

Post-transcriptional global regulation by CsrA in bacteria

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Abstract Global regulation allows bacteria to rapidly modulate the expression of a large variety of unrelated genes in response to environmental changes. Global regulators act at different levels of gene expression. This review focuses on CsrA, a post-transcriptional regulator that affects translation of its gene targets by binding mRNAs. CsrA controls a large variety of physiological processes such as central carbon metabolism, motility and biofilm formation. The activity of CsrA is itself tightly regulated by the CsrB and CsrC small RNAs and the BarA-UvrY two-component system.

Keywords CsrB · CsrC · Central carbon metabolism · Motility · Biofilm formation

Multiple levels of global regulation in bacteria

Bacteria have evolved complex and efficient mechanisms for responding to their ever-changing lives. As an example, *Escherichia coli* is able to respond to a sudden depletion in amino acids by synthesizing the ppGpp alarmone which will redirect transcription toward biosynthetic operons and indirectly lead to polyphosphate-dependent degradation of several free ribosomal proteins (for review see [1]). It can also adapt to heat shock by inducing the production of chaperones and proteases that will help to refold and/or get rid of damaged proteins (for review see [2]), it can respond

to oxidative stress by inducing expression of antioxidant enzymes (for review see [3]) and it can use the most energetic carbon sources among a mixture of carbon sources (for review see [4]). Examples are multiple and are reviewed in depth in a variety of recent reviews [5–8]. Adaptation to these environmental changes reflects gene expression reprogramming. This is achieved by so-called global regulators that allow bacteria to coordinately control the expression of genes or operons encoding unrelated functions and scattered over the genome (for review see [9]). It has been recognized that such global regulators act at the level of transcription. Recently, global post-transcriptional regulators have been characterized and shown to influence globally gene expression (for review see [10]) (Fig. 1). Global transcriptional regulators are themselves regulated by post-transcriptional regulators. Therefore, global regulation implies a cascade of regulations. The starting point is an extracellular signal that is transduced into the cell, the ultimate point being the phenotypic traits resulting from differential gene expression.

Transcriptional regulation

Among global transcriptional regulators, sigma (σ) factors are well documented (for reviews see [11, 12]). They allow RNA polymerase to be recruited at specific DNA sequences in gene promoter regions at which they initiate transcription. In addition to the major housekeeping σ^{70} factor, *E. coli* contains six other σ factors. Changes in their synthesis and/or degradation rates as well as competition between the different σ factors for the RNA polymerase core will lead to differential gene expression. In species such as *Bacillus subtilis* that undergo development, the interplay between multiple σ factors controls sporulation (for review see [13]).

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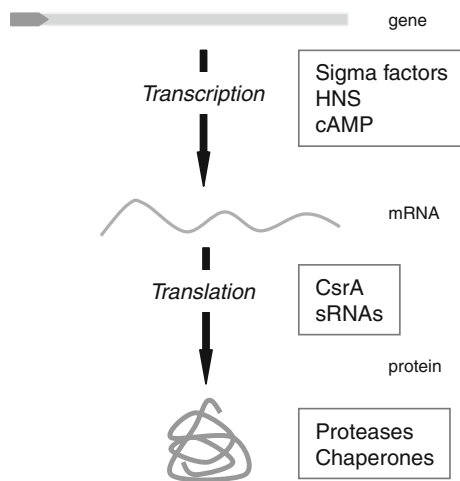


Fig. 1 Regulation of gene expression by global regulators. Different levels of gene expression regulation by global regulators are represented. Sigma factors, H-NS and cAMP control transcription. CsrA and sRNAs regulate translation by acting on mRNAs at the post-transcriptional level. Proteases and chaperones modulate stability and availability of functional protein at the post-translational level

H-NS (histone-like nucleoid structuring protein) is another type of global transcriptional regulator found in enterobacteria (for review see [14]). It is a small DNA-associated protein that binds preferentially to curved AT-rich DNA without showing sequence preferences [15–17]. H-NS regulates mostly negatively a variety of physiological pathways such as metabolism [18], fimbriae expression [19, 20], virulence [21], flagellum synthesis and proper function [22–24]. H-NS is thought to downregulate functions needed in the host when bacteria thrive outside it. These functions are positively regulated by environmental conditions.

Other types of global regulators are signalling molecules such as cyclic-AMP (cAMP) and cyclic-di-GMP (see below) [25, 26]. Synthesis of cAMP is negatively regulated by glucose uptake. cAMP binds to CRP (cAMP receptor protein), also known as CAP (catabolite activator protein), which is the effector protein. While CRP alone has no activity, the CRP-cAMP complex forms an active transcription factor that positively regulates gene expression. Recent microarray data highlight the global regulatory activity of cAMP. Directly or indirectly cAMP controls 380 *E. coli* genes (about 12% of the entire gene pool) [27]. Notably, cAMP regulates carbon source metabolism, flagellum synthesis, biofilm formation, quorum sensing and nitrogen assimilation [27–30].

Post-transcriptional regulation

Small noncoding RNAs (sRNAs) are major players in post-transcriptional global regulation (for review see

[31]). A single sRNA can affect multiple targets and drastically modify cell physiology. These sRNAs are involved in stress response regulation as well as pathogenesis and virulence. More than 70 sRNAs have been identified in *E. coli*. The vast majority belong to the bona fide class of sRNAs that act by base pairing with their mRNA targets. They modify the translation or stability of the targets and most of them act together with the Hfq RNA chaperone. As an example, in *E. coli*, SgrS is a sRNA induced during the so-called sugar phosphate stress due to cytoplasmic accumulation of hexose sugar phosphate as a consequence of glycolysis inhibition. SgrS binds to the mRNA of *ptsG*, whose product is involved in glucose uptake, leading to its degradation [32]. In addition to its regulatory role, SgrS encodes a small protein called SgrT which is also involved in glucose uptake inhibition and appears to regulate PtsG activity [33]. This avoids new supply of glucose and allows depletion of the sugar phosphate pool [32]. Furthermore, some sRNAs regulate other global regulators, making them post-transcriptional global regulators according to the definitions proposed above. As an example, the *E. coli* DsrA sRNA regulates σ^{38} expression (for review see [34]). The second group of sRNAs binds to proteins. The best-studied is the *E. coli* CsrB and its homologues in closely related bacteria (for review see [35]). These sRNAs regulate the activity of the CsrA global regulator. This is developed in detail below.

Post-translational regulation

Another level of post-transcriptional regulation is control of protein stability and folding carried out by ATP-dependent proteases and chaperones [36–38]. Proteases and chaperones act together to regulate the amount of many proteins in the cell, among them global regulators such as σ factors. As an example, the *E. coli* Lon ATP-dependent proteases regulate flagella expression by degrading the specific σ flagella factor (σ^{28}) [39] as well as the acid shock tolerance regulon by regulating the amount of the GadE master transcriptional regulator [40], *gadE* expression being itself under the σ^{38} transcriptional control. As mentioned above, σ^{38} plays an essential role in the general stress response. Post-translation control is mediated by the ClpXP ATP-dependent protease which degrades σ^{38} under steady-state conditions. Under certain types of stress or upon entry into the stationary phase, σ^{38} is no longer degraded and interacts with the RNA polymerase core and transcribes σ^{38} regulon genes (for review see [41]). Proteolysis relies on intricate interactions between protease subunits and adaptor or antiadaptor proteins depending on the growth conditions [42] (for review see [43]).

Post-transcriptional global regulation by CsrA

In a screen set up to identify *trans* factors that regulate biosynthesis of glycogen using an *E. coli* library of transposon mutants, Romeo and coworkers [44] discovered the *csrA* (carbon storage regulator A) gene. A transposon insertion in *csrA* (*csrA::kan*) was isolated and showed pleiotropic phenotypes, which led the authors to propose that CsrA is a global regulator. Cell size and adherence were affected in the *csrA* insertion mutant as well as glycogenesis and gluconeogenesis (see below). It was later shown that the *csrA* gene is essential for viability in rich medium and on minimal media supplemented with glycolytic carbon sources [45]. Moreover, the *csrA::kan* mutation was shown to be leaky, explaining the viability of the *csrA::kan* on these culture media (see below) [45].

The *csrA* gene encodes the CsrA protein which is composed of 61 amino acids. It is a post-transcriptional regulator that binds to mRNAs and regulates mRNA stability and translation [46–62]. CsrA acts mostly negatively leading in most cases to the decay of the negatively regulated mRNAs [46–59]. Some cases of positive regulation mediated by CsrA have also been described in the literature [60–62]. CsrA is regulated at the post-translational level by two sRNAs called CsrB and CsrC in *E. coli* [47, 63–65]. These sRNAs are composed of multiple CsrA binding sites that bind and sequester CsrA, thereby inhibiting its activity (for review see [35]).

CsrA is a global regulator. It regulates multiple unrelated pathways such as central carbon metabolism, motility and biofilm formation, virulence and pathogenesis, quorum sensing and oxidative stress response (Fig. 2), although some subtle differences exist depending on the bacterial species studied [44, 52, 58, 59, 62, 66–81]. Several

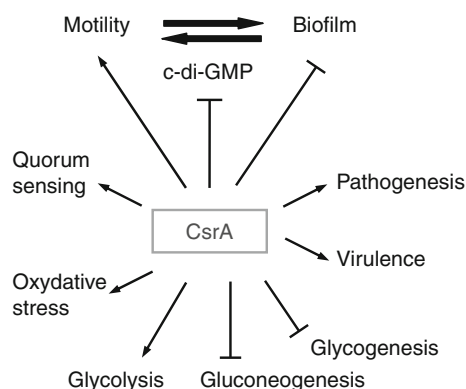


Fig. 2 CsrA regulates numerous unrelated biological pathways. CsrA positively regulates glycolysis, acetate metabolism, mobility, pathogenesis, virulence, quorum sensing and oxidative stress response. It negatively controls biofilm formation, glycogenesis, gluconeogenesis and c-di-GMP synthesis, which is involved in the switch between planktonic and sessile life-styles

homologues have been identified in other bacterial species [82]. In related species such as *Erwinia carotovora* and *Pseudomonas aeruginosa*, CsrA is called RsmA or RsmE (repressor of secondary metabolites) [83, 84]. *Pseudomonas fluorescens* contains two homologues of CsrA (RsmA and RsmE). They appear to be redundant since mutation of both is necessary to derepress production of antifungal secondary metabolites and exoenzymes [85]. In *Pseudomonas syringae* pv. *tomato*, four CsrA homologues have been identified [86], as in the case of *P. fluorescens*, the authors proposed that they might have redundant functions.

Molecular mechanisms of CsrA-mediated regulation of gene expression

Structure of CsrA

CsrA is a small homodimeric RNA-binding protein (about 7 kDa per monomer) [56, 86, 87]. It contains a degenerated KH (human heterogeneous nuclear ribonucleoprotein, hnRNP, K homology) domain [50]. Two types of structural KH domain exist, one found typically in eukaryotes and the other in prokaryotes (for review see [88]). The proteins containing a KH domain are involved in RNA or ssDNA recognition and contain a typical GxxG loop (where x is preferentially a basic amino acid) (for review see [88]) (Fig. 3a). The structure of CsrA is not similar to that of other proteins containing a KH domain although it does contain the GxxG motif (Fig. 3a) [87]. The three-dimensional structure of CsrA was solved by X-ray radiography (from *P. putida* CsrA) and by NMR (*E. coli* and *P. fluorescens* CsrA), giving very similar structures. CsrA is composed of five β -strands followed by an α -helix (Fig. 3a). The dimer is obtained by interdigitation of five β -strands of each monomer [56, 86, 87]. Binding of CsrA on a mRNA target (*glgC*, see below) leads to large conformational changes [87]. This was not observed upon binding to the CsrB-negative regulator most likely because of the structure of the CsrB RNA [87]. The loops connecting β -sheets 1 and 2, and 3 and 4 (containing the GxxG motif) as well as β -sheet 4 and the C-terminus are involved in RNA recognition (Fig. 3a, sequence shaded in grey) [87]. Comprehensive alanine-scanning mutagenesis gave different indications and revealed that the two distal regions (in β -strand 1 and in β -strand 5) are involved in RNA recognition [55] (Fig. 3a, sequence boxed in black). The β -strand 1 of one monomer is located near the β -strand 5 of the other monomer, suggesting that they form a functional domain [55]. The *csrA::kan* mutation is due to transposon insertion at the 51st codon of *csrA* [89] (Fig. 3a, arrow). This leads to the disruption of the C-terminal α -helix which is involved in RNA recognition as proposed by Mercante and coworkers [55]. Although both RNA

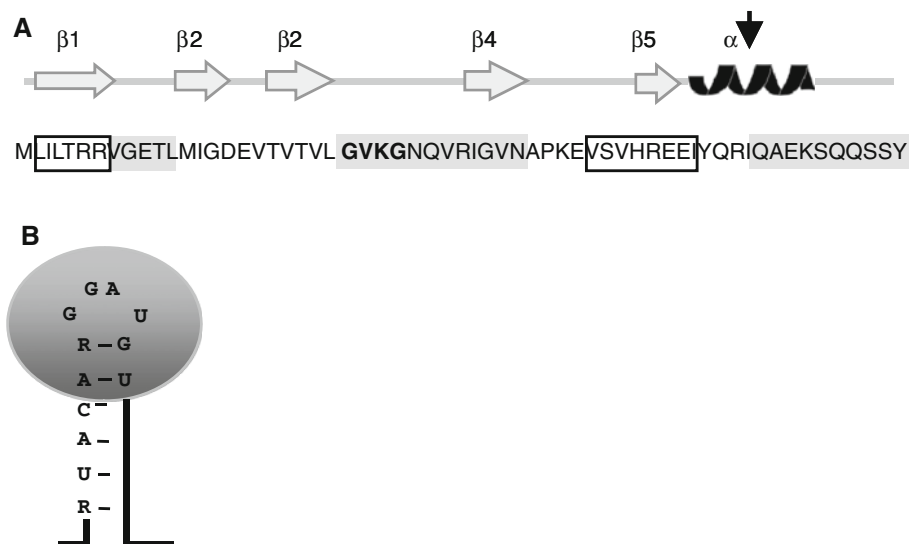


Fig. 3 Secondary structure of CsrA and RNA sequence and structure recognition. **a** CsrA is composed of five β -sheets (grey arrows) followed by an α -helix (black). The amino acid sequence of CsrA from *E. coli* is indicated. Domains involved in RNA recognition/binding based on the 3-D structure are shaded in grey [87] and on mutagenesis

studies are boxed in black [55]. The KH domain is indicated in bold. Point of transposon insertion in the *csrA::kan* mutant is indicated by an arrow [44]. **b** The CsrA consensus recognition sequence is 5'-RUACARGGAUGU-3' that is part of a stem-loop, the GGA motif being in the loop. CsrA is represented in grey

recognition domains of CsrA are necessary for proper function [90], this mutant retains partial activity [44, 45].

CsrA binding sites

The consensus sequence of the *E. coli* CsrA binding site has been identified using the SELEX method (systematic evolution of ligands by the exponential enrichment). This method is based on the high-affinity isolation of ligands to a randomized nucleic sequence (for review see [91]). The consensus binding sequence is 5'-RUACARGGAUGU-3', where R is a purine. Furthermore, the secondary structure of the RNA target is also important for CsrA recognition. Using in silico RNA structure prediction and experimental RNase and Pb²⁺ digestions, it has been shown that CsrA recognizes this sequence in a hairpin in which the middle GGA motif is located in the loop (Fig. 3b) [46, 47]. The *P. fluorescens* CsrA (RsmE) recognizes very similar sequences to that recognized by the *E. coli* CsrA (5'-A/UCANGGANGU/A-3', where N is any nucleotide). The structure is also similar: the middle ANGGA motif is located in the hairpin loop [56]. The CsrA dimer binds to two adjacent binding sites [55, 90]. It has recently been proposed, based on mRNA shift assay and *glgC* S30 transcription-translation, that CsrA binds first to a high-affinity site which enables CsrA binding to lower affinity sites [90].

Molecular mechanisms of regulation

Negative regulation is mediated by CsrA binding on specific sites which are located nearby or overlapping the

Shine-Dalgarno sequence. Binding leads to inhibition of translation initiation and generally to mRNA degradation [46–59]. The RNase(s) involved are still unknown. In the case of the *hfq* mRNA (a target of CsrA in *E. coli*), no degradation is observed. Whether it reflects another mode of regulation is unknown [53].

The number of CsrA binding sites depends on the target (Table 1). The number varies between one (*hfq* mRNA) and six (*pgaABCD* mRNA) [53, 58]. Whether the number of CsrA binding sites on a target influences the tightness of the regulation is not known. However, deletion of one of the CsrA binding sites in the *glgC* transcript enhances its translation [90].

Regulation of glycogen storage by CsrA in *E. coli* is well documented (see below). CsrA negatively regulates glycogen accumulation by controlling the expression of the *glgCAP* operon and *glgB* of the *glgBX* operon [46, 50, 52]. While *glgBX* operon regulation has not been extensively studied, four CsrA binding sites have been identified in the 5' untranslated region of the *glgCAP* mRNA, one of them overlapping the Shine-Dalgarno sequence [46, 90]. CsrA binding on these sites induces translation inhibition and rapid mRNA decay [46, 50]. *glgCAP* transcripts are rapidly degraded in the wild-type strain (half-life of about 4 min) and they are stabilized in the *csrA::kan* mutant [50]. Positive regulation by CsrA has been less investigated; only three cases have been reported in the literature. CsrA positively regulates expression of *flhDC* (encoding the master transcriptional regulator of flagellum synthesis in *E. coli*), *sepLspADB* (involved in pedestal formation induced by enteropathogenic *E. coli*) and *stm3611* (encoding a

Table 1 Direct targets of CsrA

Target genes	Function	Regulation	No. of CsrA box	Species	References
<i>glgCAP</i>	Glycogen metabolism	Negative	4	<i>E. coli</i>	[46, 90]
<i>pgaABCD</i>	Biofilm attachment phase	Negative	6	<i>E. coli</i>	[58]
<i>hfq</i>	RNA-binding protein	Negative	1	<i>E. coli</i>	[53]
<i>cstA</i>	Peptide transport	Negative	4	<i>E. coli</i>	[48]
<i>ydeH</i>	c-di-GMP metabolism; diguanylate cyclase	Negative	Unknown	<i>E. coli</i>	[54]
<i>ycdT</i>	c-di-GMP metabolism; diguanylate cyclase	Negative	Unknown	<i>E. coli</i>	[54]
PA0081	Scaffolding protein	Negative	Unknown	<i>P. aeruginosa</i>	[67]
PA0082	Hypothetical protein	Negative	Unknown	<i>P. aeruginosa</i>	[67]
PA4492	Hypothetical protein	Negative	Unknown	<i>P. aeruginosa</i>	[67]
STM1987	c-di-GMP metabolism; diguanylate cyclase	Negative	Unknown	<i>S. typhimurium</i>	[60]
STM3375 (CsrD homologue)	c-di-GMP metabolism; no diguanylate cyclase nor phosphodiesterase activities	Negative	Unknown	<i>S. typhimurium</i>	[60]
STM1344	c-di-GMP metabolism; no diguanylate cyclase nor phosphodiesterase activities	Negative	Unknown	<i>S. typhimurium</i>	[60]
STM1697	c-di-GMP metabolism; probably no phosphodiesterase activity	Negative	Unknown	<i>S. typhimurium</i>	[60]
<i>flhDC</i>	Motility regulator	Positive	Unknown	<i>E. coli</i>	[62]
<i>sepLsepADB</i>	Virulence factors	Positive	Unknown	<i>E. coli</i>	[61]
STM3611	c-di-GMP metabolism; phosphodiesterase activity	Positive	Unknown	<i>S. typhimurium</i>	[60]

CsrA was shown to bind to the mRNAs indicated, although CsrA binding sites were not systematically experimentally determined.

phosphodiesterase that degrades c-di-GMP in *Salmonella typhimurium*) [60–62] (for review see [92]). As for negative regulation, positive regulation mediated by CsrA is based on CsrA binding on the 5' untranslated region [46, 61, 62], leading to a stabilization of the mRNA [62]. The amount of their cognate mRNA is decreased in the *csrA* mutant [60, 62].

Physiological roles of CsrA

CsrA regulates central carbon metabolism

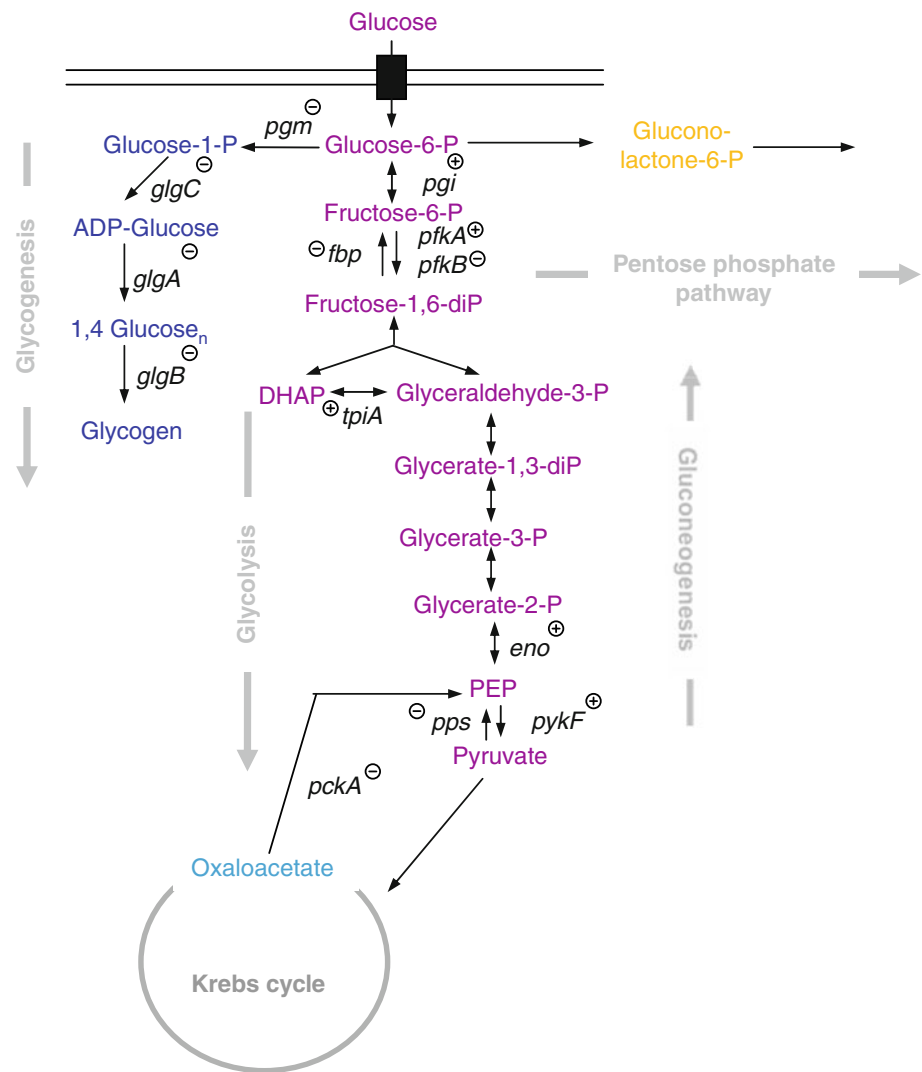
Central carbon metabolic pathways comprise glycolysis, the Krebs cycle and the pentose phosphate pathway (Fig. 4) (for review see [93]). These pathways are interconnected and rely on glucose entry by the phosphoenolpyruvate/carbohydrate phosphotransferase system (PTS) and its conversion into glucose-6-phosphate. Glycogenesis represents a fourth metabolic pathway and converts glucose-6-phosphate into glycogen (Fig. 4). In *E. coli*, the so-called *glg* genes are involved in glycogen metabolism. The *glgA*, *glgC* and *glgB* genes encode enzymes responsible for glycogen anabolism (for review see [94]). The *glgP* (also called *glgY*) (for review see [94]), *glgX* [95] and *glgS* gene products are involved in glycogen catabolism [96, 97]. CsrA regulates different steps in central carbon metabolic pathways. It negatively regulates glycogenesis as well as

gluconeogenesis, although no data on CsrA binding sites are available for gluconeogenesis genes [52, 80]. As mentioned above, CsrA inhibits translation and induces *glgCAP* transcript degradation [44, 46, 49, 50]. For the other *glg* genes (except for *glgX*, that is not regulated by CsrA), although it has been shown that CsrA regulates their expression, it is not known whether or not CsrA binding sites are present [52]. CsrA also regulates expression of the *cstA* gene encoding a putative peptide transporter [48]. Expression of this gene is induced during carbon starvation [98].

Differences are observed between closely related species. In *S. typhimurium*, CsrA does not appear to regulate glycogen synthesis. However, it regulates other metabolic pathways such as maltose and acetate metabolism [99]. CsrA also positively regulates acetate metabolism in *E. coli* [81].

Interestingly, CsrA regulates antagonistic pathways in opposite ways; for example, glycolysis (energy consumption) is positively regulated, while gluconeogenesis and glycogenesis (energy storage) are negatively regulated [80]. Thus, inactivation of the *csrA* gene leads to imbalanced metabolic fluxes towards glycogen accumulation [44, 46, 49, 50, 52]. Due to the positive effect of CsrA on glycolysis, an *E. coli* $\Delta csrA$ strain is unable to grow on glycolytic carbon sources but is able to grow on gluconeogenic carbon sources such as pyruvate. Deletion of the *glgCAP* operon restores viability of the $\Delta csrA$ deletion

Fig. 4 CsrA regulates central carbon metabolism and glycogenesis. The pentose phosphate pathway, gluconeogenesis, glycolysis, glycogenesis and the Krebs cycle are indicated in grey. Genes whose expression is regulated by *csrA* are indicated (+ positive regulation, – negative regulation). The different steps of glycolysis, gluconeogenesis and glycogenesis are indicated. Since CsrA does not regulate the pentose phosphate pathway or the Krebs cycle, these pathways are not shown in detail (DHAP dihydroxyacetone-3-P, PEP phosphoenolpyruvate). Adapted from reference [80]



strain on glycolytic carbon sources [45]. This indicates that in the absence of *csrA*, carbon fluxes are directed towards glycogenesis which impairs glycolysis, even at a basal level [45].

CsrA regulates group behaviour

Bacterial communication Quorum sensing is a cell-to-cell communication that allows bacteria to sense the size of their population and to coordinate their behaviour (for review see [100]). Quorum sensing relies on intercellular communication using signal molecules and signal transduction systems to regulate gene expression. The nature of signal molecules and of transduction systems varies among species. In gram-negative bacteria, the most common signal molecules are homoserine lactones (AHL) that are synthesized by LuxI-type enzymes. At high cell densities, AHL concentrations exceed a certain threshold, bind to

LuxR-type transcriptional regulators and induce a positive feed-back. In several species including *Vibrio* sp. and *Pseudomonas* sp., CsrA negatively regulates the production of AHL, although the underlying molecular mechanism is still unknown [79, 101]. In *E. carotovora* subsp. *carotovora*, quorum sensing molecules downregulate the expression of RsmA, the CsrA homologue [102].

Biofilm and motility Bacteria often form sessile multicellular communities associated on a surface that are called biofilms (for review see [103]). This life-style protects them against harsh environmental conditions such as predators and antibiotics. Biofilm formation comprises different steps, one of the earliest being attachment of bacteria to a substrate. In *E. coli*, numerous bacterial factors are involved in this phase, notably PGA (a linear homopolymer of poly- β -1,6-*N*-acetyl-D-glucosamine) [104–106]. The *pgaABCD* operon is involved in PGA

production and excretion and CsrA negatively regulates its expression [58]. In an *E. coli* strain containing *csrA* under the control of a riboswitch, CsrA depletion leads to autoaggregation in a PGA-dependent manner [107]. A similar phenotype is observed with the Δ glgCAP Δ csrA strain (Seyll et al., unpublished data). In *Vibrio vulnificus* and *Campylobacter jejuni*, CsrA also negatively regulates biofilm formation, although the underlying molecular mechanisms have not been identified [108, 109]. In *E. coli*, CsrA directly regulates the production of c-di-GMP (bis-(3'-5')-cyclic-dimeric guanosine monophosphate) [54], a second messenger involved in the control of biofilm formation and motility (for a recent review see [110]). Enzymes responsible for c-di-GMP synthesis and degradation are ubiquitous in the bacterial world, and many species encode a large number of these proteins. Diguanylate cyclases with a conserved GGDEF motif are responsible for c-di-GMP synthesis and phosphodiesterases with a conserved EAL motif are responsible for its degradation. In *E. coli*, expression of two GGDEF proteins (*ycdT* and *ydeH*) is downregulated directly by CsrA [54]. In a recent study, expression of two GGDEF proteins, two GGDEF-EAL proteins and four EAL proteins have been shown to be under CsrA control in *S. typhimurium* [60].

High levels of c-di-GMP favour the sessile state, whereas low levels promote motility [60]. Thus, CsrA acts at two parallel levels to downregulate biofilm formation: it negatively controls PGA and c-di-GMP production. While being sedentary in a biofilm is certainly advantageous, it is sometimes necessary to be able to quit the biofilm and move to colonize new niches. *E. coli* is able to swim using flagella. In *E. coli*, flagella production is controlled by the FlhDC transcriptional activator which is itself positively regulated by CsrA [62]. In *S. typhimurium*, the flagella class III gene STM3611 is both directly and indirectly regulated by CsrA. STM1344 a regulator of class II genes for flagella synthesis is directly regulated by CsrA [60]. Thus, CsrA appears to be a master regulator involved in the motile/sessile switch, by regulating directly and indirectly expression of behaviour genes.

CsrA regulates pathogenicity and virulence

Virulence and pathogenicity are associated with the ability to adhere to host tissue, to colonize it and to secrete virulence factors. A large amount of evidence that CsrA is involved in virulence control has been obtained in different bacterial species [61, 74, 101, 108, 111–117]. For example, CsrA directly activates expression of genes necessary for pedestal formation in enteropathogenic *E. coli* [61] and for type III secretion system in *P. aeruginosa* [77]. In vivo data in mouse models suggest that CsrA is involved in the

control of different steps in host colonization and virulence [78, 118].

Regulation of CsrA activity

CsrA is regulated by σ^{38}

In *E. carotovora*, *csrA* expression is positively regulated by the homologue of *E. coli* σ^{38} but direct regulation has not been shown [119]. In *E. coli*, σ^{38} is known to regulate genes during the onset of the stationary phase and in stress conditions (for review see [41]). It has been shown that *csrA* expression is under the positive control of σ^{38} when *E. coli* is grown in synthetic medium containing glucose as the sole carbon source [120]. However, in rich medium, this does not seem to be the case [121, 122].

Regulation by the CsrB and CsrC sRNAs

The activity of CsrA is tightly regulated by the sequestration activity of CsrB and CsrC sRNAs. CsrB sRNA has been found to copurify with CsrA [63]. This 366-nucleotide noncoding RNA is composed of 18 imperfect CsrA binding sites (CAGGAUG). Thus, CsrB sequesters CsrA and therefore inhibits its regulatory activity (Fig. 5). The 245-nucleotide CsrC sRNA regulates CsrA activity in a similar manner, although CsrC contains only nine CsrA-binding sites [65]. Functional homologues of CsrB and CsrC have been identified in other bacterial species. These sRNAs contain variable numbers of CsrA binding sites

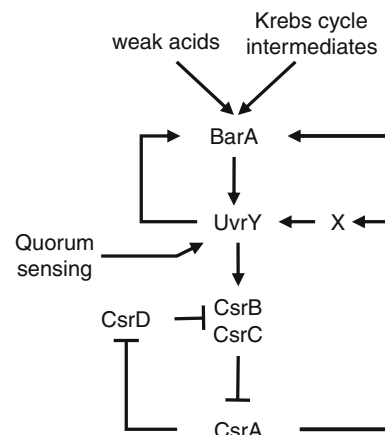


Fig. 5 Regulation of CsrA. The BarA-UvrY two-component system is activated by weak acids as well as Krebs cycle intermediates. This two-component system positively regulates expression of the sRNAs CsrB and CsrC. They sequester CsrA, thereby negatively controlling its activity. Expression of the sRNAs is negatively regulated by CsrD. In *S. typhimurium* and in *E. coli*, the CsrD homologue is negatively regulated by the CsrA homologue. CsrA also directly or indirectly regulates (via a putative X factor) the BarA-UvrY two-component system. Expression of UvrY is under the positive control of quorum sensing

(from 5 to 30) (for review see [35]). Furthermore, the number of functional homologues of CsrB and CsrC varies between species. Up to four CsrB homologues have been predicted in *Photobacterium profundum* which has the most in silico predicted CsrB homologues [123].

The CsrB and CsrC sRNAs are unstable due to their degradation by RNaseE [124]. The CsrD membrane-bound protein binds with high affinity to CsrB and CsrC and stimulates their degradation (Fig. 5). Interestingly, CsrD contains characteristic GGDEF and EAL motifs involved in c-di-GMP metabolism (see above), although these motifs are degenerated for key residues. Overproduction of CsrD does not modify c-di-GMP concentrations showing that CsrD is inactive for c-di-GMP synthesis and degradation. However, the EAL domain seems to be necessary for CsrB degradation [124]. Interestingly, in *S. typhimurium* as well as in *E. coli*, CsrA negatively regulates expression of the *csrD* homologue (STM3375) [60, 125]. Although it has been shown that the *E. carotovora* CsrB is stabilized in presence of CsrA [102], the *E. coli* CsrB decay is not affected by CsrA [126].

The expression level of CsrB and CsrC depends on the culture medium. In nutrient-poor medium, in the absence of amino acids, CsrB and CsrC are highly expressed, while in the presence of amino acids, their expression is reduced [60]. It is not clear yet what causes the suppression of CsrB and CsrC expression by amino acids. The authors proposed that the expression of these sRNAs is under the control of a signal molecule induced by amino acid starvation, although mutations in the genes necessary for ppGpp synthesis (*relA* and *spoT*) had no effect on *csrB* expression in LB medium [125]. This has still to be tested in nutrient-poor medium.

The BarA-UvrY two-component system

The BarA-UvrY two-component system (TCS) is composed of the BarA sensor kinase and its cognate response regulator UvrY (for review see [127]). Homologues of BarA-UvrY have been found in other bacterial species (called GacS-GacA, SirA or LetS-LetA) (for review see [128]). The BarA-UvrY TCS is triggered by an imbalance in the Krebs cycle intermediates and by weak acids such as formate and acetate [129, 130]. In *E. coli*, UvrY is regulated by the quorum sensing molecule AI-2 [64]. The ability of *E. coli* *barA* and *uvrY* mutants to grow on different carbon sources has been analysed. Interestingly, the mutants have an advantage on gluconeogenic carbon sources and a disadvantage on glycolytic sources compared to the wild-type strain, showing that the BarA-UvrY system is necessary for the switch between glycolysis and gluconeogenesis [131]. The activation of this TCS leads to the overproduction of CsrB and CsrC (Fig. 5).

Furthermore, expression of CsrB and CsrC is upregulated in a UvrY-dependent manner at the onset of the stationary phase [64, 65, 126, 131]. This overproduction leads to sequestration of CsrA, favouring glycogenesis.

Homologues of the BarA-UvrY TCS system also regulate CsrA activity in other bacterial species via sRNAs [85, 127, 132–135]. Microarray analysis was performed in *P. luminescens* to identify genes controlled by this TCS [133]. Motility and biofilm formation are respectively under negative and positive control of UvrY. UvrY positively regulates iron uptake as well as quorum sensing, by affecting production of the signal molecule. UvrY is also involved in the regulation of the expression of sugar and peptide transporters as well as that of virulence factors.

Expression of CsrB and CsrC is also positively regulated by CsrA. Because of this feed-back regulation, loss of one of these sRNAs results in an increase of CsrA activity. As a consequence, the remaining sRNA is upregulated [65]. By analysing *csrB* expression in Csr mutants and by complementation tests, it has been shown that CsrA regulates *csrB* transcription via UvrY and independently of it (Fig. 4) [64, 126, 127].

Concluding remarks and perspectives

CsrA regulates gene expression by modulating translation and stability of its mRNA targets. It acts together with other global regulators on several pathways such as central carbon metabolism and biofilm formation. This highlights intricate networks of regulation that allow bacteria to adapt to changing environments. Interestingly, CsrA often shows opposite regulation for antagonistic pathways, for example it positively regulates motility and negatively biofilm formation. CsrA regulation is thus involved in a switch between two different modes. Moreover, inside a specific pathway, it regulates several steps, for example for motility, it enhances the synthesis of flagellum components and decreases the synthesis of c-di-GMP and PGA. As we might expect for a global regulator, expression and activity of CsrA is also tightly regulated. It involves several regulatory systems notably the CsrB and CsrC sRNAs that control CsrA activity by sequestration. Expression of these regulators is itself positively controlled by the BarA-UvrY TCS. This TCS appears to be activated by metabolic intermediates, thereby providing a feed-back control of carbon fluxes in the cell.

Although negative regulation mediated by CsrA is well understood, the RNase involved in the degradation of the targeted mRNA is still unknown. What dictates the positive or negative effect of CsrA on translation also remains to be elucidated.

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