The Csr/Rsm system of *Yersinia* and related pathogens: a post-transcriptional strategy for managing virulence

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Abstract

This review emphasizes the function and regulation of the Csr regulatory system in the human enteropathogen *Yersinia pseudotuberculosis* and compares its features with the homologous Csr/Rsm systems of related pathogens. The Csr/Rsm systems of eubacteria form a complex regulatory network in which redundant non-translated Csr/Rsm-RNAs bind the RNA-binding protein CsrA/RsmA, thereby preventing its interaction with mRNA targets. The Csr system is controlled by the BarA/UvrY-type of two-component sensor-regulator systems. Apart from that, common or pathogen-specific regulators control the abundance of the Csr components. The coordinate control of virulence factors and infection-linked physiological traits by the Csr/Rsm systems helps the pathogens to adapt individually to rapidly changing conditions to which they are exposed during the different stages of an infection. As Csr/Rsm function is relevant for full virulence, it represents a target suitable for antimicrobial drug development.

Introduction

The ability of invading pathogens to alter their physiology and virulence properties to tolerate, cope with and exploit conditions in different host tissues is a crucial feature in any infection. Most pathogens alternate between environmental reservoirs (surrounding biosphere or vector reservoirs) and their mammalian hosts and are confronted by a global change of surrounding parameters. Subsequently, they encounter a rapidly changing spectrum of environments during their migration through different host tissues and/or body fluids. Furthermore, the infected niches inside the host become progressively altered through the combined effects of bacterial colonization and host responses.

It has become evident that pathogens evolved sophisticated perception and signal transduction systems to sense crucial parameters of their environments (e.g. temperature, oxygen, nutrients) and reprogram virulence gene expression to allow a successful infection. Much is known about the molecular structures and mechanisms of the individual systems, their environmental regulation and participation in global regulatory networks. However, it still needs to be analyzed when and where certain virulence factors are expressed during pathogenesis. It is also important to know how and with which other virulence-linked traits their expression is coordinated to optimize the overall biological fitness during infection. Over the last few years, a global regulatory system implicating small regulatory RNAs and post-transcriptional RNA-binding regulators has been identified in many bacterial pathogens which seems to play a central role in the adaptation to changing environments in plant, animal and human hosts. This post-transcriptional regulatory system is called Csr (carbon storage regulator) system in most animal pathogens or Rsm (regulator of secondary metabolism) system in many plant pathogens.

The present review is dedicated to the globally acting Csr system of *Yersinia* and other pathogens. It describes the function and interplay of the small RNAs (sRNAs) and the abundant post-transcriptional regulator CsrA. It further covers the environmental control mechanisms and the crucial role of the system in the coordination of metabolic, stress adaptation and virulence genes implicated in a variety of physiological processes pivotal for the virulence of *Yersinia* and other pathogens. The comparison revealed unique and common features placing the *Yersinia* system between that of plant and animal pathogens.

Life style of pathogenic yersiniae

Of the 15 species within the genus *Yersinia* only *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica* are known to cause diseases in mammals and humans. *Y. pestis* is the causative agent of bubonic, septicemic or pneumonic plague. It is genetically very closely related to the enteric pathogen *Y. pseudotuberculosis*, which similar to *Y. enterocolitica*, causes a number of gut-associated diseases, such as enteritis, colitis and mesenteric lymphadenitis, commonly called yersiniosis. All three pathogenic *Yersinia* species are zoonotic pathogens which are well-adapted for survival in external environments and persistence in various host animals. *Y. pestis* resides within the lymphatic system of many rodents and is transmitted to other mammals by fleas or direct contact. Both enteropathogenic *Yersinia* species were isolated from soil, ground water, animals (e.g. boars, deer, pigs), plants (e.g. carrots, salad) and insects. They are mainly taken up by humans through consumption of contaminated food, in particular raw or undercooked pork [1-4].

Upon oral uptake, *Y. enterocolitica* and *Y. pseudotuberculosis* first encounter the intestinal mucosa and initiate the infection by binding and invasion of M cells located in the intestinal epithelium (early stage of infection). After transmigration through the

epithelial layer the bacteria reach the underlying lymphatic tissues (Peyer's patches) where they multiply rapidly outside host cells and lead to the formation of micro-abscesses [5-7]. Following Peyer's patch colonization the bacteria disseminate into mesenteric lymph nodes, liver and spleen (later stages of the infection) [8-10]. In order to promote the first colonization steps, enteropathogenic yersiniae express a set of virulence determinants, including the invasion factor invasin (InvA). This outer membrane protein mediates tight attachment and internalization of the bacteria into M cells [11, 12]. Expression of *invA* and other early phase virulence genes is controlled by the dimeric MarR-type regulator of virulence RovA. The *rovA* gene is maximally induced at moderate temperature during stationary phase, i.e. conditions found during the free-living and food-associated life-style. Expression of RovA-dependent virulence traits in environments encountered just before and during the early stage of infection is believed to guarantee rapid and efficient colonization of the intestinal tract shortly after ingestion [13, 14]. During the ongoing infection, synthesis of this class of virulence factors is reduced, whereas synthesis of another set of pathogenicity factors is activated. This set includes the virulence plasmid-encoded type III secretion system (T3SS), the Yop effectors, and the adhesin YadA, which mediate dissemination into deeper tissues and resistance against the innate immune system [3, 15-17].

Several studies over the last few years revealed that small regulatory RNAs (sRNAs) are important for the control of *Yersinia* virulence. A deep sequencing approach led to the identification of 150 novel sRNAs in *Y. pseudotuberculosis* and confirmed the presence of 15 previously annotated sRNAs [18]. Most of the newly identified sRNAs were *Yersinia*-specific and multiple sRNAs contributed to virulence. Furthermore, it has been reported that the RNA chaperone Hfq, a factor known to control RNA-RNA and RNA-protein interactions as well as the stability and translation

of RNAs, is important for *Yersinia* virulence. A *Y. pestis hfq* mutant strain was highly attenuated after subcutaneous or intravenous injection and showed an impaired ability to resist phagocytosis by macrophages [19]. The Hfq protein is also required for *Y. pseudotuberculosis* to cause mortality in an intragastric mouse model. Loss of Hfq decreased production of the T3S effectors, intracellular survival in macrophages and reduced the persistence of the bacteria in the Peyer's patches, mesenteric lymph nodes and spleen [20]. In an earlier study, a genetic approach was used to identify regulatory components implicated in the environmental control of early phase virulence factors of *Y. pseudotuberculosis*. This work led to the identification of two regulatory RNAs of a Csr-type regulatory system controlling *Yersinia* virulence [21].

General principles and *Yersinia*-specific features of the Csr system

The first Csr-type regulatory system was identified in *Escherichia coli*, but since its discovery in 1993, homologous systems have been found in many other bacterial species [22, 23]. Similar to *E. coli* and *Salmonella enterica* serovar Typhimurium, the *Yersinia* Csr system is composed of a dimeric RNA-binding protein CsrA and two independent small non-coding RNAs, named CsrB and CsrC (Fig. **1**).

Up to date, 1257 genes encoding proteins of the CsrA/RsmA family have been identified in bacterial genomes. The CsrA proteins are highly conserved, but this class of regulators was not detected in eukaryotes and archaea [23, 24]. The *Yersinia* CsrA protein is most closely related to RsmA of the plant pathogen *Erwinia carotovora* (98% amino acid identity) and differs only slightly from the CsrA proteins of other *Enterobacteriaceae* in the C-terminal part of the protein (95% identity) (Fig. **2**). CsrA of *Vibrio cholerae, Legionella pneumophila, Borrelia burgdorferi* and both CsrA-type proteins of *Pseudomonas aeruginosa* (RsmA, RsmE), exhibit a lower amino acid identity (<88%). The *Borrelia* CsrA protein shows the lowest homology

with CsrA of *E. coli,* yet it was able to fulfill the same function - it repressed glycogen production in *E. coli* [25]. CsrA/RsmA family proteins consist of five β-strands followed by an α-helix. Interdigitation of the β-strands leads to the formation of CsrA dimers that are stabilized by a hydrophobic core and extensive hydrogen bonds [26-28]. Amino acids implicated in RNA-binding are located on the β1 and β5 strands. Specific residues (e.g. Arg^{7}, Arg^{44} and Ile^{47} of *E. coli* CsrA) of both β strands and the domain fold itself determine RNA-binding specificity [29].

The CsrA proteins affect translation and/or the stability of target mRNAs [30-35]. Studies on *E. coli* CsrA demonstrated that the regulator interacts generally with GGA-motifs in the 5'-UTR of target transcripts, whereby the number of the binding sites varies among the CsrA targets [31, 36-40]. A substitution analysis of a SELEX (systematic evolution of ligands by the exponential enrichment)-derived target revealed a high-affinity consensus sequence for CsrA-binding: RUACARGGAU**GU**. The GGA and the ACA motifs (underlined) were completely conserved and GU residues (bold) were present in all but one of the selected ligands [41]. Several GGA sequences are located within loops of short hairpin structures, which are beneficial but not essential for CsrA-binding, or in single-stranded sequences of unstructured RNA overlapping or adjacent to the Shine-Dalgarno (SD) sequence [28, 41]. CsrA-binding in the SD region represses translation initiation due to competition with the 30S ribosomal subunit and accelerates mRNA degradation [34, 36, 38, 42]. Synthetic RNA substrates, containing two-high affinity sites placed at varying distances, were used to characterize how CsrA interacts with RNAs containing multiple target sites. This study revealed that one CsrA dimer can bind simultaneously at both sites within a target. Dual site binding occurred when the two target sites located as close together as 10 nt or as distant as 63 nt, whereby optimal binding of CsrA was observed with a spacer of 18 nt [43]. Relevance of dual site binding was confirmed with the CsrA-

dependent *glgCAP* transcript of *E. coli*. Based on RNA band shifts and *glgC* S30 transcription-translation studies, the following mechanism for CsrA-mediated translation repression has been proposed. CsrA first binds to a high-affinity target site located within a hairpin structure of the 5'-leader that lies upstream of the SD sequence. An increased local concentration of CsrA allows the free RNA-binding surface to interact with the downstream low-affinity target site overlapping the SD sequence. This results in the formation of a repression loop blocking ribosomal binding for *glgC* translation initiation [43]. A recent publication demonstrated that CsrA can also interact with mRNA regions downstream of the translational start site. CsrA inhibits expression of *sdiA*, the N-acyl homoserine lactone receptor in *E. coli* by binding to two sites within the coding region of the mRNA [44]. Although the majority of target mRNAs are negatively influenced by CsrA-binding, *E. coli* CsrA was shown to have a positive effect on the *flhDC* mRNA, encoding the master regulator for flagella synthesis. CsrA binds directly within the 5' UTR of the *flhDC* mRNA and it was found to stabilize the *flhDC* transcript [32].

CsrA (RsmA) activity is generally controlled by non-coding Csr/Rsm RNAs which are able to bind and sequester multiple CsrA molecules and prevent them from binding to their target mRNAs [35, 45-47]. Bioinformatic and experimental approaches revealed that the Csr/Rsm-type small RNAs can be found or predicted in γ-proteo-bacteria, ε-proteobacteria and firmicutes, and almost all bacteria possess more than one Csr/Rsm RNA [48-50]. In *Yersinia*, two non-coding Csr/Rsm-type RNAs, named CsrB and CsrC were identified which act as antagonists of CsrA. Based on secondary structure predictions, both Csr RNAs of *Y. pseudotuberculosis* form highly structured molecules composed of multiple hairpins which contain 18 (CsrB) and 14 (CsrC) GGA motifs in the loop portions of the RNAs (Fig. **1**) [21]. Many other pathogens, including *E. coli, Salmonella, P. aeruginosa*, and *L. pneumophila* also

encode two Csr/Rsm RNAs (CsrBC/RsmYZ). However, three sRNAs (CsrBCD) have been described in *Vibrio cholerae*, and five CsrB/C-like sRNA were predicted in *Photobacterium profundum* [45, 50-58]. It has been shown in several species that the Csr-type RNAs can complement one another, and in *Salmonella* knock-outs of both RNA genes, *csrB* and *csrC*, are required to obtain phenotypes similar to that of a CsrA overexpression strain [46, 51, 57, 58]. However, a *Y. pseudotuberculosis csrB*/*csrC* double mutant does not influence the expression of the Csr-dependent gene *rovA*, in contrast to CsrA overexpression [21]. This indicates that other mechanisms compensate for the loss of *csrBC* in this pathogen.

In contrast to the CsrA-like proteins, the non-coding RNAs differ greatly in size and sequence within and between species. The CsrB and CsrC RNAs of *Y. pseudo-tuberculosis* share no homology and are only 50 to 55% identical to the equivalent RNAs of *E. coli*, *S. enterica* and *S. dysenteriae*. In comparison to *Yersinia*, both RNA sequences are much more conserved in *E. coli* and *Salmonella*, but their overall structures are predicted to be slightly different (Fig. **2**, **4**). Interestingly, similar to CsrA, the *Yersinia* CsrB RNA is most homologous to the single known Csr-type RNA (RsmB) of *E. carotovora* (59%) placing the *Yersinia* Csr system between that of plant and animal pathogens. The CsrBCD RNAs of *V. cholerae* represent a more separate group of Csr RNAs with higher identity to the Csr RNAs of *Yersinia* than to *Salmonella* and *E. coli* (Fig. **2**). RsmY and RsmZ of *L. pneumophila* and *P. aeruginosa* display the most distinct cluster of characterized Csr-like RNAs due to their reduced length and their low homology compared to other Csr-RNAs. Presence of many different Csr/Rsm-like RNAs raises the question why multiple RNAs are produced when they seem to fulfill the same function – sequestration of CsrA? One important functional difference between the different Csr/Rsm RNAs seems to reside in their capacity to bind different numbers of CsrA molecules. *E. coli* CsrB exhibits 18

potential CsrA binding sites in the hairpin loop structures and is capable of sequestering ~ 9 CsrA dimers, whereas *E. coli* CsrC possesses only 9 potential binding sites [45, 58]. Furthermore, possession of multiple redundant Csr-RNAs allows differential regulation of their synthesis and/or stability in response to different environmental stimuli. Moreover, the binding affinity of CsrA to the individual GGA motifs of the RNAs can be different and/or altered by assisting RNA-binding proteins, e.g. the RNA chaperone Hfq [59]. Finally, it is also possible that Csr/Rsm-type RNAs accomplish individual functions independently from CsrA. To address this question, we are currently investigating whether variations of CsrB and CsrC levels affect the *Yersinia* transcriptome even in the absence of CsrA.

Pleiotropic effects of the Csr system in *Yersinia* and other pathogens

First from work on *E. coli*, but soon also from following studies on other bacteria, it became apparent that the Csr system controls a multitude of different physiological as well as stress- and virulence-associated processes within the bacterial cell [60]. A global influence of CsrA on the bacterial transcriptome has been described for *P. aeruginosa* and *S. typhimurium,* and multiple CsrA mRNA targets have been identified in other pathogens [53, 61-63]. Similarly, a microarray analysis revealed that CsrA influences the expression of about 500 open reading frames in *Y. pseudotuberculosis* (Fig. **3A**; AK Heroven, unpublished data). The CsrA regulon includes genes and operons encoding a large variety of metabolic functions, transcription and translation factors, signal transduction pathways, motility/chemotaxis, stress and virulence traits. The global influence of the Csr system is reflected in many different physiological changes of which some are similar between pathogens, but others are unique or even opposite to phenotypical changes observed in related pathogens (Fig. **3**):

Growth and Cell Shape

There is strong evidence that growth and cell shape are affected by the Csr system, although the overall outcome seems to vary significantly between the different bacterial pathogens. Growth of a *Y. pseudotuberculosis csrA* mutant strain (doubling time of ~3.7 hours) is strongly reduced compared to wildtype (doubling time of ~1.3 hours). Scanning electron microscopy further revealed smaller-sized and round-shaped bacteria (~1.4 µm) in contrast to the typical ~2.2 µm rod-shaped wildtype cells (Fig. **3B**). CsrA-overexpressing bacteria formed elongated cells [21]. Similar to *Yersinia*, *S. typhimurium* and *L. pneumophila* converted into coccoid-shaped cells in the absence of CsrA [62, 64]. In fact, CsrA seems to be essential for *L. pneumophila* as only conditional knock-out mutants could be constructed – emphasizing the importance of this regulator for the overall fitness of this intracellular pathogen [64]. Conditional essentiality has been reported for the *csrA* gene of *E. coli*. CsrA was shown to be required for growth on LB and on synthetic media with glycolytic carbon sources, due to an excess of glycogen accumulation in absence of *csrA*, but it is not necessary for growth on synthetic media containing pyruvate [65]. In contrast, loss of *csrA/rsmA* had no or only a minor effect on growth of *P. aeruginosa* [53, 66].

Motility

Another striking phenotype of a *Yersinia csrA* mutant is that they are aflagellated and non-motile (Fig. **3B**). Expression analysis using translational reporter fusions demonstrated that CsrA activates expression of the master regulator operon *flhDC* required for flagellin production, swimming and swarming motility of *Yersinia* [21]. Specific and direct binding of the *Yersinia* CsrA protein to the *flhDC* mRNA [21] suggests that similar to what has been shown in *E. coli* [32], CsrA might activate FlhDC

synthesis through CsrA-mediated stabilization of the *flhDC* transcript. Knock-outs of the CsrA-antagonists genes *csrB* and *csrC* conversely resulted in an increase of *fliA* transcription in *Salmonella* [67]. Interestingly, CsrA in *S. typhimurium* does not only control expression of flagella via the FlhDC/FliA pathway, it also inhibits the production of a protein termed STM1344, a negative regulator of *fliA* gene expression [67]. In opposite to enteric pathogens, flagella expression is inhibited by CsrA in the plant pathogen *E. carotovora*, in which RsmA destabilizes the mRNA transcripts of *flhDC* or *fliA* [68], and in the lung pathogen *L. pneumophila* in which reduced levels of FliA and the flagellar subunit FlaA are produced when CsrA production is enhanced [64, 69].

Metabolism

The CsrA protein was first identified in *E. coli* as a regulator that negatively affects glycogen biosynthesis and gluconeogenesis [22, 36]. CsrA also inhibits glycogen synthesis in *Yersinia* (Fig. **3B;** K Böhme, M Palela, unpublished results), but it does not influence this metabolic process in *S. typhimurium* [62]. However, CsrA controls expression of multiple genes involved in other metabolic processes of *S. typhimurium,* which are beneficial for the pathogen within the intestinal tract. This includes genes responsible for the utilization of 1,2 propanediol and ethanolamine, synthesis of vitamin B_{12} as well as transport and metabolism of maltose/maltodextrins [62, 70]. Noteworthy, also the CsrA homologue RsmA of *P. aeruginosa* was found to control multiple functions of the primary metabolism. Transcriptome analyses revealed that RsmA represses genes of the fatty acid and phospholipid metabolism. Furthermore, RsmA induces genes responsible for the uptake and utilization of certain sugars, and it regulates genes of iron storage and acquisition processes which seem to enable the bacteria to colonize new niches in the human lung [53, 61]. A

broad impact of the Csr system on the overall metabolism was also observed in *Yersinia*; about 20% of the CsrA-dependent genes are involved in metabolic processes (Fig. **3A**; AK Heroven unpublished results). Global influence is partly achieved through the influence of the Csr system on global regulatory factors. In *Pseudomonas*, RsmA was found to activate expression of *vfr*, a homologue of the cAMP-recognizing protein Crp, and in *E. coli,* CsrA was shown to reduce the stability of the *relA* transcript leading to the repression of (p)ppGpp synthesis involved in the stringent response [53, 63].

In summary, global impact of the Csr system on the primary metabolism of pathogens illustrates that this regulatory complex fulfills a pivotal function adjusting metabolic functions in response to rapidly changing nutrient conditions during the course of an infection.

Stress responses

The control of stress responses by the Csr system is also common among pathogens. Drastic physiological, morphological and metabolic alterations observed in the absence of CsrA are often accompanied by changes in the expression of genes which play a key role in the general stress response (heat and cold shock, acidic resistance and periplasmic stress) and specific stresses (iron starvation) [53, 62]. The Csr-dependent stress functions vary significantly among the different bacteria and seem to form a tailor-made set of anti-stress strategies important for the adaptation of the individual microorganism to the distinct niches in their hosts. For example, in *L. pneumophila*, inhibition of CsrA function is important to adapt the bacteria to environmental stresses (osmotic, temperature and UV light) encountered during the transition from the intracellular replication phase inside macrophages to the transmissive phase [64]. CsrA-dependent stress responses of *Helicobacter pylori*

include expression of genes required to cope with acid stresses (urease operon), oxidative and heat stresses as well as iron starvation (*fur*). These physiological traits are important for the survival of *Helicobacter* in the stomach [71]. In *B. burgdorferi* as well as in *Pseudomonas* a general stress response regulator, the sigma factor RpoS (σ^S) was found to be part of the CsrA/RsmA regulon, indicating that the Csr/Rsm system plays an important role for the adaptation of the pathogens to persist in nutrient-poor environments [72, 73]. Absence of the *csrA* gene also affects expression of multiple stress resistance genes in *Y. pseudotuberculosis* (Fig. **3A**, AK Heroven, unpublished results). This suggests that the Csr system is also important for the adaptation of this pathogen to hostile conditions experienced during the infection.

Biofilm formation

Another strategy that is essential for the persistence and resistance against host-induced stresses and defense mechanisms is the formation of matrix-enclosed aggregates of bacterial communities, called biofilms. The components of the Csr system influence the establishment of biofilms in many pathogens. In *S. typhimurium*, expression of type I fimbriae which contribute to the development of biofilms, is repressed in a *csrBC* mutant strain [74]. Furthermore, CsrA was found to inhibit the synthesis of eight GGDEF and EAL-domain proteins by binding to the leader sequences of their transcripts. These proteins act as cyclases or phosphodiesterases which reversely regulate the level of the bacterial secondary messenger cyclic di-GMP and control transition from motility to sessility (biofilm formation) [67]. CsrA also serves as a repressor of biofilm formation in *E. coli* and *P. aeruginosa*. In *E. coli*, CsrA inhibits translation of the *pgaABCD* transcript encoding the adhesin PGA (poly-β-1,6-N-acetyl-D-glucosamine) which is required for cell attachment during the early stages of biofilm development [31, 75]. Furthermore, global regulation of the central

carbon flux by CsrA is an important feature of *E. coli* biofilm development [76]. In *P. aeruginosa*, RsmA blocks translation of the *psl* mRNA, encoding a major structural component (Psl) of the biofilm matrix, and induces swarming motility [77, 54]. In contrast to previous pathogens, CsrA activates biofilm formation in *V. cholerae.* In this enteric pathogen, the Csr system converges with the *V. cholerae* quorum-sensing systems, which control the formation of biofilms in response to changes in cell density through four redundant quorum regulatory RNAs (Qrr) and the master regulator HapR [51, 78]. Whether the Csr system of *Yersinia* affects biofilm formation remains to be shown. However, previous results demonstrating a CsrA-mediated activation of *flhDC* expression [21], and the observation that induction of FlhDC synthesis induced *Yersinia* biofilm formation on nematodes [79], predict an interconnection.

In summary, the Csr-type regulatory systems of *Yersinia* and all other characterized pathogens constitute important post-transcriptional control mechanisms of a large variety of metabolic activities, stress responses and physiological processes which are pivotal to the overall biological fitness of plant and animal pathogens. Interestingly, although similar cellular functions (e.g. motility, stress resistance, biofilm formation) are controlled by these highly redundant Csr-type regulatory systems, the overall outcome seems to vary significantly between the different bacterial species.

Autoregulation and environmental control of the Csr systems

Analysis of the *Yersinia* Csr system revealed that CsrA, CsrB and CsrC are tightly interconnected by autoregulatory feedback loops controlling the expression, stability and/or activity of the individual system components. For instance, upregulation of CsrB represses CsrC synthesis and *vice versa* [21]. Absence of one Csr-RNA might in-

crease the availability and sequestration of CsrA by the other Csr-RNA, which could enhance its stability. In fact, CsrA was found to be absolutely required for the presence of CsrB and CsrC [21], and recent data of our group demonstrated that both RNAs are rapidly degraded in the absence of CsrA (K Böhme, unpublished results). Interdependency of the Csr components seems to be evolutionarily conserved (Fig. **4**). For instance, RsmA of *E. carotovora* and RsmA and RsmE of *P. fluorescens* protect the associated Csr-RNAs from degradation [80-82]. In *S. typhimurium* CsrA was reported to increase the stability of CsrC, but not of CsrB [57], whereas in *E. coli* CsrA did no significantly affect the turnover of both, the CsrB and the CsrC RNA [58, 83]. However, another study described that the membrane-bound GGDEF-EAL domain protein CsrD (YhdA) of *E. coli*, which is negatively regulated by CsrA, interacts with both Csr-RNAs and converts them into substrates for RNase E [39, 84, 85]. This implies a CsrA-mediated stabilization of the CsrB and CsrC RNAs through repression of CsrD (Fig. **4**). Apart from this autoregulatory circuit, CsrA was shown to activate *csrB* transcription in *E. coli*. CsrA-mediated induction of *csrB* expression occurs through SdiA and the response regulator UvrY [86, 87] (Fig. **4**, see also below).

In *E. coli*, CsrA was also shown to regulate its own synthesis. Four CsrA binding sites were identified upstream of the start codon of the *E. coli csrA* mRNA. CsrA was shown to bind to these sites and repress translation of its own transcript by directly competing with ribosome binding [88]. However, it was also shown that *csrA* is transcribed from five promoters, and CsrA is able to indirectly activate the strong σ^S-dependent *csrA* promoter P3. Transcription of this promoter is responsible for increased *csrA* expression, as *E. coli* transitions from exponential to stationary-phase growth [88]. Similarly, CsrA synthesis of *Y. pseudotuberculosis* was shown to be induced during stationary phase [21], but whether this induction is also CsrA-dependent remains to be shown.

In addition to counter-regulation, both *Yersinia* Csr-RNAs are controlled by distinct regulatory factors and respond to different environmental stimuli and growth parameters. Transcription of *Yersinia csrC* is maximally induced during stationary phase at 25°C and during late exponential phase at 37°C [21]. An increase in cell density is also an inducing signal for the synthesis of Csr components in many other pathogens, in which highest expression levels are mainly reached during transitions from exponential to stationary phase [51, 54, 55, 57, 83]. In addition, *csrC* expression in *Yersinia* is strongly affected by the composition of the growth medium. High amounts of CsrC were found in cells growing in nutrient-rich/complex media whereas *csrC* expression was strongly repressed in minimal media [21]. The media component(s) and the signal transduction mechanism(s) controlling CsrC levels are still unknown (Fig. **4**). Similarly, expression of the Csr-RNAs in *E. coli* depends on the growth medium. However, in contrast to *Yersinia*, CsrB and CsrC synthesis is highly upregulated during growth in nutrient-poor media and repressed in the presence of amino acids [89]. In comparison to CsrC, CsrB levels of *Y. pseudotuberculosis* were found to be very low throughout growth in LB medium and under all other tested *in vitro* conditions. Regulatory studies investigating the role of the two-component system (TCS) BarA/UvrY in the control of the *Yersinia* Csr system, revealed that expression of the *Yersinia* BarA/UvrY system is also very low under standard *in vitro* conditions, but artificial overexpression of UvrY leads to a strong induction of *csrB* expression [21].

The BarA/UvrY TCS is frequently used by pathogens to sense external signals to adapt bacterial fitness and virulence to changing environmental conditions. Notably, orthologues of this TCS were found to activate transcription of all Csr-like sRNAs in the pathogens summarized in Fig. **4**. For instance, expression of the CsrB and CsrC RNA in *E. coli* and *Salmonella* is activated by the BarA(SirA)/UvrY TCS, leading to a high abundance of both Csr-RNAs at 37°C under standard growth conditions [58, 86, 90-

[92]. Furthermore, all known Csr-type RNAs of *V. cholera* (CsrB, CsrC and CsrD), *L. pneumophila* (RsmY and RsmZ), and *E. carotovora* (RsmB) are induced by the homologous TCSs VarS/VarA, LetS/LetA and GacS/GacA [51, 52, 93]. Similarly, both Csr-RNAs of *P. aeruginosa* (RsmZ, RsmY) are activated by the UvrY orthologue GacA, but the response regulator is controlled by three different sensor kinases GacS (homologue of BarA), as well as RetS and LadS, which are both not present in *E. coli* or in other enterobacteria [94, 95] (Fig. **4**).

The molecular nature of the inducing environmental stimuli of the BarA/UvrY-like TCSs is mostly unknown. However, based on recent regulatory studies, the stimuli appear to include mainly host cell- or bacterial population-derived molecules which are important for the biological fitness, cell-to-cell communication or host-pathogen interactions. For instance, in *E. coli*, activity of BarA/UvrY is triggered by an imbalance of Krebs cycle intermediates, e.g. weak acids such as formate and acetate, and seems to be pH-dependent [96, 97]. The SirA/UvrY signal transduction pathway of *Salmonella* is also regulated by short-chain fatty acids, such as acetate, formate and propionate [98]. Differently from the enteric pathogens, high levels of the global alarmone (p)ppGpp (guanosine 3',5'-bispyrophosphate) were found to activate the LetS/LetA TCS of *L. pneumophila* [99]. Production of this signal molecule is part of the stringent response, which is induced upon starvation, in particular amino acid deprivation.

Several studies documented that the components of the Csr system are also controlled by important global regulatory proteins. Expression analyses of the *Salmonella* Csr system revealed a tight connection between the carbon catabolite repression (CCR) and the Csr regulatory network. The catabolite repression protein (Crp) increases the expression of both Csr-RNAs in *Salmonella.* Crp does this indirectly by activating transcription of the *uvrY* orthologous gene *sirA* [100] (Fig. **4**).

Furthermore, the general stress regulator RpoS was found to activate *csrA* transcription in *E. coli*, e. g. during growth in synthetic medium containing glucose as single carbon source, but not in rich medium [88, 101]. Similarly, RpoS was found to act as an indirect activator of *rsmA* expression in *E. carotovora* [102], whereas quorum sensing molecules seem to inhibit RsmA synthesis [68]. Furthermore, *csrA* transcription in *L. pneumophila* is activated by the response regulator PmrA which controls expression of the *Legionella* effector genes [103] (Fig. **4**). The highly conserved RNA chaperone Hfq is another global regulator implicated in Csr control. Hfq was shown to stabilize the Csr-RNA RsmY of *P. aeruginosa*, resulting in enhanced translation of all RsmA target mRNAs. Hfq and RsmA bind concurrently to RsmY which still permits RsmA sequestration and inhibits RNase E cleavage of RsmY at AU-rich RNase E recognition sequences that are protected by bound Hfq [59, 104].

In addition, species-specific regulators of the Csr systems have been identified in bacterial pathogens. This may reflect the adaptation of the Csr system to the requirements of the individual bacteria during pathogenesis. One example is the *Pseudomonas* sigma regulator PsrA of *P. fluorescens* that induces expression of *rsmZ* during stationary phase, but not of the *rsmY* RNA gene [105]. PsrA was previously shown to activate *rpoS,* modulate the type III secretion *exsCEBA* operon and inhibit expression of a fatty acid degradation operon in *P. aeruginosa* [106, 107].

A comparison of the Csr/Rsm system of the different pathogens illustrates that the *Yersinia* Csr system has many conserved but also distinct properties (Fig. **4**). Similar to *E. coli* and *Salmonella*, two Csr-RNAs are produced by *Yersinia*, but the sequence and predicted structure of the Csr-RNAs are very different. All known components of the regulatory system in *Yersinia* are tightly autoregulated, whereby the Csr-RNAs control CsrA and vice versa, similar to what has been described for closely related

pathogens. Furthermore, expression of both Csr-type RNAs is controlled by the TCS BarA/UvrY. However in contrast to all other Csr-RNAs, expression of the *Yersinia csrC* gene was not induced by the BarA/UvrY system. On the contrary, UvrY-mediated upregulation of CsrB levels resulted in a downregulation of CsrC levels [21]. This inhibition occurred exclusively through the negative autoregulatory feedback loop and was eliminated in the absence of CsrB [21]. Up to date, no signal transduction system controlling the *Yersinia csrC* gene has been identified, but *csrC* expression is highly induced during growth in complex media. On the other hand, expression of the *Yersinia* BarA/UvrY system is very low under all tested conditions. As a consequence, the CsrB RNA is only very weakly expressed in *Yersinia*. This is very different to *E. coli* and *Salmonella* in which both Csr-RNAs are abundant under standard growth conditions [57, 58, 89].

Impact of the Csr system of *Yersinia* and other pathogens on virulence

One important feature for a successful bacterial infection is the expression of multiple virulence determinants, such as cell adhesion and invasion factors, exo- and cytotoxins as well as host defense factors by the pathogen. These factors promote efficient colonization, dissemination to deeper tissues and long-term persistence within hosts. Recent work demonstrated that the Csr/Rsm system plays a central role coordinating the synthesis of these virulence functions with virulence-related physiological traits described above [49].

In *Y. pseudotuberculosis*, the Csr system seems to control switching between different infection phases. Expression of the Csr-RNAs is crucial for the initial phase of the infection as it allows efficient production of the primary cell adhesion and invasion factor InvA [21]. Presence of higher amounts of the CsrC RNA during standard culture conditions, but also induction of CsrB synthesis is sufficient to sequester CsrA

and allows expression of *invA* via a complex regulatory cascade, implicating multiple transcriptional regulators, including the MarR-type regulator RovA [17, 21, 108]. Transcriptional profiling further revealed that CsrA manipulates the expression of many other colonization factors (Fig. **3A**, AK Heroven, unpublished data). In summary, CsrA appears to control switching of virulence gene expression from early (colonization) to later stages (persistence) of the infection. This would also explain why both loss and overproduction of CsrA have a negative influence on cell adhesion/invasion [21].

Importance of the Csr system for the synthesis of colonization factors that trigger initial attachment to cells of the intestinal epithelium has also been demonstrated in several other gastrointestinal pathogens. A *csrA* mutant of enteropathogenic *E. coli* (EPEC), a causative agent of diarrhea in humans, fails to form actin pedestals important for the intimate attachment of the pathogen to intestinal cells [109]. Also *S. enterica* serovar Typhimurium, another diarrhea-causing enteric pathogen, and *Shigella flexneri*, a facultative intracellular pathogen that invades and disrupts the colonic epithelium use the Csr system to adjust their colonization properties [57, 110]. A comparative analysis revealed that the Csr system affects pathogenicity in all these pathogens by controlling the expression and function of their T3SSs. T3SSs of these pathogens are essential for virulence as they are used for secreting infection-related effector proteins, which subvert host cell signaling pathways and manipulate the host cytoskeleton to promote adhesion and uptake of the pathogens by the host cell. For instance in EPEC, synthesis of the T3S components EspABD and secretion of the effector Tir was substantially reduced in the absence of CsrA [109]. In *Shigella*, production of the main virulence regulators VirB and VirF of the T3SS system secreting the Ipa effectors for invasion is CsrA-dependent [111], and in *Salmonella* it was found that CsrA represses translation of the virulence regulator gene *hilD* [90].

HilD itself regulates expression of the virulence regulator HilA and the TCS SsrAB controlling the expression of crucial T3SSs for invasion and intracellular replication encoded on the *Salmonella* pathogenicity islands (SPI) I and II [112, 113]. A different type of virulence factors is affected by the Csr system in the non-invasive pathogen *V. cholerae* causing severe watery diarrhea. In this pathogen, CsrA controls biofilm production and the synthesis of the hemagglutinin/protease A (HapA), which helps *V. cholerae* to trespass the mucus layer of the gastrointestinal epithelium. This occurs through regulation of the master regulator HapR [51, 114]. It is interesting to note that also *Helicobacter pylori,* a pathogen causing peptic ulceration and gastric cancer, requires the Csr system for the successful and persistent colonization of the gastric mucosa. *H. pylori csrA* mutant strains show strongly attenuated virulence, most likely because CsrA is crucial for the adaptation of *H. pylori* to heat, oxidative and acidic stress [71].

Role of CsrA in host-pathogen interactions has also been studied in great detail in two pathogens causing acute and chronic infections of the respiratory tract. The Csr system of the pneumonia inducing lung pathogen *L. pneumophila* was identified to trigger the switching of the bacterium's lifestyle from the intracellular, replicative form within protozoa or human alveolar macrophages to the extracellular, highly virulent transmissive form. When *csrA* was overexpressed in *L. pneumophila*, cells in the transmissive state were less cytotoxic to bone marrow-derived macrophages. High *csrA* expression also lowered the infectivity of *Legionella* in the extracellular, normally virulent phase, whereas loss of *csrA* led to a highly infectious phenotype of replicative bacteria – i.e. CsrA seems to act as an inhibitor of the transmissive phase [64]. In agreement, a deletion of both Csr-RNA genes (*rsmY* and *rsmZ*) was found to inhibit intracellular growth of *L. pneumophila* within *Acanthamoeba castellanii* and monocyte-derived macrophages, because the bacteria were partially blocked from

entering the transmissive phase. This can be explained by the fact that the RsmYZ-CsrA signal cascade controls the expression of several effectors (e.g. YlfA, YlfB and VipA) secreted by the Icm/Dot type 4 secretion system (T4SS). The Icm/Dot T4SS is the major virulence system of *L. pneumophila* shown to translocate effectors which manipulate vasicular trafficking, host protein degradation as well as cell exit and cell death [52, 103]. The Csr/Rsm system of the opportunistic human pathogen *P. aeruginosa*, which causes severe pneumonia and chronic respiratory infections (in particular in patients with cystic fibrosis), promotes switching between the acute and the chronic phase of the infection. This complex adaptation process implicates multiple different classes of Csr-dependent virulence traits. For instance, it involves a change from the planktonic to the sessile lifestyle in biofilms. Presence of both regulatory RNAs RsmZ and RsmY and knock-out of the *rsmA* gene repress motility of *P. aeruginosa*, which favors biofilm formation and the persistent form of the infection [54, 95, 115]. In addition, RsmA regulates the expression of the *Pseudomonas* T3SS and secreted effectors (e. g. exoenzymes S and Y), which affect expression of host proteins (Kruppel-like factors 2 and 6), actin depolymerisation and cell viability of airway epithelial cells [61, 116, 117]. Furthermore, the Rsm system controls the synthesis of components of the type 6 secretion system (T6SS), which is closely associated with the chronic infection phase of *P. aeruginosa* [61].

Finally, evidence exists that the Csr/Rsm system affects the pathogenicity of animal and plant pathogens that enter their hosts through external surfaces (skin, leaves). A recent study showed that CsrA modulates virulence of *B. burgdorferi,* a spirochete that is found in ticks and causes Lyme disease. CsrA was found to be important for the colonization of mice following intradermal infection, most likely because the borrelial CsrA protein plays a key role in the regulation of crucial pathophysiological determinants, including the outer surface protein OspC, the

decorin binding protein DbpA, and the fibronectin binding protein BBK32 [73]. Several studies have also shown that the regulatory protein RsmA controls the pathogenicity in soft-rotting *Erwinia* species. In *E. carotovora,* RsmA suppresses the tissue macerating ability by repressing the production of glycolytic and proteolytic exoproteins, such as the pectate lyase, polygalactonurase, cellulase and proteases, whereas in *E. amylovora* RsmA inhibits the elicitation of the hypersensitive reaction in tobacco leaves and the production of disease symptoms in apple shoots [118, 119].

In summary, manipulation of the Csr system in infectious bacteria clearly demonstrated its importance for the pathogenicity of human, animal and plant pathogens. Loss of the conserved CsrA/RsmA protein can lead to a strong attenuation or a complete loss of virulence and/or it can change the course and character of an infection process. This is caused by a strong deregulation of a varying set of crucial virulence factors (e.g. secretion systems/translocated effectors, secreted virulence determinants) important for colonization or long-term persistence.

Concluding remarks

The Csr/Rsm system of *Yersinia* and other pathogens constitutes a very important post-transcriptional control mechanism that enables the bacteria to coordinate expression of specific virulence factors with certain infection-associated physiological attributes. This interconnection is crucial for the successful establishment of an infection as it provides a tailor-made, fine-tuned adjustment to the environments within hosts and allows the pathogen to cope with host-induced stresses and rapidly changing metabolic demands during the course of the infection. The ability of CsrA/RsmA to manipulate the expression profile of a large variety of virulence-related genes makes the Csr/Rsm system ideal for the control of major system changes, which occur: (i) during the shift from the early colonization to later persisting stages of

the infection, (ii) in the course of a switch from the acute to the chronic form of infection, or (iii) during transmission between hosts.

Moreover, conserved “GGA” sequences in the SD region are used by CsrA/RsmA to manipulate their mRNA targets. Variations in the surrounding sequences that define the specificity of CsrA-binding can result in the loss but also in the generation of a new CsrA target during evolution. As a consequence, a wide variety of common and specific virulence-related determinants are controlled by the Csr/Rsm systems which allow the best-possible adaptation to host environments faced by the individual pathogens. Complex autoregulated control of CsrA/RsmA function by redundant antagonistic RNAs ensures a fine-tuned adjustment of CsrA/RsmA levels. Moreover, differential regulation of the individual RNAs permits the system to respond to different environmental signals and facilitates adaptation of the signal-sensing and transduction process to specific requirements. In summary, the Csr/Rsm system represents a perfect strategy for evolving pathogens to manage their biological fitness and virulence.

Although the molecular functions of individual components of the Csr/Rsm system are characterized, other important characteristics of the system are still unknown. For instance: What distinguishes a typical CsrA target mRNA from a non-regulated mRNA also containing GGA-motifs? What are the features of the target mRNA and which molecular mechanisms lead to direct activation of protein synthesis by CsrA? Which RNases are involved in the degradation of CsrA-dependent mRNAs? Do the Csr/Rsm-RNAs have individual functions independent of CsrA/RsmA? - and finally - Which external signals and internal regulators are used to control the Csr/Rsm systems? Despite the fact that these challenges must still be addressed, it is evident that the Csr/Rsm system is pivotal for virulence. Whenever the equilibrium of CsrA/RsmA becomes unbalanced, the bacteria are strongly virulence-attenuated.

Based on this fact and the high homology between the CsrA/RsmA regulators, this protein could be a suitable target for antimicrobial drug development. Future work characterizing expression and function of CsrA during the infection process, as well as identification of CsrA target genes in pathogens and harmless commensals will help us to evaluate the potential of CsrA as a drug target.

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Figure Legends

Fig. 1. Molecular function of the Csr system in *Yersinia*.

The dimeric RNA-binding protein CsrA (green) binds to one or more GGA-motifs which overlap or are located adjacent to the ribosome-binding site (RBS). This represses translation initiation due to competition with the 30S ribosomal subunit and accelerates mRNA degradation by RNases (brown). Production of the regulatory RNAs CsrB and CsrC antagonizes the CsrA function. According to the secondary structure predictions, both RNAs form highly structured molecules composed of multiple hairpins with 18 (CsrB) and 14 (CsrC) GGA motifs in the single-stranded loop structures. The GGA motifs are able to bind CsrA molecules and prevent them from binding to their target mRNAs. This results in upregulation of *rovA* transcription. Secondary structures of the CsrB and CsrC RNA of *Y. pseudotuberculosis* were predicted using the RNA folding software Mfold (http://mfold.rna.albany.edu).

Fig. 2. Phylogenetic trees of homologues of the Csr system components of *Yersinia*.

Phylogram trees were created from multiple alignments of gene sequences of the *csrB* (**A**) and *csrC* (**B**) gene family. (**C**) The phylogram tree represents an alignment of amino acid sequences of the CsrA/Rsm protein family. The identity of the respective CsrA/Rsm-like proteins relative to CsrA of *Y. pseudotuberculosis* is given in percentage at the end of the lines. The alignments were performed using ClustalW2 [120] (http://www.ebi.ac.uk/Tools/msa/clustalw2/) with sequences representing the mentioned species *Borrelia burgdorferi* strain B31 (*B. burgdorferi*), *Escherichia coli* (*E. coli*), *Erwinia carotovora* (*E. carotovora*), *Legionella pneumophila* strain Paris (*L. pneumophila*), *Pseudomonas fluorescence* (*P. fluorescence*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enterica* serovar Typhimurium

(*S. typhimurium*), *Shigella flexneri* (*S. flexneri*), *Shigella dysenteriae* (*S. dysenteriae*), *Vibrio cholerae* (*V. cholerae*) and *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*). The identity of the respective CsrA-like proteins relative to CsrA of *Y. pseudotuberculosis* is given in percentage at the end of the lines (C).

Fig. 3. Pleiotropic effects of a *csrA* mutation in *Y. pseudotuberculosis*.

(**A**) Functional classification of genes that were differentially expressed in the *csrA* mutant compared to the parent *Y. pseudotuberculosis* strain YPIII. Strains were grown at 25°C to late stationary phase in LB medium, total RNA of 16 independent cultures was prepared and used for microarray analysis (AK Heroven, unpublished data). (**B**) Effect of CsrA on *Y. pseudotuberculosis* virulence (invasion into M-cells of the intestinal epithelial layer), metabolism (glycogen accumulation), flagella production, cell shape and growth. Comparison was performed with the *Y. pseudotuberculosis* wildtype strain YPIII and the isogenic *csrA* mutant YP53 grown to late exponential phase. Bars represent 1 µm (flagella production) and 10 µm (morphology), respectively. To determine glycogen synthesis, cultures were streaked onto Kornberg medium and incubated for two nights at 25°C before staining with iodine.

Fig. 4. Models of the Csr/Rsm system in *Yersinia* and other pathogenic bacteria. Expression of the Csr/Rsm-RNAs in *Y. pseudotuberculosis* (grey box), *E. coli*, *V. cholera*, *S. typhimurium*, *E. carotovora*, *P. aeruginosa* and *L. pneumophila* is under the positive control of the TCS BarA/UvrY and its homologues VarS/VarA, BarA/SirA, GacS/GacA and LetS/LetA (sensor kinase: dark green; response regulator light green). Activation of the mRNA-binding protein CsrA (RsmA/E) is inhibited by Csr/-Rsm-RNAs. Although the function of the BarA/UvrY-Csr signal transduction cascade is conserved in all depicted pathogenic bacteria, a range of different incoming signals

and participating regulators has been identified, and diverse consequences for the physiology and virulence of the different pathogens have been described. In contrast to the other pathogens, *Y. pseudotuberculosis* CsrC synthesis is not activated by the BarA/UvrY TCS and requires other regulatory factors. Further explanation is provided in the text. *Blue lines*, transcriptional regulation; *red lines*, sequestration; *green lines*, stability; *grey lines*, translation; *solid lines*, direct regulation; *dashed lines*, indirect control; *arrows*, positive control; *perpendicular lines*, negative control; *curved arrow*, phosphorelay.

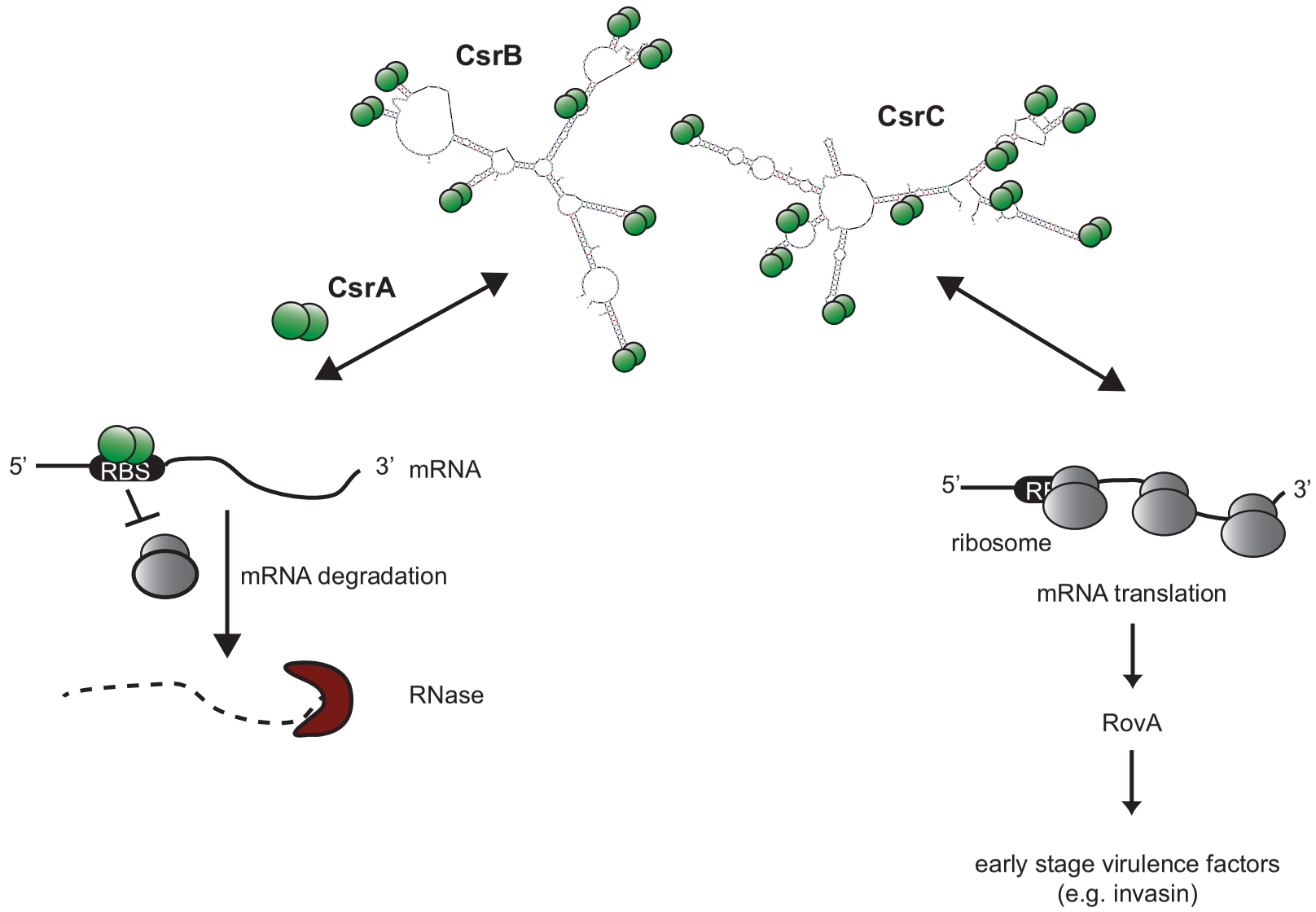
CsrB
CsrC
CsrA
5’
RBS
3’ mRNA
mRNA degradation
RNase
5’
3’
ribosome
mRNA translation
RovA
early stage virulence factors
(e.g. invasin)

Fig. 1

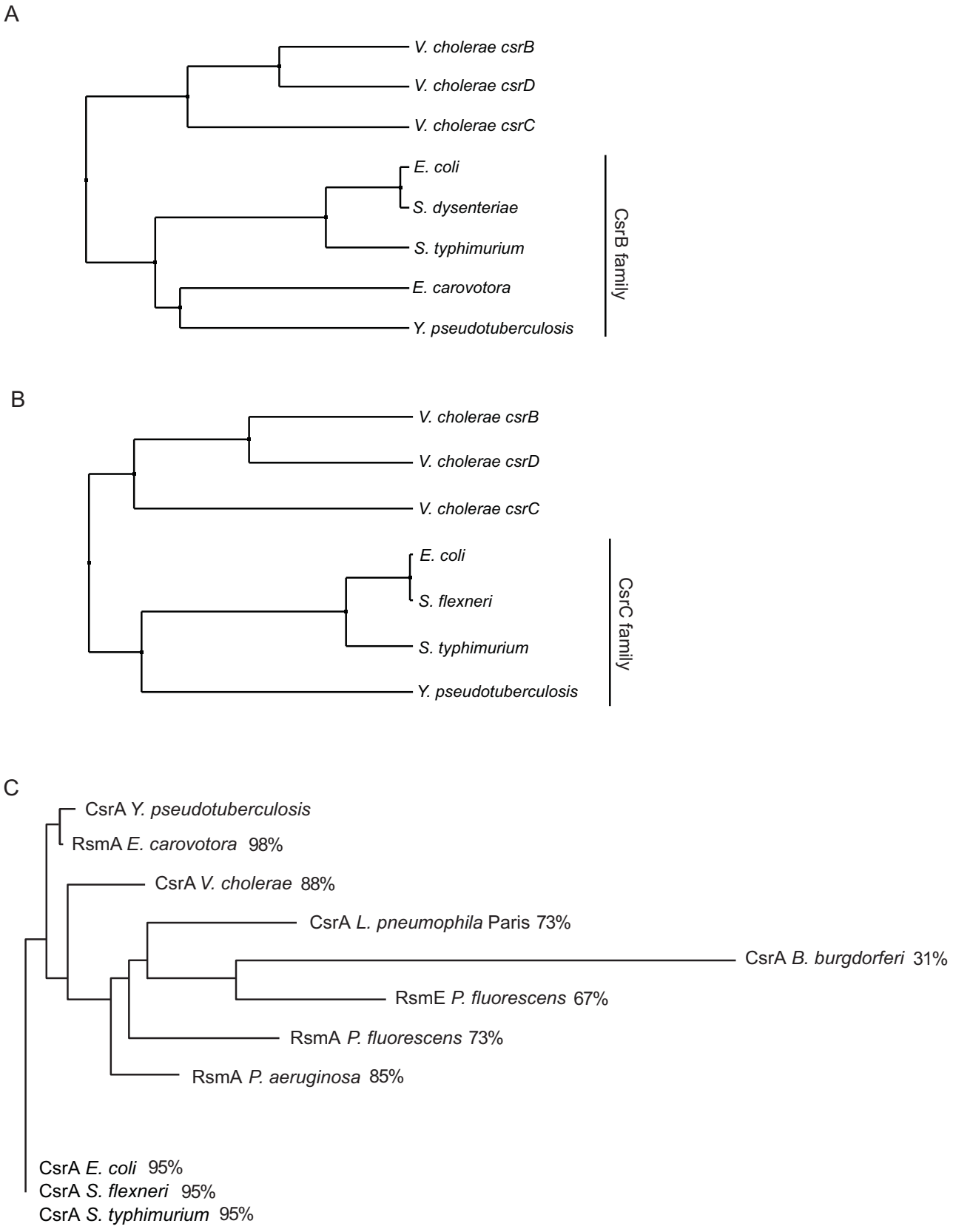
A
V. cholerae csrB
V. cholerae csrD
V. cholerae csrC
E. coli
S. dysenteriae
S. typhimurium
E. carovotora
Y. pseudotuberculosis
CsrB family
B
V. cholerae csrB
V. cholerae csrD
V. cholerae csrC
E. coli
S. flexneri
S. typhimurium
Y. pseudotuberculosis
CsrC family
C
CsrA Y. pseudotuberculosis
RsmA E. carovotora 98%
CsrA V. cholerae 88%
CsrA L. pneumophila Paris 73%
CsrA B. burgdorferi 31%
RsmE P. fluorescens 67%
RsmA P. fluorescens 73%
RsmA P. aeruginosa 85%
CsrA E. coli 95%
CsrA S. flexneri 95%
CsrA S. typhimurium 95%

Fig. 2

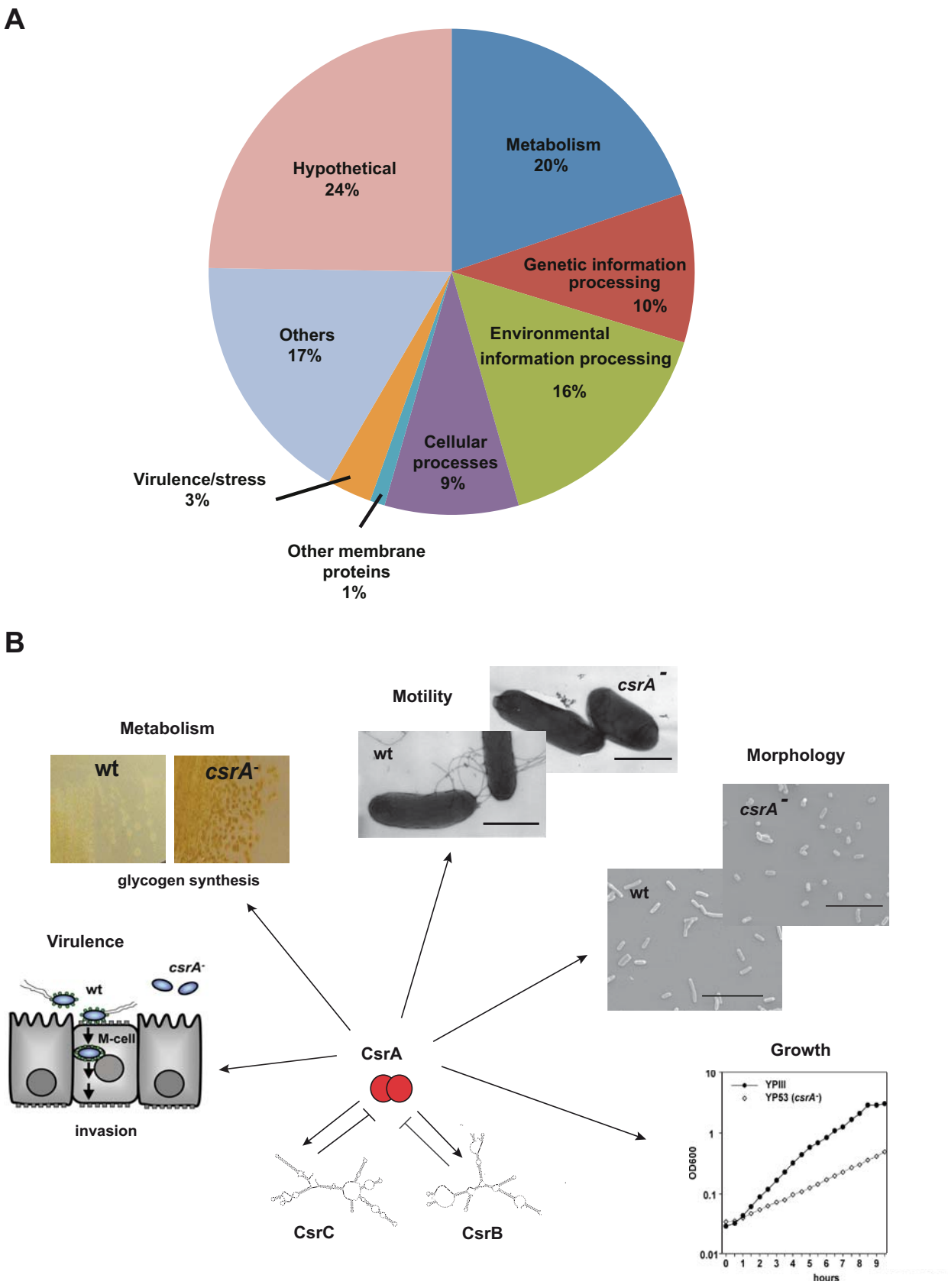
A
Hypothetical 24%
Metabolism 20%
Genetic information processing 10%
Environmental information processing 16%
Cellular processes 9%
Other membrane proteins 1%
Virulence/stress 3%
Others 17%
B
Metabolism
wt
csrA-
glycogen synthesis
Motility
wt
csrA-
Morphology
wt
csrA-
Virulence
wt
csrA-
M-cell
invasion
CsrA
CsrC
CsrB
Growth
YPIII
YP53 (csrA-)
OD600
hours

Fig. 3

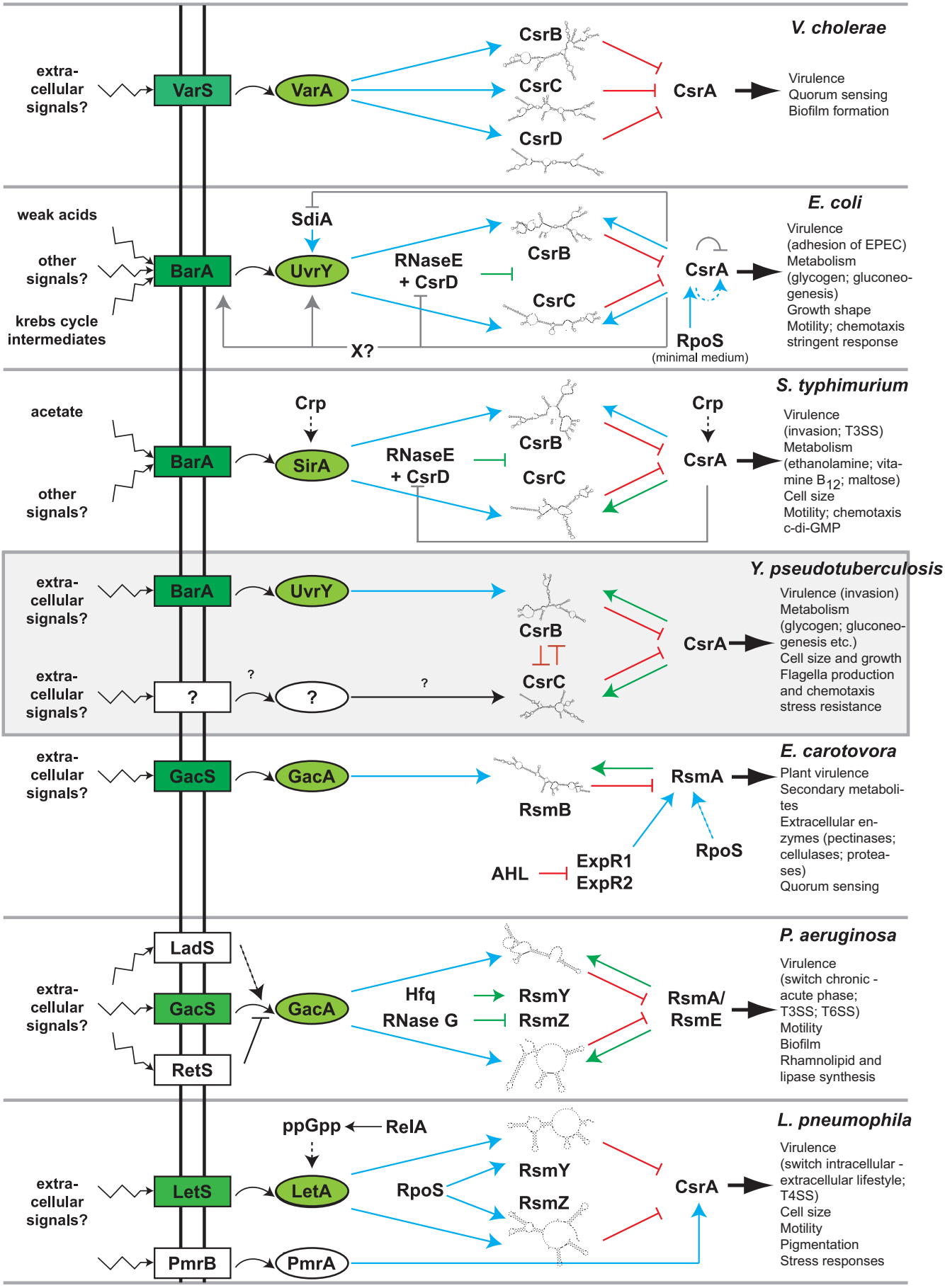
V. cholerae
extra-cellular signals?
VarS
VarA
CsrB
CsrC
CsrD
CsrA
Virulence
Quorum sensing
Biofilm formation
E. coli
weak acids
other signals?
krebs cycle intermediates
BarA
SdiA
UvrY
RNaseE + CsrD
CsrB
CsrC
CsrA
RpoS
(minimal medium)
X?
Virulence (adhesion of EPEC)
Metabolism (glycogen; gluconeo-genesis)
Growth shape
Motility; chemotaxis
stringent response
S. typhimurium
acetate
other signals?
BarA
Crp
SirA
RNaseE + CsrD
CsrB
CsrC
CsrA
Virulence (invasion; T3SS)
Metabolism (ethanolamine; vita-mine B12; maltose)
Cell size
Motility; chemotaxis
c-di-GMP
Y. pseudotuberculosis
extra-cellular signals?
BarA
UvrY
extra-cellular signals?
?
?
?
CsrB
CsrC
CsrA
Virulence (invasion)
Metabolism (glycogen; gluconeo-genesis etc.)
Cell size and growth
Flagella production and chemotaxis
stress resistance
E. carotovora
extra-cellular signals?
GacS
GacA
RsmB
RsmA
RpoS
AHL
ExpR1
ExpR2
Plant virulence
Secondary metaboli-tes
Extracellular en-zymes (pectinases; cellulases; protea-ses)
Quorum sensing
P. aeruginosa
LadS
extra-cellular signals?
GacS
RetS
GacA
Hfq
RNase G
RsmY
RsmZ
RsmA/ RsmE
Virulence (switch chronic - acute phase; T3SS; T6SS)
Motility
Biofilm
Rhamnolipid and lipase synthesis
L. pneumophila
ppGpp
RelA
extra-cellular signals?
LetS
LetA
RpoS
RsmY
RsmZ
CsrA
PmrB
PmrA
Virulence (switch intracellular - extracellular lifestyle; T4SS)
Cell size
Motility
Pigmentation
Stress responses

Fig. 4