>NA</td>
</tr>
<tr>
<td>BA1111</td>
<td><em>S. typhimurium</em> rck/100</td>
<td>315</td>
<td>11,350</td>
</tr>
<tr>
<td>BA1112</td>
<td>No homology</td>
<td>477</td>
<td>NA</td>
</tr>
</tbody>
</table>

- **a**: Size of genomic DNA portion of inverse PCR product.
- **b**: Nucleotide preceding Mutl insertion site according to the sequence reported in GenBank accession no. L08613. NA, not applicable.
ized ORF (gyk) in the chromosome of *E. coli* that shares 32% amino acid identity.

Rck is an outer membrane protein that is known to confer two phenotypes when expressed in *E. coli*: adhesion to eukaryotic cells and resistance to human serum (9, 19–21). During formation of the membrane attack complex on a bacterial membrane, complement component C9 is able to attach to the O antigen of LPS, but Rck prevents C9 polymerization into a complete membrane attack complex (20). Other homologs of this family include all of *Yersinia enterocolitica*, *pagC* of *S. typhimurium*, *ompX* of *E. coli* and *Enterobacter cloacae*, and *lom* of bacteriophage lambda (35, 38, 45, 57).

The final gene product, SrgC (ORF11), is homologous to several regulatory proteins of the AraC family including EnvY, AppY, and Rns (from *E. coli*) and VirF (from *Y. enterocolitica*) (12). However, a function for *srgC* in *S. typhimurium* has not been reported.

The environmental signal that SdiA recognizes is unknown, although sequence homology and evidence in *E. coli* (53) suggest that SdiA is a quorum sensor. The fact that *E. coli* does not encode a known family of autoinducer synthase makes the *E. coli* quorum-sensing system unique among gram-negative bacteria. It is possible that *S. typhimurium* also lacks an autoinducer synthase. In agreement with this, no complementation of *N*-acylhomoserine lactone biosensors has been seen in dichloromethane-extracted culture supernatants of *E. coli* and *S. typhimurium* (57a). One possible explanation for this is that *E. coli* and *S. typhimurium* simply produce a novel type of autoinducer. Another possibility is that *E. coli* and *S. typhimurium* sense autoinducers produced only by other species of bacteria.

We recently performed Northern blot experiments and gene fusion studies of *S. typhimurium* sdiA, and we know that it is expressed from the chromosome under laboratory conditions (unpublished data). So why can SdiA expressed from the chromosome not activate the genes identified in this study? One possibility is that the genes regulated by SdiA are also under the control of another regulatory protein(s). In the absence of the correct conditions for expression by these other proteins, chromosomally encoded SdiA may not be able to activate the promoters, while overexpressed SdiA is able to effectively compete with these other influences and activate transcription. Another possibility is that chromosomally encoded SdiA was unable to activate the genes because an autoinducer was not present in the assays. SdiA expressed under arabinose control may have bypassed a signal requirement by overexpression. Precedence for this hypothesis is provided by the fact that overexpression of *V. fischeri* LuxR in *E. coli* results in activation of luminescence genes in the absence of autoinducer (8).

While our hypothesis is speculative, *E. coli* and *S. typhimurium* may use quorum sensing to detect the transition from a free-living state to the intestinal environment. If this is true, detection of heterologous bacterial pheromones in the intestine would seem sufficient, and *E. coli* and *S. typhimurium* would have no need to produce their own autoinducers. The activation of rck in the intestinal environment could presumably confer adhesion to intestinal epithelium and/or prepare the bacterium for complement attack during subsequent steps of infection. We hope to use the fusions identified in this study to screen heterologous bacterial pheromones for sdiA-dependent activation of these genes.

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**FIG. 6.** ORF and mutation map of the 5’ end of the pef region (12). Black bars, ORFs (orientation is left to right for all genes); arrows, sdiA-regulated Mfd insertions.


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