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The csr regulatory system of *Salmonella* regulates the expression of the genes of *Salmonella* pathogenicity island 1 (SPI1) required for the invasion of epithelial cells. This system consists of the posttranscriptional regulator CsrA and an untranslated regulatory RNA, CsrB, that opposes the action of CsrA. Here we identify and characterize the role of a second regulatory RNA, CsrC, whose ortholog was discovered previously in *Escherichia coli*. We show that a mutant of *csrC* has only mild defects in invasion and the expression of SPI1 genes, as does a mutant of *csrB*, but that a double *csrB csrC* mutant is markedly deficient in these properties, suggesting that the two regulatory RNAs play redundant roles in the control of invasion. We further show that CsrC, like CsrB, is controlled by the BarA/SirA two-component regulator but that a *csrB csrC* mutant exhibits a loss of invasion equivalent to that of a barA or sirA mutant, indicating that much of the effect of BarA/SirA on invasion functions through its control of CsrB and CsrC. In addition to their control by BarA/SirA, each regulatory RNA is also controlled by other components of the csr system. The loss of *csrB* was found to increase the level of CsrC by sevenfold, while the loss of *csrC* increased CsrB by nearly twofold. Similarly, the overexpression of *csrA* increased CsrC by nearly 11-fold and CsrB by 3-fold and also significantly increased the stability of both RNAs.

Invasion is the process by which *Salmonella* penetrates the barrier of the intestinal epithelial layer. It requires the functions of a type III secretion system, encoded within *Salmonella* pathogenicity island 1 (SPI1) (6, 16, 17, 21, 25, 30). This island encodes a multiprotein secretion apparatus, termed a needle complex, as well as secreted effector proteins that are delivered to the cytoplasm of the epithelial cells via the needle complex (15, 19, 22). These effector proteins thus induce changes in the cell cytoskeleton that cause the bacterium to be engulfed (8, 14, 38), causing both the signs of enteric salmonellosis and the potential for the organism to reach deeper tissues (16, 21, 29, 35).

The control of the SPI1 type III secretion system is complex, with several transcriptional regulators present within the island. HilD and HilC are activators of the regulator *hilA* (13, 20, 33). HilA itself can control invasion directly by inducing expression of the secretion apparatus through control of the *inv/spa* operon and by inducing the genes that encode secreted effector proteins through control of the *sip* operon (3, 4). HilA also induces invasion indirectly by its activation of another regulator, *invF*, which itself induces the *sip* operon (3, 4, 9, 12).

Control of SPI1 also extends to global regulators encoded outside the island. One such regulator is the posttranscriptional regulatory protein CsrA. First identified in *Escherichia coli* as a regulator of carbon storage and metabolism, CsrA binds to the messages of its targets and acts to alter mRNA stability (27, 28, 32). CsrA has been shown to reduce the half-life of at least one target message and to increase the half-life of another, indicating that mRNA can be made more or less stable as the result of this interaction, depending upon the specific target (5, 34, 36). The binding site for CsrA has been shown to overlap the ribosome binding site, suggesting that CsrA may act by altering the efficiency of translation (5, 11). Although such a mechanism of regulation might appear to be generic, CsrA presumably recognizes and binds a limited set of targets, as it controls only specific functional classes of genes. In *Salmonella*, *csrA* regulates invasion and the expression of SPI1 genes as well as those required for the production of flagella and for certain pathways of carbon metabolism (1, 23). It appears that the level of CsrA must be tightly controlled to allow optimal invasion, as both loss of *csrA* and its overexpression are detrimental to invasion (1).

A second part of the csr regulatory system in *E. coli* consists of two untranslated regulatory RNA molecules, CsrB and CsrC. The two have similar predicted structures, with multiple stem-loops, and carry similar sequence motifs in the unpaired loop regions that resemble the sequence of a ribosome binding site. It has thus been proposed that CsrB and CsrC bind CsrA, titrating it from its targets (26, 37). In *Salmonella*, CsrB has been identified previously and has been shown to have 16 predicted stem-loops, each carrying the consensus sequence GWGGRHG (2). The loss of CsrB, however, produced a reduction in SPI1 gene expression much milder than that achieved through alterations in *csrA* expression and showed no discernible change in the penetration of epithelial cells (1). These findings thus suggested that there exist other methods by which CsrA is controlled, in addition to its titration by CsrB.

A second regulator known to control invasion in *Salmonella* is BarA/SirA. BarA and SirA are the respective sensor kinase and cognate response regulator that comprise a two-component regulator required for the expression of SPI1 genes (2, 20, 34). SirA can bind to the promoters of the SPI1 genes *hilA* and *hilC* and thus presumably directly activates these genes (34). It can, however, also bind to *csrB*, and both BarA and SirA have been...
shown to be required for the expression of csrB (24, 34). Thus, BarA/SirA works, at least in part, through its control of the csr system.

Although the second regulatory RNA, CsrC, has been identified in E. coli, it has not yet been described in Salmonella. Here, we identify CsrC and describe its function. We show that the cumulative effects of the loss of both CsrB and CsrC significantly reduce SPI2 gene expression and epithelial cell invasion. We further show that CsrB and CsrC are both controlled by BarA/SirA and that control of these two regulatory RNAs provides an important pathway by which BarA/SirA regulates invasion. We also demonstrate that control of the csr system is complex, with levels of each regulatory RNA, CsrB and CsrC, being altered by changes in expression of the other and by the expression of CsrA.

MATERIALS AND METHODS

Strains and growth conditions. Strains and plasmids used in this study are shown in Table 1. Growth conditions used for each assay are described below. When required, plasmids were maintained by the addition of ampicillin to the medium at a concentration of 100 μg/ml. The csrC mutant was constructed by first identifying a region of the Salmonella enterica serovar Typhimurium LT2 genome homologous to E. coli csrC. We then created a marked deletion of this region using a one-step inactivation method (10). PCR primers were designed to allow the amplification of the chloramphenicol resistance marker from plasmid pKD3 and with 40 bases of homology to the regions immediately flanking the predicted csrC region. Primers used were 5'-GATACTTTGAGTACCCCAAAAGGATCAAAGTATGCTAGGCTGGAGCTGCTTC and 5'-GGTATCTTGAGTTTACCCCA and were cloned into the pKD46, which encodes λ Red recombination, which provides for allelic exchange. This created a 253-bp deletion encompassing positions 1191 to 1443 inclusive of GenBank sequence AE088887 and the replacement of this region with a chloramphenicol resistance marker. Candidate mutants were tested for loss of the appropriate region by PCR amplification. All strains carrying combinations of mutations were constructed by P22 transduction. The csrC mutant was complemented in single copy by integration of the wild-type gene under P<sub>barA</sub> and by the expression of CsrC.

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<th>Strain or plasmid</th>
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Plasmids

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<td>csrB&lt;sup&gt;+&lt;/sup&gt;; Ap&lt;sup&gt;+&lt;/sup&gt;</td>
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Strains and plasmids used described previously (28a). Cultures were grown in LB medium supplemented with 100 mM HEPES, pH 8.

Northern analysis and RNA stability assays. All strains were grown overnight with aeration in LB broth. Bacteria were then subcultured 1:50 and grown to late log phase (optical density at 600 nm [OD<sub>600</sub>] of 0.8) in LB supplemented with 100 mM HEPES, pH 8. Strains with the plasmid pCA114, which carries csrC under the control of the araBAD promoter on pBAD18, were, in addition, grown with ampicillin and either 0.2% glucose for promoter repression or 0.2% arabinose for promoter induction. To 1 ml of each culture was added 100 μl of stop solution (10% buffer-saturated phenol in ethanol), and total RNA was isolated using an SV total RNA isolation system kit (Promega) according to the manufacturer's protocol. RNA concentration was determined by measuring the OD<sub>260</sub> and 5 μg of total RNA from each sample was separated by electrophoresis using an agarose gel containing 9.25% formaldehyde. RNA was transferred to a nylon membrane (Roche) and fixed by UV cross-linking. Prehybridization and hybridization were performed using Roche Easy Hyb granules at 42°C with gentle agitation. The membrane was hybridized overnight with digoxigenin-dUTP-labeled probes from either the 289-bp csrB region (2) or a 325-bp region that encompasses csrC, created using the primers 5'-CCGCAATCTGGTGCGCATG and 5'-GGTATCTTGAGTTTACCCCA and was cloned into the pKD46, which encodes λ Red recombination, which provides for allelic exchange. This created a 253-bp deletion encompassing positions 1191 to 1443 inclusive of GenBank sequence AE088887 and the replacement of this region with a chloramphenicol resistance marker. Candidate mutants were tested for loss of the appropriate region by PCR amplification. All strains carrying combinations of mutations were constructed by P22 transduction. The csrC mutant was complemented in singly copy by integration of the wild-type gene under P<sub>barA</sub> and by the expression of CsrC.

β-Galactosidase assays. Triplicate cultures of each bacterial strain to be assayed were grown standing at 37°C and assayed for β-galactosidase activity as

after rifampin treatment, and 100 μl of stop solution was added to each culture. RNA isolation and detection were performed as described above using 5 μg of total bacterial RNA for each time point.

**RNA half-life determination.** Wild-type and ΔcsrA strains were grown overnight with aeration in LB broth. Bacteria were then subcultured 1:50 and grown to stationary phase (OD600 of 1.2) in LB broth. The production of new RNA was halted with rifampin (500 μg/ml). Samples were collected at 0, 1, 3, 5, 8, and 15 min after rifampin treatment, and 100 μl of stop solution was added to 1-ml aliquots of culture. Total RNA was isolated as above, and samples were treated with DNase according to the manufacturer’s directions (Promega). cDNA was produced by reverse transcription using Superscript II (Invitrogen) from equal concentrations of RNA. To detect CsrC, multiplex PCR amplification was performed with primers specific to csrC, 5′-GCCGTAAGGTCACAGGAAAA and 5′-AAATCTGGCGAGAATAA, and to the control gene icd, 5′-CCGGTAAGGTCACAGGAAAA and 5′-GCGCTGAAACCCTTGATTTA and 5′-ATTCAATTCGGCGTAGATG, in the same reaction. The products were measured by densitometry using a Lumi-Imager, and decay was plotted using linear regression (Microsoft Excel 2003). The mean of three independent trials was used to determine the half-life of CsrC.

**RNA secondary structure prediction.** RNA secondary structure was analyzed with the program RNADraw 1.01, which uses the algorithm of Zuker and Stiegler (39).

**Statistical analysis.** For β-galactosidase and invasion assays, a one-way analysis of variance was used to determine whether the mean of at least one strain differed from that of any of the others. Then, multiple comparison tests (least square difference t test at a P level of ≥0.05) were used to determine which means differed (SAS System for Windows 8).

**RESULTS**

**Effects of CsrC and CsrB on Salmonella invasion.** The csr regulatory system, comprised of the posttranscriptional regulator CsrA and the untranslated regulatory RNA CsrB, has been shown to control the invasion of intestinal epithelial cells in *Salmonella enterica* serovar Typhimurium (1, 2, 34). Recently, a second regulatory RNA, termed CsrC and unlinked to CsrB, was identified in *E. coli* and was postulated to play a role similar to that of CsrB (37). To determine whether *Salmonella* carries a csrC ortholog, we examined the sequence of *Salmonella* serovar Typhimurium strain LT2 for nucleotide similarities to *E. coli* csrC. We first identified a region at centisome 87 of the chromosome with 88% identity to the reported *E. coli* csrC sequence. In *E. coli*, RNA secondary structure analysis predicted a CsrC molecule with nine stem-loops having conserved sequence within the unpaired portions of the loops (37). A similar analysis of the *Salmonella* serovar Typhimurium CsrC predicted it to have eight such structures, along with a rho-independent transcriptional terminator (Fig. 1). Of these eight stem-loops, seven had a pair of guanine residues in the loop portion, and four of these carried the sequence AGGA, a motif found in *E. coli* CsrC and in CsrB from both *E. coli* and *Salmonella*. This motif is also similar to a ribosome binding site thought to be the recognition site of CsrA for its target messages.

To investigate whether CsrC played any role in the control of *Salmonella* invasion, we made a deletion of csrC in the virulent strain ATCC 14028s and tested the ability of the resulting mutant to penetrate cultured epithelial cells. We found that invasion of HEp-2 cells was significantly reduced in the csrC mutant but that the reduction was modest, to a level 54% of that of the wild type (Fig. 2). This invasion defect was similar in magnitude to that produced by the loss of csrB. However, the invasion of HEp-2 cells was significantly reduced in the csrC mutant but that the reduction was modest, to a level 54% of that of the wild type (Fig. 2). This invasion defect was similar in magnitude to that produced by the loss of csrB. However, the invasion of HEp-2 cells was significantly reduced in the csrC mutant but that the reduction was modest, to a level 54% of that of the wild type (Fig. 2).
A wild-type strain carrying the plasmid pCA114, which

mutant with this construct was equal to that

using the wild-type csrC (data not shown), indicating that produc-

duction of this protein was not required for the activity of CsrC and

supporting the role of CsrC as a functional RNA.

Control of CsrC and CsrB. It has previously been shown that

csrB is induced by both BarA and SirA (24, 34). As our findings

indicated that both CsrB and CsrC participate in the control of

SPI1, we next determined whether the BarA/SirA two-compo-

nent regulator also controls csrC expression. Northern analysis

showed that the level of CsrC was reduced fivefold in a barA

mutant or in a sirA mutant (Fig. 4A). Consistent with previous

findings, levels of CsrB were also reduced fivefold in mutants

of barA and sirA (Fig. 4B). Thus, the BarA/SirA two-compo-

nent regulator positively controls both of the known regulatory

RNAs of the csr system.

CsrA is a posttranscriptional regulator that binds to its target

messages and alters their half-lives. It has been previously

shown in E. coli that CsrA positively regulates CsrC, but the

method of this action is postulated to be indirect, rather than

by alteration of the stability of the CsrC regulatory RNA by

CsrA (37). To determine whether CsrA could control the level of

CsrC in Salmonella, we next examined the level of CsrC in response

to altered expression of csrA using Northern analysis. Using a wild-type strain carrying the plasmid pCA114, which

has csrA under the control of the arabinose-inducible araBAD

promoter, we found that overexpression of csrA significantly

increased the levels of both CsrC and CsrB. When csrA was

expressed from this arabinose-inducible promoter, the addition

of arabinose to the culture medium resulted in a 10.8-fold

increase in CsrC (Fig. 4A) and a 3-fold increase in CsrB (Fig.

4B). Repression of csrA from this same plasmid by growth in

glucose produced a level of CsrC indistinguishable from that of

wild type and a level of CsrB approximately 60% of that found

in the wild type.
Because CsrA is known to bind to its target messages and alter their half-lives, we next sought to determine whether the stability of CsrB or CsrC could be changed in response to altered levels of CsrA. We identified changes in the stability of CsrB and CsrC in cultures grown to mid-log by halting the production of all RNA with the RNA polymerase inhibitor rifampin and then measuring the levels of each at time points thereafter using Northern analysis. As shown in Fig. 5A, the concentration of CsrC was reduced in the csrA null mutant, but there was no detectable change in the rate of decay of CsrC. Overexpression of csrA, however, did significantly increase the longevity of CsrC. The induction with arabinose of csrA on pCA114 under the control of the arabinose-inducible promoter caused a significant increase in the stability of CsrC, while repression of csrA by growth in glucose produced CsrC with a stability no different from that of wild type (Fig. 5A). Similarly, the stability of CsrB was also increased by csrA overexpression but was not reduced in the csrA null mutant (Fig. 5B). Thus, csrA, at least when it is overexpressed, can lead to increased stabilities of both CsrC and CsrB.

As CsrA can bind to its two regulatory RNAs, presumably titrating the protein, the levels of CsrB or CsrC might also be affected by the loss of the other regulatory RNA by providing a greater concentration of unbound and active CsrA. To test this prediction, we examined the effects of deletions of csrB or csrC on the level of the other RNA. We found that the loss of csrB increased the concentration of CsrC by 7.1-fold (Fig. 4A), while the loss of csrC increased CsrB by 1.9-fold. Therefore, levels of the two regulatory RNAs can each be changed by altering the concentration of free CsrA both directly, through its overexpression, and indirectly, by elimination of an RNA to which CsrA can bind.

One plausible explanation for these findings is that CsrA binds to and stabilizes CsrB and CsrC. Thus, the loss of CsrA would reduce the levels of both CsrB and CsrC, while the loss of either CsrB or CsrC would increase the pool of CsrA available to bind to the remaining regulatory RNA, further stabilizing it. As a null mutation of csrA had little effect on either CsrB or CsrC stability, we postulated that the level of CsrA present in the mid-log culture (OD_{600} of 0.8) from which the

![Figure 3](image_url)
RNA was extracted might be too low to allow an observable change. Indeed, in *E. coli*, levels of CsrA and CsrB increase as cultures reach stationary phase (18), and we found by reverse transcription-PCR that expression of *csrA* in *Salmonella* increases with increasing culture density (data not shown). We therefore next measured changes in the half-lives of CsrB and CsrC due to the loss of *csrA* at a later point in the growth cycle (OD$_{600}$, 1.2) and using reverse transcription-PCR, a more sensitive means to detect alterations in RNA levels. We halted RNA production in cultures with rifampin, isolated total RNA at time points, reverse transcribed the RNA pool to create cDNA, and then PCR amplified using primers specific to either *csrB* or *csrC*. As a control, we similarly amplified *icd*, encoding isocitrate dehydrogenase, a gene we had previously determined by microarray analysis not to have altered expression in a *csrA* mutant (not shown). Using this assay, we found that the stability of CsrC in the *csrA* mutant was greatly reduced (Fig. 6). The half-life of CsrC in the wild type was calculated to be 10.9 min but was reduced to 0.5 min in the *csrA* mutant. We could not, however, detect a difference in the half-life of CsrB due to the loss of *csrA* (not shown). These findings therefore suggest that CsrA stabilizes CsrC, but they fail to show the means by which CsrA affects the concentration of CsrB.

**DISCUSSION**

The csr regulatory system of *Salmonella* controls a number of functions, including the expression of SPI1 genes and the invasion of epithelial cells, the production of flagella, and the utilization of specific nutrient sources (23). This system was previously known to consist of the protein regulator CsrA and the untranslated regulatory RNA CsrB, which opposes the action of CsrA (1, 2). Here we have shown that a second regulatory RNA previously identified in *E. coli*, CsrC, exists in *Salmonella* and is an equally important component of the csr system for the control of epithelial cell invasion. The loss of either of the two regulatory RNAs alone produced only mild defects in invasion, but the loss of both together resulted in a much more severe defect. This suggests that the two have similar functions and play redundant roles in the bacterium. Supporting this contention is the proposed structure of the two molecules. Each is predicted to consist of multiple stem-loop structures that may present binding sites for CsrA, titrating it from its intended targets.

Both csrB and csrC are positively controlled by BarA/SirA, a two-component regulator required for invasion, suggesting the following model for control by the csr system (Fig. 7). Within the intestinal tract of an animal host, BarA/SirA is activated, either in response to high concentrations of acetate or by an as-yet-unidentified signal for BarA activation (24). SirA then activates both csrB and csrC. The activation of csrB by SirA is likely to be direct, as SirA has been shown to bind to csrB DNA (34). It is not yet known, however, whether SirA activates csrC by the same means. Once produced, the two regulatory RNAs bind and titrate CsrA. The reduction in free CsrA leads to the
induction of SPI1 genes, although the direct target, or targets, of CsrA in the invasion pathway are not known. It is possible that CsrA has direct targets within SPI1, but it is also possible that CsrA affects the message stability of one or more regulators outside SPI1 that affect the expression of invasion genes. Thus, it is likely that conditions that induce invasion cause a reduction in the concentration of free CsrA within the bacterium. It is also known, however, that both the loss of CsrA and its overproduction can reduce the expression of SPI1 genes (1), suggesting that the concentration of CsrA must be tightly controlled to produce maximal invasion.

In addition to its control of the csr system, BarA/SirA likely also directly activates the expression of SPI1 genes. SirA has been shown to bind to two central regulators of SPI1, hilA and hilC, and so presumably induces the expression of numerous SPI1 genes through their control (34). Results shown here, however, suggest that a large part of the control of invasion by BarA/SirA is manifested through control of the csr system, rather than by the direct control of SPI1. A mutant of both csrB and csrC had a defect in invasion and SPI1 gene expression similar to that of either a barA or sirA mutant, and the loss of barA in a csrB csrC double mutant engendered no additional invasion defect. As the other known targets of SirA in the control of invasion, hilA and hilC, were intact in these tests, these findings suggest that control of the csr system provides the primary route for control of invasion by BarA/SirA.

This work also shows that each of the two regulatory RNAs can be controlled by other components of the csr system. The overproduction of CsrA increased both the levels and the stability of CsrC and CsrB. Further, the loss of either CsrB or CsrC increased the concentration of the other regulatory RNA. One model by which these findings can be explained holds that CsrA binds to and stabilizes each of the regulatory RNAs. Thus, overexpression of CsrA provides a greater binding capacity, while the loss of one of the RNAs allows greater binding of CsrA to the remaining RNA. Consistent with this model is the finding that the loss of CsrA greatly reduced the half-life of CsrC. As CsrA is known to affect the stability of its targets, the change of stability of CsrC would suggest a direct method of control by CsrA. We were, however, not able to demonstrate a similar change in stability of CsrB, perhaps suggesting that its control by CsrA is instead not the result of direct binding. This work also shows that the concentration of CsrC changes more in response to alterations in CsrA and CsrB than does the concentration of CsrB in response to changes in CsrA and CsrC. It is possible that CsrB exists in higher concentrations in the wild-type bacterium, and so its loss has more profound effects on other elements of the system. Alternatively, CsrB may exert a greater influence by the nature of its structure. It carries a predicted 16 stem-loops for CsrA binding, while CsrC has only 8 (Fig. 1), and thus its loss

**FIG. 6.** Half-life of CsrC. Production of RNA was halted with rifampin in wild-type and csrA mutant strains grown to an OD_{600} of 1.2. Samples were collected at 0, 1, 3, 5, 8, and 15 min after rifampin treatment, total RNA was isolated and treated with DNase, and cDNA was produced by reverse transcription. To detect CsrC, multiplex PCR amplification was performed with primers specific to csrC and to the control gene icd in the same reaction. The products were measured by densitometry using a Lumi-Imager, and decay was plotted using linear regression (Microsoft Excel 2003). The lower panel shows the mean intensity at each time point for three independent trials, with error bars representing standard errors of the means. Half-life was calculated using the 0- and 15-min time points for the wild type and the 0- and 3-min time points for the csrA mutant, the latest time at which a product could be detected. The upper panel shows a representative PCR.

**FIG. 7.** Model of SPI1 regulation. SirA is activated either by the sensor kinase BarA or independently of BarA by acetate. SirA activates transcription of the regulatory RNAs CsrB and CsrC, which titrate CsrA and thus oppose its action. CsrA can act either positively or negatively on SPI1 genes, depending upon its concentration, and serves to increase the concentrations of both CsrB and CsrC. In addition to its control of the csr system, SirA also likely has direct effects on SPI1 gene expression.
may lead to a higher concentration of free CsrA and consequent effects. Finally, it is possible that the interaction between CsrA and the regulatory RNA molecules affects changes in their concentrations. CsrA alters the half-life of CsrC (Fig. 6) but does not appear to do so for CsrB. Thus, it may be that CsrA regulates CsrC by binding and stabilizing it, while CsrA regulates CsrB by an indirect mechanism.

The csr system was first identified in E. coli, and all three of the essential components, CsrA, CsrB, and CsrC, have close homologs in Salmonella serovar Typhimurium. In E. coli, the system is known to function in the control of carbon metabolism, motility, and cell surface properties (31). In Salmonella, the csr system controls motility as well but has also adapted to regulate functions not found in E. coli, including invasion and the metabolism of specific carbon sources (23). Regulation of the system also has elements in common between the two species. In both, the two-component regulator BarA/SirA (BarA/UvrY in E. coli) is required for expression of CsrB and CsrC, and the levels of both of the regulatory RNAs are affected by CsrA. In E. coli, however, this control of CsrB and CsrC is proposed to be at the level of transcription, as CsrA has not been found to alter the stability of either regulatory RNA but does alter the expression of lacZ transcriptional fusions to each (37). In contrast, we have reported here that the loss of CsrA reduced the half-life of CsrC, but not CsrB. In remains possible that Salmonella CsrA also regulates CsrB and CsrC by controlling their transcription, but these findings indicate as well that CsrA increases the longevity of CsrC by stabilizing it.

The csr system provides a complex means to control the expression of invasion and important metabolic functions in Salmonella. It remains unknown, however, why this system would require two regulatory RNAs, CsrB and CsrC, that have such similar structures, functions, and mechanisms of control. It is possible that, in addition to control by BarA/SirA, each also responds to additional genetic regulators, allowing the integration of multiple environmental signals. It is also plausible that the integrated effects of these two regulators could produce a finely controlled level of CsrA required for the differential expression of target genes. It is known that invasion is repressed both by the loss of csrA and its overexpression, suggesting that levels of CsrA must be tightly controlled (1). It is therefore possible that other members of the csr regulon require differing levels of CsrA to achieve optimal expression and that the concentrations of CsrC and CsrB within the bacterium provide this fine control.

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