Post-Transcriptional Regulation by the Csr Global Regulatory System in *Escherichia coli*

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Summary

In many species of bacteria, the Csr (carbon storage regulator) global regulatory system coordinates the expression of various genes. In *Escherichia coli*, the central component of this system, CsrA, is a RNA-binding protein. The CsrA is a homodimer and binds to leader segments of target mRNAs, affecting their translation and stability. CsrA activity is regulated by two small non-coding RNAs, CsrB and CsrC. These RNAs contain multiple CsrA-binding sequences and act by sequestering CsrA. In this system, CsrA indirectly activates transcription of *csrB* and *csrC* through a two-component signal transduction system, BarA/UvrY. Another component of this system, CsrD, controls the degradation of CsrB and CsrC RNAs. CsrD contains GGDEF and EAL signaling domains, however, unlike typical GGDEF and EAL domain proteins, its activity does not involve cyclic di-GMP metabolism. The dramatic stabilization of CsrB and CsrC RNAs in a *csrD* mutant altered the expression of CsrA-regulated genes. The Csr components, CsrA, CsrB and CsrC RNAs, and CsrD, interact within the autoregulatory circuit that provides a homeostatic mechanism for control of CsrA activity.

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Bacteria possess regulatory systems that permit them to recognize and adapt to environmental changes such as nutrient availability. In *Escherichia coli* and related species, the transition from exponential growth into stationary-phase growth is accompanied by striking physiological changes (Hengge-Aronis, 1996 ; Huisman *et al.*, 1996). In many species of bacteria, the Csr (carbon storage regulator) and homologous Rsm (repressor of stationary phase metabolites) systems coordinate the expression of various genes that facilitate adaptation among major physiological phases of growth ; e.g., exponential versus stationary phase, planktonic versus biofilm, and ostensibly acute versus chronic states of infection (Romeo, 1998 ; Wei *et al.*, 2001 ; Jackson *et al.*, 2002 ; Goodman *et al.*, 2004 ; Majdalani *et al.*, 2005). Csr (Rsm) systems are characterized by an RNA-binding protein that post-transcriptionally activates or represses expression of target mRNAs and small non-coding RNAs that function as antagonists of the protein (Romeo, 1998).

In *E. coli*, three major components of the Csr system include the RNA-binding protein CsrA and two small non-coding RNA molecules, CsrB and CsrC (Romeo *et al.*, 1993 ; Liu *et al.*, 1997 ; Liu and Romeo, 1997 ; Weilbacher *et al.*, 2003). The central component of this system, CsrA, is a 61-amino-acid RNA-binding protein. CsrA represses gluconeogenesis, glycogen biosynthesis and catabolism, peptide transport, and biofilm formation (Romeo *et al.*, 1993 ; Sabnis *et al.*, 1995 ; Yang *et al.*, 1996 ; Jackson *et al.*, 2002 ; Dubey *et al.*, 2003), while it activates glycolysis, acetate metabolism, and flagellum biosynthesis (Sabnis *et al.*, 1995 ; Wei *et al.*, 2000 ; Wei *et al.*, 2001). The Pfam database (http://www.sanger.ac.uk/Software/Pfam/) includes 198 CsrA homologues (accession number, PF02599) among 141 different eubacterial species and one *Pseudomonas* phage with several species encoding more than one homologue. CsrB and CsrC function as antagonists of CsrA by sequestering this protein and preventing its ability to interact with mRNA targets. The multiple imperfect repeat sequences (18 in CsrB and nine in CsrC) primarily within the loops of predicted stem-loop structures in these regulatory RNAs function as CsrA-binding sites (Liu *et al.*, 1997 ; Gudapaty *et al.*, 2001 ; Weilbacher *et al.*, 2003). Interaction of CsrA with these sites leads to its sequestration (Liu *et al.*, 1997 ; Weilbacher *et al.*, 2003 ; Dubey *et al.*, 2005).

The Csr components in *E. coli* interact within an autoregulatory circuit that provides a homeostatic mechanism for control of CsrA activity. In this system, CsrA regulates the transcription of *csrB and csrC* via a two-component signal transduction system, BarA/UvrY (Gudapaty *et al.*, 2001 ; Suzuki *et al.*, 2002 ; Weilbacher *et al.*, 2003). A previous study had suggested the presence of at least one additional undefined regulator of *csrB* expression (Suzuki *et al.*, 2002). A mutation that decreased *csrB-lacZ* expression was isolated within a gene, *csrD* (formerly *yhdA*) (Suzuki *et al.*, 2006). The *csrD* gene encodes a member of a large family of proteins that contain GGDEF and EAL signaling domains (for reviews, see D'Argenio and Miller, 2004 ; Jenal, 2004 ; Römling *et al.*, 2005). In various species, GGDEF and EAL proteins affect production of exopolysaccharides and surface proteins, and influence adhesion, motility, biofilm formation, and host-pathogen interactions (D'Argenio and Miller, 2004 ; Hisert *et al.*, 2005). A number of GGDEF and EAL domain proteins are known to synthesize and hydrolyze, respectively, bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), a secondary messenger (e.g., see Hickman *et al.*, 2005 ; Hisert *et*

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al., 2005 ; Ryjenkov *et al.*, 2005 ; Schmidt *et al.*, 2005 ; Simm *et al.*, 2005 ; Camilli and Bassler, 2006). CsrD regulated the degradation of CsrB and CsrC RNAs. The dramatic stabilization of CsrB and CsrC RNAs in a *csrD* mutant altered the expression of CsrA-controlled genes via the Csr regulatory circuitry (Suzuki *et al.*, 2006). The new member of the Csr system CsrD is not involved in c-di-GMP metabolism, but rather appears to target the CsrB and CsrC RNAs for degradation by RNase E (Suzuki *et al.*, 2006).

This review will present information about the function and mechanism of the Csr system in *E. coli*.

Discovery of CsrA as a repressor of glycogen synthesis in *E. coli*

The ability of microorganism to respond rapidly to changes in the environment is crucial for their survival. During the transition from exponential growth to stationary phase, a few global regulatory factors mediate many extensive changes in gene expression. One of the metabolic pathways activated in the stationary phase is the glycogen biosynthesis pathway, which may provide carbon and energy to promote survival in the stationary phase (Preiss and Romeo, 1989 ; Preiss and Romeo, 1994). Glycogen biosynthesis is regulated via several global systems, including cyclic AMP (cAMP), the cAMP receptor protein (CRP), guanosine 3'-bisphosphate 5'-bisphosphate (ppGpp), and stationary-phase sigma factor (σ^S) encoded by *rpoS* (Romeo and Preiss, 1989 ; Romeo *et al.*, 1990 ; Hengge-Aronis and Fischer, 1992). The cAMP-CRP and ppGpp are positive regulators of *glgC* (encoding ADPglucose pyrophosphorylase) expression (Romeo and Preiss, 1989 ; Romeo *et al.*, 1990). Romeo et al. (1993) found that the glycogen excess mutation TR1-5 led to increased expression of the *glgC* gene. The mutated gene was designated *csrA* for carbon storage regulator (Romeo *et al.*, 1993). The effect of *csrA* on glycogen biosynthesis was mediated independently of cAMP-CRP and ppGpp. The *csrA* gene encoded a global regulator and was originally shown to have pleiotropic effects on glycogen biosynthesis, gluconeogenesis, cell size, and surface properties (Romeo *et al.*, 1993).

The *csrA* gene encodes a 61-amino-acid polypeptide (Romeo *et al.*, 1993). The *in vivo* studies indicated that CsrA facilitates the decay of *glgCAP* mRNA, which encodes two enzymes required for glycogen biosynthesis and the glycogen phosphorylase (Liu *et al.*, 1995). These transcripts exhibited rapid decay rates in the wild-type strain but were extremely stable in the *csrA* mutant. A cis-acting site for CsrA was located between nucleotides -18 and +31 of the *glgC* coding region (Liu *et al.*, 1995). The *glg* mRNA destabilization by CsrA resulted in the repression of *glgCAP*.

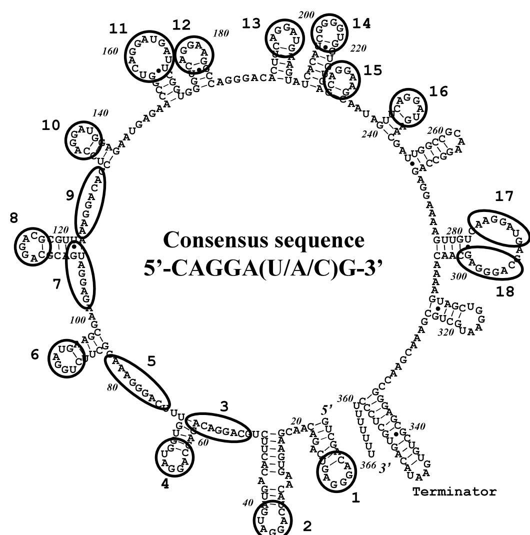

Fig. 1. Predicted secondary structure of CsrB RNA and CsrA binding sequences.

CsrB is a 366-nucleotide long, small non-coding RNA. The repeated CsrA binding sequence elements, numbered 1-18, are shown in a circle. The consensus repeated sequence is 5'-CAGGA (U/A/C) G-3'.

Small non-coding RNAs, CsrB and CsrC

Liu et al. (1997) discovered CsrB RNA when they purified a recombinant CsrA protein, CsrA-His_6. The purified protein contained approximately 18 CsrA-His_6 subunits and a single 366-nucleotide RNA, CsrB containing no open reading frame (Liu *et al.*, 1997). CsrB RNA binds to ~18 CsrA subunits, forming a large globular ribonucleoprotein complex. The 18 imperfect repeated nucleotide sequences (5'-CAGGA (U/A/C)G-3') are located in loops of predicted hairpins and other single-stranded regions between the hairpins of CsrB RNA (**Fig. 1**) (Liu *et al.*, 1997). The observation that about 18 CsrA proteins interact with a single CsrB molecule in the CsrA-CsrB complex strongly suggested that this repeated sequence constitutes the CsrA binding element. The purified CsrA-CsrB complex and RNA-free CsrA were active in regulating *glg* gene expression *in vitro* (Liu *et al.*, 1997). Overexpression of *csrB* enhanced glycogen accumulation in *E. coli*. These results have revealed that CsrB RNA functions as an antagonist of CsrA, apparently by sequestering this protein (**Fig. 2**) (Liu *et al.*, 1997 ; Romeo, 1998).

A second small non-coding RNA, CsrC, was discovered using a genetic screen for factors that regulate glycogen biosynthesis (Weilbacher *et al.*, 2003). The original *csrC* clone was isolated as a result of its stimulatory effects on glycogen accumulation and was shown to activate *glgC'-'lacZ* expression (Romeo *et al.*, 1991). The 245-nucleotide long CsrC RNA also contains nine conserved sequences mainly in predicted single-stranded loops or bulges of the molecule, which resemble the repeated sequences of CsrB RNA. CsrC RNA binds multiple copies of CsrA and antagonizes the regulatory effects of CsrA, presumably by sequestering this protein (Weilbacher *et al.*, 2003). Quantitative gel mobility shift assays with CsrB and CsrC transcripts demonstrated that the affinity of CsrA for CsrB RNA is ~10-fold higher than that for CsrC RNA (Weilbacher *et al.*, 2003). CsrC binds specifically to CsrA, although with less affinity than CsrB,

and antagonizes the regulatory effects of CsrA.

The half-lives of both CsrB and CsrC are relatively short (~2 min) and should allow CsrA activity to respond rapidly to conditions that alter CsrB or CsrC levels (Gudapaty *et al.*, 2001 ; Weilbacher *et al.*, 2003). This system employs a mechanism distinct from that of other small RNAs such as OxyS and RyhB, which involve RNA-RNA base pairing (Gottesman, 2004).

Repression or activation of target genes by CsrA

CsrA represses gluconeogenesis, glycogen biosynthesis and catabolism, peptide transport, and biofilm formation (Romeo *et al.*, 1993 ; Liu *et al.*, 1995 ; Yang *et al.*, 1996 ; Jackson *et al.*, 2002 ; Dubey *et al.*, 2003). The mechanism of negative regulation has been examined in three cases. For the *glgC* gene, CsrA binds specifically to two RNA segments in the *glgCAP* leader transcript (Baker *et al.*, 2002). One of these binding sites overlaps the *glgC* Shine-Dalgarno (SD) sequence, whereas the other CsrA target is located further upstream within a short hairpin structure (**Fig. 2**). The short hairpin sequence with GGA in the loop is similar to the repeated CsrB sequence. Bound CsrA prevents ribosome binding and this inhibits translation. Both CsrA-RNA and CsrA-CsrA interaction are involved in the formation of the CsrA-*glgCAP* leader RNA complexes (**Fig. 2**). Two kinds of deletion mutations in the hairpin reduced the affinity for CsrA *in vitro* and CsrA-dependent regulation *in vivo*. CsrA binding to the upstream target is necessary for proper *glgC* regulation. A previous study established that CsrA promotes *glgCAP* operon mRNA decay (Liu *et al.*, 1995). The binding of CsrA to the *glgCAP* leader blocks access of the ribosome to the *glgC* ribosome-binding site. CsrA inhibits initiation of *glgC* translation. It is likely that CsrA-mediated translational control of *glgC* contributes to the accelerated decay of *glg* mRNA (**Fig. 2**).

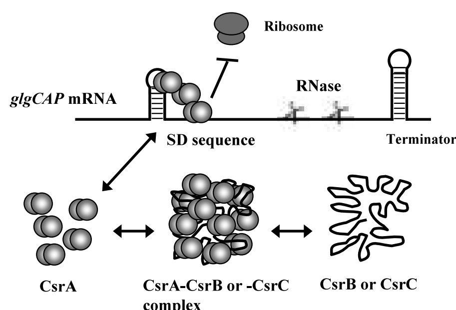

Fig. 2. Model of CsrA-mediated post-transcriptional regulation of *glgCAP*.
CsrA inhibits translation of *glgC* by binding to the *glgCAP* leader transcript. There are two binding sites, one is within the short hairpin and the other overlaps the *glgC* SD sequence. CsrB and CsrC RNAs function as an antagonist of CsrA by sequestering this protein.

The upstream CsrA binding site was used to search the *E. coli* genomic sequence for other genes that might be regulated by CsrA. This sequence (GCACACGGAU) overlapped the *cstA* SD sequence (Dubey *et al.*, 2003). *cstA* is a cAMP-dependent carbon starvation response gene and encodes a peptide transporter (Schultz and Matin, 1991). A genetic analysis of the expression of a *cstA'-'lacZ* translational fusion and the coupled-transcription-translation experiments demonstrated that expression of *cstA* is repressed by CsrA (Dubey *et al.*, 2003). Gel mobility shift results showed that CsrA binds specifically to *cstA* mRNA. There were three or four CsrA-binding sites, one of which overlaps the *cstA* SD sequence. CsrA regulates translation initiation of *cstA* by blocking ribosomal access to the *cstA* ribosome-binding site (Dubey *et al.*, 2003).

Biofilm development is guided by several regulatory systems in *E. coli*. The Csr system has a marked effect on the biofilm formation (Jackson *et al.*, 2002). CsrA represses biofilm formation. The *pgaABCD* locus affects biofilm development and all of them are required for optimal biofilm formation (Wang *et al.*, 2004). The *pga* genes encode envelop proteins involved in the synthesis, translocation, and possibly surface docking of poly-β-1,6-GlcNAc (PGA). CsrA is shown to regulate biofilm formation by post-transcriptionally repressing PGA, and PGA synthesis is necessary for CsrA (Wang *et al.*, 2005). Quantitative gel mobility shift assays demonstrated that CsrA bound specifically to *pgaA* mRNA. CsrA destabilizes the *pgaA* transcript *in vivo* (Wang *et al.*, 2005). There were six apparent CsrA-binding sites in the *pgaABCD* leader transcript. This is the most extensive arrangement of CsrA-binding sites yet observed within an mRNA leader, and provides for relatively strong repression *in vivo*. One of the CsrA-binding sites overlaps the initiation codon. Substitution mutations in CsrA-binding sites overlapping the SD sequence and the initiation codon partially relieved the repression by CsrA. It appears that CsrA inhibits translation of *pgaA* by a mechanism that is related to those used for translational inhibition of *glgC* and *cstA* (Wang *et al.*, 2005).

CsrA activates glycolysis, motility, and acetate metabolism (Wei *et al.*, 2000 ; Wei *et al.*, 2001). The mechanism of positive regulation of CsrA has been examined in one case. Motility and chemotaxis permit bacterial cells to move away from stressful environments and toward nutrients, O_2, light, or other stimuli. Motility is also required for biofilm formation. Bacteria move towards or away from specific stimuli using flagella. In *E. coli* and related species, motility is regulated by several global regulatory circuits, which converge to modulate the overall expression of the master operon for flagellum biosynthesis, *flhDC* (Liu and Matsumura, 1994). The *csrA* mutants were non-motile because of the absence of flagella and a plasmid clone of the *csrA* gene complemented the motility defect (Wei *et al.*, 2001). The *flhDC'-'lacZ* translational fusion in the chromosome was expressed at three- to four-fold higher levels in wild-type strains than in *csrA* mutants. The coupled

transcription-translation of the fusion in S-30 extracts was stimulated by purified CsrA protein. The CsrA protein specifically bound to the 5'-leader sequence of *flhDC* mRNA in RNA mobility shift assays. The *flhDC* message levels were approximately three-fold higher and were more stable in *csrA* wild-type strain than in isogenic *csrA* mutants (Wei *et al.*, 2001). The CsrA protein binds to the 5'-leader sequence of *flhDC* mRNA and stabilizes its mRNA. The precise mechanism by which CsrA binds and stabilizes *flhDC* mRNA remains to be defined.

Interaction of CsrA with high-affinity RNA ligands

CsrA negatively regulates the expression of genes by binding to multiple sites in the transcript. CsrA binds to two sites in the untranslated leader of the *glgCAP* operon transcript, to four sites in the *cstA* transcript, and to at least six sites in the *pgaABCD* operon leader transcript (Baker *et al.*, 2002 ; Dubey *et al.*, 2003 ; Wang *et al.*, 2005). In each case, one of the CsrA-binding sites overlaps with the SD sequence. CsrB and CsrC RNAs function as antagonists of CsrA by sequestering this protein. The multiple imperfect repeat sequences in these small RNAs function as CsrA-binding sites. There are sequence variations among these RNA targets with GGA being the most highly conserved element. Systematic evolution of ligands by exponential enrichment (SELEX) was used to identify RNA ligands containing single high-affinity CsrA-binding site (Dubey *et al.*, 2005). The consensus sequence derived from SELEX was determined as RUACARGGAUGU. The ACA and GGA motifs were 100% conserved and the GGA motif was located in the loop of a hairpin within the most stable predicted structure in the majority of the RNAs. The AC residues of the ACA motif were predicted to pair with the conserved GU residues in all but one selected ligand. This predicted structure and sequence is similar to several natural CsrA-binding sites. A mutational analysis confirmed that the conserved residues were critical for binding CsrA and that RNA secondary structure participates in CsrA-RNA recognition. However, for the residues ACA and GU, the primary sequence itself was more important for binding CsrA than the ability to form a base pair. CsrA is still able to bind a sequence with no stem loop structure, but does so with less affinity.

Three-dimensional structure and critical amino acid residues of CsrA

The three-dimensional structures of three CsrA proteins (*E. coli*, *Pseudomonas putida,* and *Yersinia enterocolitica*) were independently solved (Gutierrez *et al.*, 2005 ; Rife *et al.*, 2005 ; Heeb *et al.*, 2006). All three had the same overall structure. CsrA is a dimer of two identical subunits, as previously suggested (Dubey *et al.*, 2003). Each CsrA monomer is composed of five consecutive antiparallel β-strands followed by one α-helix and a flexible C-terminus. The strands β1 and β5 of one monomer form a hydrogen bond with β4 and β2 of the other monomer, forming an interlocking structure. The CsrA protein structure is a novel protein fold. In order to examine the amino acids required for CsrA-RNA interaction and gene regulation, comprehensive alanine-scanning mutagenesis was performed on *csrA* of *E. coli* (Mercante *et al.*, 2006). The 58 mutants were tested for the regulation of glycogen accumulation, motility, and biofilm formation, and the effects of these mutations on the regulation of the *glgCAP, flhDC*, and *pgaABCD* operons were examined using *glgCA'-'lacZ, flhDC'-'lacZ*, and *pgaA'-'lacZ* fusions. The selected mutant proteins were purified and tested for RNA binding. The minimal CsrA target sequence (16 nucleotides) for the RNA gel mobility shift assay was designed based on a previous SELEX analysis (Dubey *et al.*, 2005). There were two regions of the amino acid sequence that were essential for repression and activation of gene expression and RNA binding (Mercante *et al.*, 2006). Region 1 was located at the first N terminal β-strand (β1) and region 2 was contained in the last β-strand (β5). The β1 and β5 of opposite monomers were aligned in parallel and adjacent to each other in the CsrA dimer. These findings suggested that the symmetrical CsrA dimer contains two distinct RNA-binding surfaces or subdomains located on opposite sides of the protein.

Regulatory circuitry of the Csr system

Northern analysis indicated that intercellular CsrB RNA levels were severely decreased in a *csrA* mutant with unaltered CsrB RNA stability (Gudapaty *et al.*, 2001). The level of expression of *csrB-lacZ* was ~20-fold higher in a *csrA* wild-type strain than in an isogenic mutant. Nevertheless,

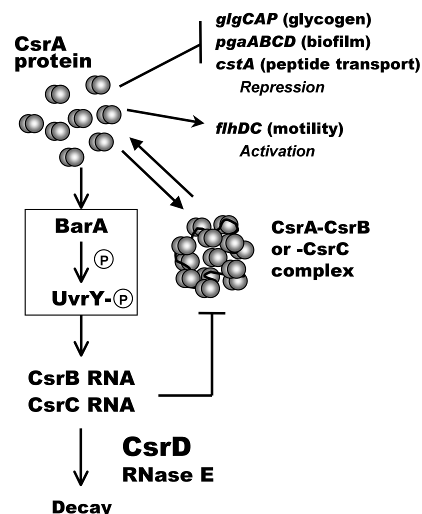

Fig. 3. Csr regulatory circuitry and target gene regulation with proposed CsrD function.
CsrA represses *glgCAP*, *pgaABCD*, and *cstA* etc., and activates *flhDC* etc. CsrA activates the transcription of *csrB* and *csrC* via the BarA/UvrY two-component signal transduction system. CsrA is associated in a ribonucleoprotein complex with either CsrB or CsrC RNA. CsrB and CsrC RNAs bind to CsrA and inhibit its action. CsrD regulates RNase E-dependent CsrB and CsrC RNA decay.

the coupled transcription–translation of the *csrB–lacZ* plasmid in S-30 extracts was not stimulated by the purified recombinant CsrA protein. These results indicate that CsrA indirectly regulates the transcription of *csrB*, either activating an activator or inhibiting a repressor, and autogenously controls its own activity in the cell by stimulating the transcription of *csrB* (Gudapaty *et al.*, 2001).

Mutations affecting the BarA/UvrY two-component signal transduction system (Pernestig *et al.*, 2001) decreased *csrB* transcription but did not affect *csrA'–'lacZ* expression and CsrA stimulated *barA–lacZ* expression (Suzuki *et al.*, 2002). Homologous BarA/UvrY systems in gram-negative pathogens control a variety of virulence functions (Pernestig *et al.*, 2001 ; Heeb *et al.*, 2002).The coupled transcription-translation of a *csrB-lacZ* fusion in S–30 extracts was stimulated by purified recombinant UvrY protein indicating that *csrB* transcription is directly activated by UvrY. Like CsrB, transcription of *csrC* is indirectly activated by CsrA via UvrY (Weilbacher *et al.*, 2003). The environmental sensors, BarA sensor kinase and the LuxR homolog SdiA, also regulate *csrB* and *csrC* transcription, apparently through effects on UvrY phosphorylation and *uvrY* expression, respectively (Suzuki *et al.*, 2002). These effects on *csrB* and *csrC* transcription should modulate the intracellular activity of CsrA. **Fig. 3** shows the summary of the regulatory interaction of Csr system. Interestingly, either *csrB* or *csrC* mutations cause a compensatory increase in the remaining RNA because of the antagonization of CsrA activity by CsrB and CsrC RNAs (Weilbacher *et al.*, 2003).

CsrD, specificity factor for CsrB and CsrC RNA decay

To identify additional genes that affect the expression of *csrB*, a strain containing a chromosomal *csrB-lacZ* fusion was mutagenized with a mini–Tn*10cam* transposon (Suzuki *et al.*, 2006). A mutation in the *csrD* gene resulted in decreased *csrB-lacZ* expression, no change in *csrA'-'lacZ* expression, and modestly increased glycogen accumulation. CsrD is predicted to be a cytoplasmic membrane protein that contains GGDEF and EAL domains (**Fig. 4A**) (Römling *et al.*, 2005 ; Suzuki *et al.*, 2006). Further studies showed that *csrC-lacZ* expression was also decreased in the *csrD* mutant. The effects of *csrD* on genes regulated by CsrA, including genes for motility, biofilm formation, and glycogen biosynthesis, indicated that *csrD* disruption decreases CsrA activity in the cell. Nevertheless, CsrA expression levels were unaltered in this mutant. The stability of CsrB and CsrC RNAs was dramatically increased in the *csrD* mutant (**Fig. 4B**). Thus, the *csrD* gene is required for functional inactivation of CsrB and CsrC RNAs, which should in turn decrease CsrA activity in the mutant (**Fig. 3**). RNase E which is the primary endonuclease involved in mRNA decay (Kushner, 2002) was found to be essential for the decay of CsrB and CsrC RNAs (Suzuki *et al.*, 2006). The C–terminus of RNase E provides a scaffold for assembly of the degradosome, which also includes PNPase (Carpousis, 2002). CsrB decay was highly compromised in a *pnp* mutant and modestly decreased in a strain that contained both catalytic activities but could not assemble the degradosome. In contrast, RNase E-dependent turnover of the *rpsO* and *rpsT* mRNAs and RyhB RNA was unaffected by *csrD*, indicating that CsrD does not regulate bulk RNase E activity in the cell. Analysis of the decay of CsrB RNA suggested that CsrD is not a ribonuclease and serves as a specificity factor for the degradosome-mediated decay of CsrB and CsrC RNAs (Suzuki *et al.*, 2006).

CsrD consists of two predicted membrane-spanning regions, as well as HAMP-like, GGDEF, and EAL domains (**Fig. 4A**). The latter three domains were found to be required for CsrD activity. The GGDEF and EAL domain proteins typically synthesize (diguanylate cyclase ; DGC) and degrade (phosphodiesterase ; PDE) c–di–GMP, respectively (Römling *et al.*, 2005). CsrD does not possess the GGDEF signature motif that contains critical residues for DGC activity. Amino acid substitutions in the region corresponding to the GGDEF motif did not affect CsrD activity. In addition, the EAL motif that is typically required for PDE activity is only partially conserved in CsrD, and other motifs conserved in typical EAL proteins are lacking. The substitution E430A in the ELM (corresponding to EAL) sequence of CsrD did not affect its activity. Cyclic-di-GMP was not detected in wild type, *csrD* mutant, or *csrD*–overexpressing strains by 2D–TLC. Overexpression of AdrA (DGC) or YhjH (PDE) did not affect the Csr regulatory circuitry. These results provide substantial evidence that CsrD activity is mediated

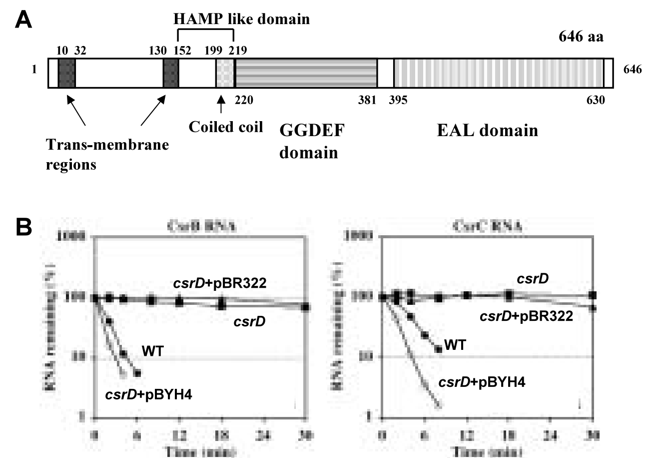

Fig. 4. Domain structure of CsrD and effect of *csrD* on CsrB and CsrC RNA decay.
(A) Domain structure of CsrD with predicted trans-membrane regions, HAMP-like domain, GGDEF domain, and EAL domain. (B) Effect of *csrD* on CsrB and CsrC decay. WT; wild type (MG1655), *csrD* ; *csrD* mutant, *csrD*+pBR322; *csrD* mutant harboring control vector, *csrD*+pBYH4; *csrD* mutant harboring *csrD*-overexpressing plasmid. The CsrB half-life in WT, *csrD, csrD*+pBR322, and *csrD*+pBYH4 was 1.4, >30, >30, and 0.9 min, respectively. The CsrC half-life in the same strains was 2.2, >30, >30, and 1.1 min, respectively.

independently of c-di-GMP (Suzuki *et al.*, 2006). This is the first report of RNA turnover being selectively controlled by a signaling protein containing GGDEF and EAL domains.

CsrD orthologues are present in many gram-negative species and exhibit conserved elements that are absent from c-di-GMP-metabolizing proteins (Suzuki *et al.*, 2006). It is likely that a CsrD-RNase E-mediated pathway of CsrB and CsrC RNA decay regulates CsrA activity and influences metabolism, motility, biofilm formation, and the expression of various genes (**Fig. 3**).

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大腸菌の Csr グローバル制御システムによる転写後調節

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要　約

Csr（carbon strage regulator）グローバル制御システムは多くの細菌に存在し、様々な遺伝子の発現を調節している。大腸菌において、Csr システムの活性の中心は RNA 結合蛋白質 CsrA である。CsrA は二量体を形成し、標的となる mRNA のリーダー部位に結合することで mRNA の安定性と翻訳に影響を与える。CsrA の活性は２つの非翻訳型の small RNA、CsrB 及び CsrC、によって制御される。これらの small RNA は複数の CsrA 結合配列をもっており、CsrA を捕らえる働きをする。Csr システムには自己調節回路が存在し、CsrA は二成分制御系の BarA/UvrY を介して間接的に *csrB* と *csrC* の転写を活性化させる。Csr システムの新たな構成成分 CsrD タンパク質は CsrB 及び CsrC RNA の分解を制御している。CsrD は GGDEF と EAL の２つのドメインをもっているが、典型的な GGDEF-EAL タンパク質とは異なり、cyclic di-GMP の代謝に関与していない。*csrD* 変異株において、CsrB 及び CsrC RNA は劇的に安定となり、それは CsrA が制御する遺伝子の発現を変化させた。Csr システムの構成成分、CsrA、CsrB と CsrC の small RNA、及び CsrD は、恒常的な CsrA 活性制御のメカニズムを備えた自己調節回路内で相互に作用している。

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キーワード：EAL ドメイン , CsrA, Csr システム , GGDEF ドメイン , small RNA

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