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# Regulation of σ factors by conserved partner switches controlled by divergent signaling systems

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**Running title:** Many sensing roads lead to partner switches.

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## Abstract

Partner-Switching Systems (PSS) are widespread regulatory systems, each comprising a kinase-anti-σ, a phosphorylatable anti-σ antagonist and a phosphatase modules. The anti-σ domain quickly sequesters or delivers the target σ factor according to the phosphorylation state of the anti-σ antagonist induced by environmental signals. The PSS components are proteins alone or merged to other domains probably to adapt to the input signals. PSS are involved in major cellular processes including stress response, sporulation, biofilm formation and pathogenesis. Surprisingly, the target σ factors are often unknown and the sensing modules acting upstream from the PSS diverge according to the bacterial species. Indeed, they belong to either two-component systems or complex pathways as the stressosome or Chemosensory Systems (CS).

Based on a phylogenetic analysis, we propose that the sensing module in Gram-negative bacteria is often a CS.

## Introduction

Bacteria have to face constantly many environmental changes in their ecological niches. Their ability to rewire rapidly the expression of genes involved in the response to these alterations is crucial for their survival and is mediated by the use of different alternative σ factors. The σ factors target the core RNA polymerase to the promoter of genes required to adapt to new cell surroundings. All bacteria possess a primary housekeeping σ factor ($\sigma^{70}$) that insures the transcription of the majority of genes, but most bacteria own additional σ factors that coordinate the transcription of many genes involved in response and adaptation of bacteria to changing environmental or cellular conditions. The number of these alternative σ factors varies depending on the lifestyle of the bacteria (Österberg *et al.*, 2011).

Except for *Escherichia coli* $\sigma^{54}$ and homologs, all σ factors belong to the $\sigma^{70}$ family, divided itself into four subfamilies based on phylogenetic and structural properties (Paget and Helmann, 2003; Helmann, 2011). The group 1 of $\sigma^{70}$ family is composed of the whole housekeeping σ factors, $\sigma^{70}$ also called $\sigma^{A}$ in Gram-positive bacteria. The group 2 gathers σ factors that resemble to that of group 1 except that they are not essential in laboratory conditions, as $\sigma^{S}$ factor involved in the General Stress Response (GSR) of proteobacteria (Helmann, 2011; Battesti *et al.*, 2011). The group 3 contains more diverse σ factors, which have roles in various cellular processes as $\sigma^{32}$ that regulates genes involved in response to heat shock, $\sigma^{28}$ in flagella synthesis and also $\sigma^{E}$, $\sigma^{F}$, $\sigma^{G}$, $\sigma^{K}$, of *Bacillus subtilis* that control different stages of sporulation. The group 4 contains very diverse σ factors called $\sigma^{ECF}$ (for ExtraCytoplasmic Function) that have been classified into at least 56 subgroups (Staroń *et al.*, 2009; Huang *et al.*, 2015; Sineva *et al.*, 2017).

Sigma factors play crucial roles to conduct the cellular processes and in turn they have to be tightly regulated. In many cases, sigma factors are controlled at several levels, from transcription to post-translation. At the protein level, we can distinguish three main modes of regulation, the proteolysis of the sigma factor as for $\sigma^{S}$ in *E. coli* (Becker *et al.*, 1999; Hengge, 2009, 2011;

Battesti *et al.*, 2011)*;* the cleavage of the σ factor from an inactive to an active form, including pro-$\sigma^E$ and pro-$\sigma^K$ in *B. subtilis* (Hilbert and Piggot, 2004; Higgins and Dworkin, 2012; Fimlaid and Shen, 2015) and the inactivation of the sigma factor activity by an anti-σ factor (Hughes and Mathee, 1998; Helmann, 1999; Österberg *et al.*, 2011; Feklístov *et al.*, 2014; Paget, 2015). The anti-σ factors sequester their target σ factor, disabling it to interact with the core RNAP and thus to transcribe genes of its regulon (Campbell *et al.*, 2002; Sorenson *et al.*, 2004; Feklístov *et al.*, 2014). In doing so, anti-σ factors could protect their σ factor partner from proteolysis (Barembruch and Hengge, 2007; Mao *et al.*, 2013, 2014; Bouillet *et al.*, 2017). Thus, anti-σ factors enable the cell to maintain a pool of σ factor molecules that can be rapidly released to act without *de novo* synthesis when suddenly required. Most of the $\sigma^{ECF}$ are regulated by anti-σ factors and are usually co-transcribed into the same transcriptional unit to keep a 1:1 stoichiometry of the two proteins (Brooks and Buchanan, 2008; Campagne *et al.*, 2015). The release of the σ factor is also precisely driven to ensure the transcription of its regulon in response to specific signals. Different strategies are thus employed including cell-surface signaling (regulation of $\sigma^{24}$ also called $\sigma^E$ in *E. coli*) (Brooks and Buchanan, 2008; Ho and Ellermeier, 2012), the secretion of the anti-σ factors (FliA/FlgM in *E. coli*) (Hughes *et al.*, 1993; Kutsukake, 1994; Smith and Hoover, 2009), the direct sensing of redox state by cysteine residues of the anti-σ factors (RsrA/$\sigma^R$ in *Streptomyces coelicolor*) (Ilbert *et al.*, 2006; Jung *et al.*, 2011) as well as the involvement of an additional protein called anti-σ factor antagonist in a mechanism known as the partner-switching system (PSS).

**Partner-switching systems**

The term “partner-switch” has been defined by Alper and colleagues in 1994 to describe the mechanism that regulates $\sigma^F$, crucial in the sporulation process of *B. subtilis* (Alper *et al.*, 1994).

The nature of the PSS components as well as their function is a common feature among typical PSS. Indeed, PSS are made up with an anti-σ factor having a serine kinase activity (HATPase domain), a phosphorylatable STAS anti-σ antagonist, a PP2C serine phosphatase and a target σ factor (Alper *et al.*, 1994; Mittenhuber, 2002). Anti-σ factors are constituted of a dimerization

interface and of a HATPase domain. The latter harbors conserved motifs for phosphorylation and is found in many kinase families (Dutta and Inouye, 2000). STAS stands for Sulfate Transporter and Anti-Sigma antagonist and proteins containing this domain play a role in either of these two processes and are phosphorylatable on a specific serine residue (Sharma *et al.*, 2011). The PP2C domain characterizes a family of serine/threonine phosphatases that need metallic ion for their activity (Shi, 2009; Pereira *et al.*, 2011; Bradshaw *et al.*, 2017).

In a partner-switching mechanism, the anti-σ factor binds to its target σ factor disabling it to recruit the core RNAP. The release of the σ factor is mediated by the anti-σ factor antagonist, also called anti-anti-σ factor. Indeed, when no specific signal is transduced to the regulatory system, the anti-σ factor binds to the σ factor and phosphorylates its partner the anti-σ factor antagonist. When the specific signal arises, the PP2C-type phosphatase is activated and dephosphorylates the anti-σ factor antagonist that becomes thus efficient to interact with the anti-σ, leading to the release of the σ factor by a competition effect. Anti-σ factors have usually a better affinity for the unphosphorylated form of the anti-anti-σ factor than for the σ protein allowing the binding of the σ factor only when the anti-σ factor antagonist is phosphorylated but not when it is dephosphorylated (Duncan *et al.*, 1996; Masuda *et al.*, 2004; Bouillet *et al.*, 2016).

A 3D-structure of the anti-σ factor SpoIIAB bound to its partners showed that two monomers of anti-σ factors interact with only one monomer of $\sigma^F$ factor but with two monomers of anti-σ factor antagonist SpoIIAA (Campbell *et al.*, 2002; Masuda *et al.*, 2004). This mode of binding is probably similar for other PSS. Moreover, anti-σ proteins contain a dimerization interface suggesting their ability to dimerize. However, additional biochemical characterization of the interactions of the PSS partners is needed to confirm their mode of action.

Numerous PSS have been discovered in many bacteria including Gram-negative bacteria whereas they were initially thought to be restricted to Gram-positive bacteria (Shi *et al.*, 1999; Mittenhuber, 2002; Kozak *et al.*, 2005; Morris and Visick, 2010; Houot *et al.*, 2012; Morris and Visick, 2013b; Eshghi *et al.*, 2014; Mercer and Lang, 2014; Lambert *et al.*, 2015; Thompson *et al.*, 2015; Bouillet *et al.*, 2016; Gebhardt and Shuman, 2017). Although PSS comprises four

components (an anti-σ factor, an anti-σ factor antagonist, a phosphatase and a σ factor), the domain organization of the partners is highly diversified as depicted in Figure 1. While the anti-σ antagonist usually remains as a one-domain protein, the anti-σ factor can be a domain of a complex protein. Indeed, it can be associated with other domains including receiver domain of typical response regulator, PP2C-type phosphatase domain or unknown function domain. Interestingly, the phosphatase is usually associated with a signaling domain such as receiver, HAMP or detection domains. These data show that PSS have evolved probably according to the detected stresses and to the target σ factor.

In conclusion, although the core domain composition is conserved, the domain organization frequently varies and, consequently, PSS often comprise additional domains (Figure 1) (Mittenhuber, 2002; Galperin, 2006).

Nevertheless, the presence of PSS encoded within bacterial genomes remains yet poorly studied. Phylogenetic analyses of PSS are thus needed to evaluate how widespread are these systems in particular in Gram-negative bacteria. In fact, the presence of HATPase and STAS domains in other proteins and complex domain organizations make difficult the characterization of anti-σ and anti-anti-σ factors in bacteria by using basic bioinformatics.

However, anti-σ factor antagonist and phosphatase homologs have been found in Gram-positive bacteria, as well as cyanobacteria, *Deinococcus* species and proteobacteria including *Pseudomonas, Vibrio* and *Shewanella* species (Mittenhuber, 2002). For example, *B. subtilis* and *B. cereus* possess 16 σ factors and two known PSS targeting $\sigma^F$ and $\sigma^B$, *M. tuberculosis* harbors 13 σ factors, one of which is subjected to two PSS ($\sigma^F$ is submitted to UsfX/RsfAB and to Rv1364c regulation). In *S. oneidensis*, 10 σ factors are present with a known and a putative PSS. Surprisingly, some species including *E. coli* do not seem to possess PSS. In contrast, the Gram-positive bacterium *S. coelicolor* is one of the organisms that contain the highest number of σ factors (60 to 65) and its chromosome has been predicted to encode many PSS partners with 45 anti-σ factors, 18 anti-anti-σ factors and 44 PP2C proteins (Bentley *et al.*, 2002; Mittenhuber, 2002; Martínez *et al.*, 2009). Among them, few have been identified but their study appears

complicated because of cross-talks between several PSS, and anti-σ factor antagonists could have more than one associated anti-σ factor. If we consider that each anti-σ factor antagonist is the output protein from distinct sensory modules, this suggests that the release and thus the activation of the targeted σ factor might be induced by many transducing pathways in response to various signals.

**Role of the Partner-switching systems**

The role of PSS is to control the availability of specific σ factors. However, various PSS have been discovered in many bacteria but the targeted σ factor has not been found in some cases (Figure 1).

PSS signal transduction pathways seem to be implicated in various major cellular processes including the control of type III secretion system synthesis, virulence, chemotaxis, biofilm formation, exoprotein production, stress responses and also metabolism (Shi *et al.*, 1999; Mattoo *et al.*, 2004; Kozak *et al.*, 2005; Bordi *et al.*, 2010; Bhuwan *et al.*, 2012; Houot *et al.*, 2012; Morris and Visick, 2013b, 2013a; Eshghi *et al.*, 2014; Mercer and Lang, 2014; Lambert *et al.*, 2015; Bouillet *et al.*, 2016; Gebhardt and Shuman, 2017). For instance, the spore formation of *B. subtilis* is a complex multi-step mechanism under the control of many regulators (Higgins and Dworkin, 2012; Fimlaid and Shen, 2015). Notably, four main σ factors act successively during the sporulation process. Each of them is thus tightly regulated but has to be also quickly freed to make sure that all genes are correctly expressed in time. $\sigma^F$ is active during the first stage of sporulation only in the forespore compartment. The anti-σ factor SpoIIAB interacts with $\sigma^F$ disabling it to recruit the core RNAP. The release of $\sigma^F$ is permitted by the anti-σ factor antagonist SpoIIAA. When no signal is transduced to the regulatory system, SpoIIAB binds to $\sigma^F$ and also phosphorylates SpoIIAA. When a signal is launched, the PP2C-type phosphatase SpoIIE, which is a membrane-anchored protein that perceives the signal dephosphorylates specifically SpoIIAA. The anti-σ antagonist becomes thus efficient to interact with SpoIIAB, leading to the release of $\sigma^F$ (Diederich *et al.*, 1994; Duncan *et al.*, 1996; Magnin *et al.*, 1997; Campbell *et al.*, 2002; Masuda *et al.*, 2004; Levdikov *et al.*, 2012).

The Rsb partner-switching system regulating the availability of the $\sigma^B$ factor of Bacillales is another PSS that has been extensively studied. $\sigma^B$, the general stress response (GSR) σ factor, is inhibited during growth conditions without stress by the anti-σ factor RsbW and $\sigma^B$ becomes active during stress conditions due to the binding of the dephosphorylated anti-σ factor antagonist RsbV on RsbW (Figures 1 and 2) (Price, 2011).

Beside Gram-positive bacteria PSS models, it has been recently shown in *Shewanella oneidensis*, an aquatic bacterium from the γ-proteobacteria class, $\sigma^S$, the GSR-σ factor, is post-translationally regulated by a PSS (Figures 1 and 2E). This system is composed of the two main proteins CrsA and CrsR, an anti-σ factor antagonist and a three-domain response regulator, respectively. CrsR contains a N-terminal receiver, a central PP2C and a C-terminal HATPase domains. The latter is able to phosphorylate efficiently CrsA whereas the phosphatase domain dephosphorylates it. In addition, direct interactions of CrsR-CrsA and of CrsR-$\sigma^S$ combined with *in vivo* data have revealed that $\sigma^S$ is the target of the PSS. The CrsRA-$\sigma^S$ is the first system that links GSR regulation to PSS in a γ-proteobacterium as it is the case in many other bacterial species (Bouillet *et al.*, 2016, 2017).

In *P. aeruginosa*, the PSS protein HsbR comprises a receiver, a PP2C phosphatase and a HATPase domains. It has been proposed that the last domain could constitute a simple kinase and not an anti-σ factor (Figure 1). The anti-σ factor antagonist HsbA can bind to the anti-σ factor FlgM, inducing the release of the σ factor FliA (or $\sigma^{28}$) (Bhuwan *et al.*, 2012). Furthermore, HsbA is subjected to phosphorylation control by HsbR that, consequently, modulates the activity of HsbA. Contrarily to typical PSS, HsbA acts thus downstream HsbR. This system controls swimming motility through the regulation of the flagella synthesis mediated by FliA. Furthermore, HsbA interacts with the diguanylate cyclase HsbD stimulating therefore the c-di-GMP production (Valentini *et al.*, 2016). This original partner-switch has thus crucial functional implications in both motility control and biofilm development.

The Syp system formed by SypE and SypA of *Vibrio fisheri* as well as the Btr system composed of BtrW and BtrV of *Bordetella pertussis* have important roles in biofilm formation and

pathogenesis through type III secretion system control, respectively (Figure 1). The target σ factor and the mechanism of action have not been unraveled. Interestingly, as for HsbR of *P. aeruginosa*, the HATPase domains of SypE and RsbW could act solely as a kinase and not as an anti-σ factor (Kozak *et al.*, 2005; Morris and Visick, 2013b, 2013a).

Usually, PSS regulate the activity of alternative σ factors but one example of a housekeeping sigma factor regulation by a PSS has been recently brought to light (Figure 1). Indeed, the primary σ factor $\sigma^{66}$ of *Chlamydia trachomatis* is controlled by PSS partners (Hua *et al.*, 2006; Thompson *et al.*, 2015).

**Atypical partner-switching modules**

In *E. coli*, $\sigma^{70}$ activity is also modulated by the anti-σ factor Rsd and the histidine phosphorylatable HPr that acts as an anti-σ factor antagonist. However, even though the mechanism of sequestration and release of the σ factor is alike that of PSS, the two partners Rsd and HPr are not homologous to typical PSS proteins with HPr playing a primary role in the translocation of several sugars across the membrane (Mitchell *et al.*, 2007; Yuan *et al.*, 2008; Hofmann *et al.*, 2011; Park *et al.*, 2013, 2015).

In Gram-negative α-proteobacterial species, the σ factor controlling the GSR called $\sigma^{ECFG}$, RpoE or SigT depending on the bacterium, is controlled by an atypical PSS (Figure 2F) (Francez-Charlot *et al.*, 2009; Staroń *et al.*, 2009; Campagne *et al.*, 2012; Kaczmarczyk *et al.*, 2014; Kim *et al.*, 2014; Francez-Charlot *et al.*, 2015; Fiebig *et al.*, 2015; Herrou *et al.*, 2015; Francez-Charlot *et al.*, 2016). Indeed, the PSS partners are not homologous to canonical PSS proteins. Nevertheless, they use a similar mechanism of sequestration and release of the σ factor as typical PSS. The PSS module of GSR regulation is mostly shared by α-proteobacterial species with little divergences. In all cases, two proteins are involved: the anti-σ factor NepR and the two-domain PhyR. The latter acts as an anti-σ factor antagonist and contains a N-terminal σ-like factor and a C-terminal receiver domains (Figure 2F). NepR possesses homologies with $\sigma^{ECF}$ factors. Phosphorylation of the receiver domain increases the affinity between the σ-like domain of PhyR and NepR so that the $\sigma^{ECF}$ is freed whereas unphosphorylated PhyR has almost no affinity for NepR that in turn binds

to $\sigma^{ECFG}$. The phosphorylation state of PhyR is controlled by various histidine kinases that detect and transduce signals including blue light and osmolytes. As for GSR regulation of Gram-positive bacteria, the composition and the number of the sensory inputs vary greatly from a bacterium to another depending on their lifestyle (Fiebig *et al.*, 2015; Francez-Charlot *et al.*, 2015).

**Activation of partner-switching systems**

Diversified sensory and transducing modules appear to converge to PSS, although the mechanisms of transduction have not been unraveled in most cases. Input modules could be two-component systems, chemosensory systems or might be directly integrated in PSS modules via the addition of sensing domain(s) in PSS proteins (Figures 1 and 2) (Hsu *et al.*, 2008; Morris and Visick, 2013a; Lambert *et al.*, 2015; Norsworthy and Visick, 2015; Chambonnier *et al.*, 2016). In the Gram-positive bacteria *Bacillus, Listeria* and *Staphyloccoccus,* the stress responsive σ factor $\sigma^B$ is submitted to a PSS. The PSS module formed by the anti-σ factor RsbW and the anti-σ factor antagonist RsbV is conserved but the RsbV phosphatases as well as the mechanisms of signal transduction to the PSS module diverge from one species to another (Figure 2A and 2B).

In *B. subtilis*, two sensing modules linked to two independent PP2C-containing phosphatases (RsbU and RsbP) converge to the PSS module. Environmental stresses including blue light, heat shock and osmolytes are detected by a protein complex called the stressosome that subsequently transduces signals to the PP2C phosphatase RsbU. The energetic level of the cell is perceived by the PAS domain containing phosphatase RsbP associated with the hydrolase RsbQ (Figure 2A) (Benson and Haldenwang, 1993; Boylan *et al.*, 1993; Voelker *et al.*, 1996; Kim *et al.*, 2004; Marles-Wright *et al.*, 2008; Marles-Wright and Lewis, 2010; Price, 2011; Gaidenko and Price, 2014; Guldimann *et al.*, 2016). RsbU, RsbP and a stressosome are absent from other *Bacillales* as *B. cereus*. They are replaced by the RsbY protein composed of a receiver fused to a PP2C domain and the complex histidine kinase RsbK, which is able to detect internal and environmental stresses and thus transduces the signal to RsbY (Figure 2B) (van Schaik *et al.*, 2005; de Been *et al.*, 2010, 2011). Interestingly, genes coding for a stressosome-like complex are also found in many species in particular among the proteobacteria, cyanobacteria and

actinobacteria phyla (Pané-Farré *et al.*, 2005; Jia *et al.*, 2016). Likewise, homologs of the histidine kinase RsbK have been found in many species including the proteobacterial genus *Vibrio, Pseudomonas, Magnetoccocus* or *Myxococcus* but their roles are still unknown (de Been *et al.*, 2011).

*Streptomyces coelicolor* PSS regulation of $\sigma^B$ resembles that of *B. subtilis* but possesses an additional PSS absent from other species (Lee *et al.*, 2004): the Osa system that regulates $\sigma^B$ under "back to normal" conditions after an osmotic shock. OsaA is a RsbK homolog that may detect signals from a GAF domain, OsaB is a two-domain protein with a N-terminal receiver and an unknown C-terminal domain, and OsaC contains an anti-σ factor, a PAS, two GAF and a PP2C-type phosphatase domains (Figure 2C) (Martínez *et al.*, 2009; Price, 2011). The regulatory cascade that regulates $\sigma^B$ availability is still unknown but the domain composition of OsaC suggests a direct additional signal sensing by the phosphatase protein. In the Actinomycetales *Mycobacterium tuberculosis*, the GSR σ factor called $\sigma^F$ is also mediated by two PSS (DeMaio *et al.*, 1997). Notably, the protein Rv1364c is a PSS module organized in four domains corresponding to a PAS, a phosphatase, an anti-σ factor and an anti-σ factor antagonist domain (Parida *et al.*, 2005; Sachdeva *et al.*, 2008; Greenstein *et al.*, 2009; Malik *et al.*, 2009; Jaiswal *et al.*, 2010; King-Scott *et al.*, 2011). It has been shown that its anti-σ factor domain can bind to $\sigma^F$ whereas its anti-σ factor antagonist domain antagonizes the action of the anti-σ domain. However, the complex network of $\sigma^F$ post-translational regulation has not been completely unraveled yet, but Rv1364c seems to detect signals itself without upstream sensing module.

The PSS composed of HsbR and HsbA from *P. aeruginosa* is activated by a complex phosphocascade. The histidine phosphotransfer protein HptB constitutes the module activating HsbR. HptB acts in the GacA-GacS two-component pathway regulating sRNA involved in *P. aeruginosa* biofilm formation and pathogenesis (Lin *et al.*, 2006; Hsu *et al.*, 2008; Bordi *et al.*, 2010; Bhuwan *et al.*, 2012; Houot *et al.*, 2012; Chambonnier *et al.*, 2016). Moreover, other histidine kinases have been shown to detect specific signals and transduce them by

phosphorylating HptB that in turn phosphorylates the receiver domain of HsbR, activating the phosphatase domain of HsbR (Hsu *et al.*, 2008).

**Activation of partner-switching modules in various proteobacteria: a role for chemosensory systems**

Chemosensory systems (CS) are complex signal transduction pathways mainly involve in the regulation of the flagella rotation necessary for swimming motility in most bacteria. Since then, many CS that do not control bacterial motility have been spotted in various bacteria. They were shown to play a role in the cellular differentiation of *Myxococcus xanthus* and *Rhodospirillum centenum*, in the production of molecules involved in biofilm formation as c-di-GMP or EPS in *P. aeruginosa* and *Azospirillum brasilense* or in the type IV pili based motility in *P. aeruginosa*. In fact, a large number of bacteria possess more than one CS coding locus in their genome, but their roles are still largely unknown (Kirby, 2009; Wuichet and Zhulin, 2010; He and Bauer, 2014).

In *S. oneidensis*, the genes coding for the two PSS proteins CrsA and CrsR are located in the *che1* locus, composed of 10 genes with 8 of them coding for classical CS components (Che proteins). This system is not involved in swimming motility and could form a chemosensory system (Armitano *et al.*, 2013). This gene organization strongly suggests that the chemosensory system Che1 contains the signal sensing machinery that regulates the activity of the CrsR-CrsA PSS.

Interestingly, it appears that chemosensory machinery adapted to regulate a partner-switch is a common feature of aquatic proteobacteria (Figure 3). Indeed, *crsR* gene homologs are widespread among those bacteria and an analysis of the genes surrounding *crsR* in 59 bacterial genomes was carried out for this review (Bouillet *et al.*, 2017). This phylogenetic analysis clearly shows that the CrsR-CrsA PSS is most of the time genetically related to a *che* locus. As shown in Figure 3, the *crsR-crsA* genes are located in the vicinity of a central histidine kinase *cheA* gene in 71% of these bacteria (32 out of 45), or of two-component histidine kinase(s). This indicates that the sensing modules of the CrsR-CrsA partner-switch could predominantly be a chemosensory system. Interestingly, two genes coding for detectors MCP (Methyl-accepting Chemotaxis Proteins) are comprised in the *che1* locus of *S. oneidensis*. One of them is predicted

to be anchored to the membrane whereas the other seems to be located in the cytosol and possesses two PAS domains (Figure 2). The two MCP could thus allow the detection of different kinds of signals: one from outside and the other from inside the cell cytoplasm. Interestingly, many PSS controlling the GSR including those in *B. cereus* and in some alpha-proteobacterial species often comprise two sensory detectors presenting similar sensing domains as those found in MCP (Figure 2 and Figure 3). It has been shown that the RsbK histidine kinase from *B. cereus* that controls the downstream RsbVWY PSS is subjected to the methylation by the methyltransferase RsbM, as usually seen in MCP (Chen *et al.*, 2012, 2015). The methylation of RsbK by RsbM leads to the inhibition of $\sigma^B$. As the *che1* locus of *S. oneidensis* contains a gene encoding the methyltransferase CheR1, we propose that $\sigma^S$ sequestration by CrsR could also be modulated by the methylation level of the two MCP.

PSS imbedded in a CS operon has been recently described in *Leptospira interrogans*. This operon contains genes encoding a response regulator composed of a receiver and an anti-σ factor domains, an anti-σ factor antagonist and chemosensory proteins (CheA, CheY, CheW, CheD, CheB, MCP). This PSS that controls a still unknown σ factor could be regulated by the chemosensory system (Eshghi *et al.*, 2014; Lambert *et al.*, 2015).

Two-component systems or signal transduction coding genes have also been found in the neighborhood of *crsR* homologs and could thus be the sensor that detects signals and transduces them to the PSS. Genes encoding Hpt proteins are also found near to *crsR-crsA* genes in *Hahella ganghwensis* and *Marinobacter lipolyticus* (Figure 3). This illustrates that the sensing modules acting upstream PSS diverge from one species to another.

**The case of GSR regulation in bacteria**

The main strategy commonly developed by bacteria to respond, defend and adapt to general stresses is to modify its transcriptional program in order to express appropriate genes. This ability is mediated by the use of a specific σ factor. Gram-positive bacteria as *Bacillales* and *Actinomycetales* possess a type-3 σ factor named $\sigma^B$ or $\sigma^F$, whereas β- and γ-proteobacteria hold the type-2 σ factor $\sigma^S$ and α-proteobacteria use a type-4 $\sigma^{ECF}$ often called $\sigma^{ECFG}$ (Boylan *et al.*,

1993; Battesti *et al.*, 2011; Hengge, 2011; Price, 2011; Fiebig *et al.*, 2015; Francez-Charlot *et al.*, 2015). Despite the fact that all these σ factors are not homologous and present large differences in terms of sequence and structure, they control analogous processes in the cells (Alvarez-Martinez *et al.*, 2006; Sauviac *et al.*, 2007; Gourion *et al.*, 2009; Martínez-Salazar *et al.*, 2009; Britos *et al.*, 2011; Hengge, 2011; Foreman *et al.*, 2012; Jans *et al.*, 2013; Kim *et al.*, 2013; Landini *et al.*, 2014; Guldimann *et al.*, 2016). Moreover, although their global regulation is highly divergent, the presence of a PSS is a relatively common feature for their post-translational regulation (Figure 2).

Strikingly, although *B. cereus* and *S. oneidensis* are distant species and use the two non-homologous GSR σ factors $\sigma^B$ and $\sigma^S$, respectively, the pathways that control their post-translational activity are similar. Conversely, *E. coli* and *S. oneidensis* are both γ-proteobacteria and both possess $\sigma^S$ to regulate GSR, but their post-translational regulations of $\sigma^S$ are entirely different (Battesti *et al.*, 2011; Price, 2011). This strongly suggests that these regulatory pathways can be more related between bacteria that share common lifestyle than phylogenetic proximity.

**Concluding Remarks**

Since a couple of years, novel PSS have been detected in many bacteria. These PSS play a key role in major cellular processes although the partner σ factor has not been determined in several cases. PSS are common post-translational regulators for the control of the response to general stress but they are also involved in crucial cell processes as motility, biofilm formation, virulence, and cell differentiation as sporulation. Other physiological roles of PSS will be undoubtedly discovered in the next future.

In fact, based solely on basic bioinformatics, it is quite difficult to find out PSS genes within bacterial genomes mainly because their HATPase domain is common to other types of proteins. Furthermore, each species has to adapt to its own environment, doing this, it has set up dedicated regulatory pathways. PSS and the sensing modules have thus evolved to adapt to their target σ factors and to the signals this σ factor has to be responding to. As a consequence, the

domain organization of the PSS (Figures 1 and 2) and the composition of the sensing modules vary greatly (Figures 2 and 3). In other words, a common signal transduction pathway like PSS can be activated by a large range of sensing machineries.

In conclusion, recent results have revealed that PSS are found not only in Gram-positive but also in Gram-negative bacteria. Moreover, many PSS are governed by complex signaling pathways including two-component and chemosensory systems. PSS are clearly very efficient and rapid ways to trigger or stop specific σ factor responses. Therefore, future studies will most probably reveal novel module architectures for PSS and their regulatory pathways to better respond to the numberless environmental signals encountered by bacteria.

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**References**

Alper, S., Duncan, L., and Losick, R. (1994) An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in *B. subtilis*. *Cell* **77**: 195–205.

Alvarez-Martinez, C.E., Baldini, R.L., and Gomes, S.L. (2006) A *Caulobacter crescentus* Extracytoplasmic Function Sigma Factor Mediating the Response to Oxidative Stress in Stationary Phase. *J. Bacteriol.* **188**: 1835–1846.

Armitano, J., Méjean, V., and Jourlin-Castelli, C. (2013) Aerotaxis governs floating biofilm formation in *Shewanella oneidensis*. *Environ. Microbiol.* **15**: 3108–3118.

Barembruch, C. and Hengge, R. (2007) Cellular levels and activity of the flagellar sigma factor FliA of *Escherichia coli* are controlled by FlgM-modulated proteolysis. *Mol. Microbiol.* **65**: 76–89.

Battesti, A., Majdalani, N., and Gottesman, S. (2011) The RpoS-mediated general stress response in *Escherichia coli*. *Annu. Rev. Microbiol.* **65**: 189–213.

Becker, G., Klauck, E., and Hengge-Aronis, R. (1999) Regulation of RpoS proteolysis in *Escherichia coli*: The response regulator RssB is a recognition factor that interacts with the turnover element in RpoS. *Proc. Natl. Acad. Sci. U. S. A.* **96**: 6439–6444.

de Been, M., Francke, C., Siezen, R.J., and Abee, T. (2011) Novel sigmaB regulation modules of Gram-positive bacteria involve the use of complex hybrid histidine kinases. *Microbiol. Read. Engl.* **157**: 3–12.

de Been, M., Tempelaars, M.H., van Schaik, W., Moezelaar, R., Siezen, R.J., and Abee, T. (2010) A novel hybrid kinase is essential for regulating the sigma(B)-mediated stress response of *Bacillus cereus. Environ. Microbiol.* **12**: 730–745.

Benson, A.K. and Haldenwang, W.G. (1993) Regulation of sigma B levels and activity in *Bacillus subtilis*. *J. Bacteriol.* **175**: 2347–2356.

Bentley, S.D., Chater, K.F., Cerdeño-Tárraga, A.-M., Challis, G.L., Thomson, N.R., James, K.D., et al. (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**: 141–147.

Bhuwan, M., Lee, H.-J., Peng, H.-L., and Chang, H.-Y. (2012) Histidine-containing phosphotransfer protein-B (HptB) regulates swarming motility through partner-switching system in *Pseudomonas aeruginosa* PAO1 strain. *J. Biol. Chem.* **287**: 1903–1914.

Bordi, C., Lamy, M.-C., Ventre, I., Termine, E., Hachani, A., Fillet, S., et al. (2010) Regulatory RNAs and the HptB/RetS signalling pathways fine-tune *Pseudomonas aeruginosa* pathogenesis. *Mol. Microbiol.* **76**: 1427–1443.

Bouillet, S., Genest, O., Jourlin-Castelli, C., Fons, M., Méjean, V., and Iobbi-Nivol, C. (2016) The General Stress Response σS Is Regulated by a Partner Switch in the Gram-negative Bacterium *Shewanella oneidensis*. *J. Biol. Chem.* **291**: 26151–26163.

Bouillet, S., Genest, O., Méjean, V., and Iobbi-Nivol, C. (2017) Protection of the General Stress Response σ(S) by CrsR Allows a Rapid and Efficient Adaptation of *Shewanella oneidensis. J. Biol. Chem.*

Boylan, S.A., Redfield, A.R., Brody, M.S., and Price, C.W. (1993) Stress-induced activation of the sigma B transcription factor of *Bacillus subtilis*. *J. Bacteriol.* **175**: 7931–7937.

Bradshaw, N., Levdikov, V.M., Zimanyi, C.M., Gaudet, R., Wilkinson, A.J., and Losick, R. (2017) A widespread family of serine/threonine protein phosphatases shares a common regulatory switch with proteasomal proteases. *eLife* **6**:.

Britos, L., Abeliuk, E., Taverner, T., Lipton, M., McAdams, H., and Shapiro, L. (2011) Regulatory response to carbon starvation in *Caulobacter crescentus. PloS One* **6**: e18179.

Brooks, B.E. and Buchanan, S.K. (2008) Signaling mechanisms for activation of extracytoplasmic function (ECF) sigma factors. *Biochim. Biophys. Acta* **1778**: 1930–1945.

Campagne, S., Allain, F.H.-T., and Vorholt, J.A. (2015) Extra Cytoplasmic Function sigma factors, recent structural insights into promoter recognition and regulation. *Curr. Opin. Struct. Biol.* **30**: 71–78.

Campagne, S., Damberger, F.F., Kaczmarczyk, A., Francez-Charlot, A., Allain, F.H.-T., and Vorholt, J.A. (2012) Structural basis for sigma factor mimicry in the general stress response of Alphaproteobacteria. *Proc. Natl. Acad. Sci. U. S. A.* **109**: E1405-1414.

Campbell, E.A., Masuda, S., Sun, J.L., Muzzin, O., Olson, C.A., Wang, S., and Darst, S.A. (2002) Crystal Structure of the *Bacillus stearothermophilus* Anti-σ Factor SpoIIAB with the Sporulation σ Factor σF. *Cell* **108**: 795–807.

Chambonnier, G., Roux, L., Redelberger, D., Fadel, F., Filloux, A., Sivaneson, M., et al. (2016) The Hybrid Histidine Kinase LadS Forms a Multicomponent Signal Transduction System with

the GacS/GacA Two-Component System in *Pseudomonas aeruginosa. PLoS Genet.* **12**: e1006032.
Chen, J.-C., Liu, J.-H., Hsu, D.-W., Shu, J.-C., Chen, C.-Y., and Chen, C.-C. (2015) Methylatable Signaling Helix Coordinated Inhibitory Receiver Domain in Sensor Kinase Modulates Environmental Stress Response in *Bacillus Cereus. PLoS ONE* **10**:.
Chen, L.-C., Chen, J.-C., Shu, J.-C., Chen, C.-Y., Chen, S.-C., Chen, S.-H., et al. (2012) Interplay of RsbM and RsbK controls the σB activity of *Bacillus cereus. Environ. Microbiol.* **14**: 2788–2799.
DeMaio, J., Zhang, Y., Ko, C., and Bishai, W.R. (1997) *Mycobacterium tuberculosis* sigF is part of a gene cluster with similarities to the Bacillus subtilis sigF and sigB operons. *Tuber. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis.* **78**: 3–12.
Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., et al. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **36**: W465-469.
Diederich, B., Wilkinson, J.F., Magnin, T., Najafi, M., Erringston, J., and Yudkin, M.D. (1994) Role of interactions between SpoIIAA and SpoIIAB in regulating cell-specific transcription factor sigma F of *Bacillus subtilis. Genes Dev.* **8**: 2653–2663.
Duncan, L., Alper, S., and Losick, R. (1996) SpoIIAA governs the release of the cell-type specific transcription factor sigma F from its anti-sigma factor SpoIIAB. *J. Mol. Biol.* **260**: 147–164.
Dutta, R. and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem. Sci.* **25**: 24–28.
Eshghi, A., Becam, J., Lambert, A., Sismeiro, O., Dillies, M.-A., Jagla, B., et al. (2014) A putative regulatory genetic locus modulates virulence in the pathogen *Leptospira interrogans. Infect. Immun.* **82**: 2542–2552.
Feklístov, A., Sharon, B.D., Darst, S.A., and Gross, C.A. (2014) Bacterial sigma factors: a historical, structural, and genomic perspective. *Annu. Rev. Microbiol.* **68**: 357–376.
Fiebig, A., Herrou, J., Willett, J., and Crosson, S. (2015) General Stress Signaling in the Alphaproteobacteria. *Annu. Rev. Genet.* **49**: null.
Fimlaid, K.A. and Shen, A. (2015) Diverse mechanisms regulate sporulation sigma factor activity in the Firmicutes. *Curr. Opin. Microbiol.* **24**: 88–95.
Foreman, R., Fiebig, A., and Crosson, S. (2012) The LovK-LovR Two-Component System Is a Regulator of the General Stress Pathway in *Caulobacter crescentus*. *J. Bacteriol.* **194**: 3038–3049.
Francez-Charlot, A., Frunzke, J., Reichen, C., Ebneter, J.Z., Gourion, B., and Vorholt, J.A. (2009) Sigma factor mimicry involved in regulation of general stress response. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 3467–3472.
Francez-Charlot, A., Frunzke, J., Zingg, J., Kaczmarczyk, A., and Vorholt, J.A. (2016) Multiple σEcfG and NepR Proteins Are Involved in the General Stress Response in *Methylobacterium extorquens*. *PLoS ONE* **11**:.
Francez-Charlot, A., Kaczmarczyk, A., Fischer, H.-M., and Vorholt, J.A. (2015) The general stress response in Alphaproteobacteria. *Trends Microbiol.* **23**: 164–171.
Gaidenko, T.A. and Price, C.W. (2014) Genetic evidence for a phosphorylation-independent signal transduction mechanism within the *Bacillus subtilis* stressosome. *PloS One* **9**: e90741.
Galperin, M.Y. (2006) Structural Classification of Bacterial Response Regulators: Diversity of Output Domains and Domain Combinations. *J. Bacteriol.* **188**: 4169–4182.
Gebhardt, M.J. and Shuman, H.A. (2017) GigA and GigB are Master Regulators of Antibiotic Resistance, Stress Responses and Virulence in *Acinetobacter baumannii. J. Bacteriol.*

Gourion, B., Sulser, S., Frunzke, J., Francez-Charlot, A., Stiefel, P., Pessi, G., et al. (2009) The PhyR-sigma(EcfG) signalling cascade is involved in stress response and symbiotic efficiency in *Bradyrhizobium japonicum*. *Mol. Microbiol.* **73**: 291–305.

Greenstein, A.E., Hammel, M., Cavazos, A., and Alber, T. (2009) Interdomain communication in the *Mycobacterium tuberculosis* environmental phosphatase Rv1364c. *J. Biol. Chem.* **284**: 29828–29835.

Guldimann, C., Boor, K.J., Wiedmann, M., and Guariglia-Oropeza, V. (2016) Resilience in the face of uncertainty: sigma B fine-tunes gene expression to support homeostasis in Gram-positive bacteria. *Appl. Environ. Microbiol.* AEM.00714-16.

He, K. and Bauer, C.E. (2014) Chemosensory signaling systems that control bacterial survival. *Trends Microbiol.* **22**: 389–398.

Helmann, J.D. (1999) Anti-sigma factors. *Curr. Opin. Microbiol.* **2**: 135–141.

Helmann J.D. (2011) Regulation by Alternative Sigma Factors. In, *Bacterial Stress Responses, 2nd ed.* Gisela Storz and Regine Hengge, pp. 31–43.

Hengge, R. (2009) Proteolysis of sigmaS (RpoS) and the general stress response in *Escherichia coli. Res. Microbiol.* **160**: 667–676.

Hengge, R. (2011) The General Stress Response in Gram-negative Bacteria. In, *Bacterial Stress Responses, 2nd edition, ASM.* Storz, Gisela and Hengge, Regine, p. 251.

Herrou, J., Willett, J.W., and Crosson, S. (2015) Structured and Dynamic Disordered Domains Regulate the Activity of a Multifunctional Anti-σ Factor. *mBio* **6**: e00910-15.

Higgins, D. and Dworkin, J. (2012) Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiol. Rev.* **36**: 131–148.

Hilbert, D.W. and Piggot, P.J. (2004) Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol. Mol. Biol. Rev. MMBR* **68**: 234–262.

Ho, T.D. and Ellermeier, C.D. (2012) Extra cytoplasmic function σ factor activation. *Curr. Opin. Microbiol.* **15**: 182–188.

Hofmann, N., Wurm, R., and Wagner, R. (2011) The *E. coli* anti-sigma factor Rsd: studies on the specificity and regulation of its expression. *PloS One* **6**: e19235.

Houot, L., Fanni, A., de Bentzmann, S., and Bordi, C. (2012) A bacterial two-hybrid genome fragment library for deciphering regulatory networks of the opportunistic pathogen *Pseudomonas aeruginosa*. *Microbiol.* **158**: 1964–1971.

Hsu, J.-L., Chen, H.-C., Peng, H.-L., and Chang, H.-Y. (2008) Characterization of the histidine-containing phosphotransfer protein B-mediated multistep phosphorelay system in *Pseudomonas aeruginosa* PAO1. *J. Biol. Chem.* **283**: 9933–9944.

Hua, L., Hefty, P.S., Lee, Y.J., Lee, Y.M., Stephens, R.S., and Price, C.W. (2006) Core of the partner switching signalling mechanism is conserved in the obligate intracellular pathogen *Chlamydia trachomatis*. *Mol. Microbiol.* **59**: 623–636.

Huang, X., Pinto, D., Fritz, G., and Mascher, T. (2015) Environmental Sensing in Actinobacteria: a Comprehensive Survey on the Signaling Capacity of This Phylum. *J. Bacteriol.* **197**: 2517–2535.

Hughes, K.T., Gillen, K.L., Semon, M.J., and Karlinsey, J.E. (1993) Sensing structural intermediates in bacterial flagellar assembly by export of a negative regulator. *Science* **262**: 1277–1280.

Hughes, K.T. and Mathee, K. (1998) The anti-sigma factors. *Annu. Rev. Microbiol.* **52**: 231–286.

Ilbert, M., Graf, P.C.F., and Jakob, U. (2006) Zinc center as redox switch--new function for an old motif. *Antioxid. Redox Signal.* **8**: 835–846.

Jaiswal, R.K., Manjeera, G., and Gopal, B. (2010) Role of a PAS sensor domain in the *Mycobacterium tuberculosis* transcription regulator Rv1364c. *Biochem. Biophys. Res. Commun.* **398**: 342–349.

Jans, A., Vercruysse, M., Gao, S., Engelen, K., Lambrichts, I., Fauvart, M., and Michiels, J. (2013) Canonical and non-canonical EcfG sigma factors control the general stress response in *Rhizobium etli*. *MicrobiologyOpen* **2**: 976–987.

Jia, X., Wang, J., Rivera, S., Duong, D., and Weinert, E.E. (2016) An O2-sensing stressosome from a Gram-negative bacterium. *Nat. Commun.* **7**: 12381.

Jung, Y.-G., Cho, Y.-B., Kim, M.-S., Yoo, J.-S., Hong, S.-H., and Roe, J.-H. (2011) Determinants of redox sensitivity in RsrA, a zinc-containing anti-sigma factor for regulating thiol oxidative stress response. *Nucleic Acids Res.* **39**: 7586–7597.

Kaczmarczyk, A., Hochstrasser, R., Vorholt, J.A., and Francez-Charlot, A. (2014) Complex two-component signaling regulates the general stress response in Alphaproteobacteria. *Proc. Natl. Acad. Sci. U. S. A.* **111**: E5196-5204.

Kim, H.-S., Caswell, C.C., Foreman, R., Roop, R.M., and Crosson, S. (2013) The *Brucella abortus* General Stress Response System Regulates Chronic Mammalian Infection and Is Controlled by Phosphorylation and Proteolysis. *J. Biol. Chem.* **288**: 13906–13916.

Kim, H.-S., Willett, J.W., Jain-Gupta, N., Fiebig, A., and Crosson, S. (2014) The *Brucella abortus* virulence regulator, LovhK, is a sensor kinase in the general stress response signalling pathway. *Mol. Microbiol.* **94**: 913–925.

Kim, T.-J., Gaidenko, T.A., and Price, C.W. (2004) In vivo phosphorylation of partner switching regulators correlates with stress transmission in the environmental signaling pathway of *Bacillus subtilis*. *J. Bacteriol.* **186**: 6124–6132.

King-Scott, J., Konarev, P.V., Panjikar, S., Jordanova, R., Svergun, D.I., and Tucker, P.A. (2011) Structural characterization of the multidomain regulatory protein Rv1364c from *Mycobacterium tuberculosis*. *Struct. Lond. Engl. 1993* **19**: 56–69.

Kirby, J.R. (2009) Chemotaxis-like regulatory systems: unique roles in diverse bacteria. *Annu. Rev. Microbiol.* **63**: 45–59.

Kozak, N.A., Mattoo, S., Foreman-Wykert, A.K., Whitelegge, J.P., and Miller, J.F. (2005) Interactions between partner switcher orthologs BtrW and BtrV regulate type III secretion in *Bordetella*. *J. Bacteriol.* **187**: 5665–5676.

Krummenacker, M., Paley, S., Mueller, L., Yan, T., and Karp, P.D. (2005) Querying and computing with BioCyc databases. *Bioinforma. Oxf. Engl.* **21**: 3454–3455.

Kutsukake, K. (1994) Excretion of the anti-sigma factor through a flagellar substructure couples flagellar gene expression with flagellar assembly in *Salmonella typhimurium*. *Mol. Gen. Genet. MGG* **243**: 605–612.

Lambert, A., Wong Ng, J., and Picardeau, M. (2015) Gene inactivation of a chemotaxis operon in the pathogen *Leptospira interrogans*. *FEMS Microbiol. Lett.* **362**: 1–8.

Landini, P., Egli, T., Wolf, J., and Lacour, S. (2014) sigmaS, a major player in the response to environmental stresses in *Escherichia coli*: role, regulation and mechanisms of promoter recognition. *Environ. Microbiol. Rep.* **6**: 1–13.

Lee, E.-J., Cho, Y.-H., Kim, H.-S., Ahn, B.-E., and Roe, J.-H. (2004) Regulation of σB by an Anti- and an Anti-Anti-Sigma Factor in *Streptomyces coelicolor* in Response to Osmotic Stress. *J. Bacteriol.* **186**: 8490–8498.

Levdikov, V.M., Blagova, E.V., Rawlings, A.E., Jameson, K., Tunaley, J., Hart, D.J., et al. (2012) Structure of the phosphatase domain of the cell fate determinant SpoIIE from *Bacillus subtilis*. *J. Mol. Biol.* **415**: 343–358.

Lin, C.-T., Huang, Y.-J., Chu, P.-H., Hsu, J.-L., Huang, C.-H., and Peng, H.-L. (2006) Identification of an HptB-mediated multi-step phosphorelay in *Pseudomonas aeruginosa* PAO1. *Res. Microbiol.* **157**: 169–175.

Magnin, T., Lord, M., and Yudkin, M.D. (1997) Contribution of partner switching and SpoIIAA cycling to regulation of sigmaF activity in sporulating *Bacillus subtilis*. *J. Bacteriol.* **179**: 3922–3927.

Malik, S.S., Luthra, A., and Ramachandran, R. (2009) Interactions of the *M. tuberculosis* UsfX with the cognate sigma factor SigF and the anti-anti sigma factor RsfA. *Biochim. Biophys. Acta* **1794**: 541–553.

Mao, X.-M., Ren, N.-N., Sun, N., Wang, F., Zhou, R.-C., Tang, Y., and Li, Y.-Q. (2014) Proteasome involvement in a complex cascade mediating SigT degradation during differentiation of *Streptomyces coelicolor*. *FEBS Lett.* **588**: 608–613.

Mao, X.-M., Sun, N., Wang, F., Luo, S., Zhou, Z., Feng, W.-H., et al. (2013) Dual positive feedback regulation of protein degradation of an extra-cytoplasmic function σ factor for cell differentiation in *Streptomyces coelicolor. J. Biol. Chem.* **288**: 31217–31228.

Marles-Wright, J., Grant, T., Delumeau, O., van Duinen, G., Firbank, S.J., Lewis, P.J., et al. (2008) Molecular architecture of the "stressosome," a signal integration and transduction hub. *Science* **322**: 92–96.

Marles-Wright, J. and Lewis, R.J. (2010) The stressosome: molecular architecture of a signalling hub. *Biochem. Soc. Trans.* **38**: 928–933.

Martínez, L.F., Bishop, A., Parkes, L., Del Sol, R., Salerno, P., Sevcikova, B., et al. (2009) Osmoregulation in *Streptomyces coelicolor:* modulation of SigB activity by OsaC. *Mol. Microbiol.* **71**: 1250–1262.

Martínez-Salazar, J.M., Salazar, E., Encarnación, S., Ramírez-Romero, M.A., and Rivera, J. (2009) Role of the extracytoplasmic function sigma factor RpoE4 in oxidative and osmotic stress responses in *Rhizobium etli*. *J. Bacteriol.* **191**: 4122–4132.

Masuda, S., Murakami, K.S., Wang, S., Anders Olson, C., Donigian, J., Leon, F., et al. (2004) Crystal Structures of the ADP and ATP Bound Forms of the *Bacillus* Anti-σ Factor SpoIIAB in Complex with the Anti-anti-σ SpoIIAA. *J. Mol. Biol.* **340**: 941–956.

Mattoo, S., Yuk, M.H., Huang, L.L., and Miller, J.F. (2004) Regulation of type III secretion in *Bordetella. Mol. Microbiol.* **52**: 1201–1214.

Mercer, R.G. and Lang, A.S. (2014) Identification of a predicted partner-switching system that affects production of the gene transfer agent RcGTA and stationary phase viability in *Rhodobacter capsulatus. BMC Microbiol.* **14**: 71.

Mitchell, J.E., Oshima, T., Piper, S.E., Webster, C.L., Westblade, L.F., Karimova, G., et al. (2007) The *Escherichia coli* Regulator of Sigma 70 Protein, Rsd, Can Up-Regulate Some Stress-Dependent Promoters by Sequestering Sigma 70. *J. Bacteriol.* **189**: 3489–3495.

Mittenhuber, G. (2002) A phylogenomic study of the general stress response sigma factor sigmaB of *Bacillus subtilis* and its regulatory proteins. *J. Mol. Microbiol. Biotechnol.* **4**: 427–452.

Morris, A.R. and Visick, K.L. (2010) Control of biofilm formation and colonization in *Vibrio fischeri*: a role for partner switching? *Environ. Microbiol.* **12**: 2051–2059.

Morris, A.R. and Visick, K.L. (2013a) Inhibition of SypG-Induced Biofilms and Host Colonization by the Negative Regulator SypE in *Vibrio fischeri. PLoS ONE* **8**:.

Morris, A.R. and Visick, K.L. (2013b) The response regulator SypE controls biofilm formation and colonization through phosphorylation of the syp-encoded regulator SypA in *Vibrio fischeri*. *Mol. Microbiol.* **87**: 509–525.

615 NCBI Resource Coordinators (2016) Database resources of the National Center for
616 Biotechnology Information. *Nucleic Acids Res.* **44**: D7-19.
617 Norsworthy, A.N. and Visick, K.L. (2015) Signaling between two interacting sensor kinases
618 promotes biofilms and colonization by a bacterial symbiont. *Mol. Microbiol.* **96**: 233–248.
619 Österberg, S., del Peso-Santos, T., and Shingler, V. (2011) Regulation of alternative sigma factor
620 use. *Annu. Rev. Microbiol.* **65**: 37–55.
621 Paget, M.S. (2015) Bacterial Sigma Factors and Anti-Sigma Factors: Structure, Function and
622 Distribution. *Biomolecules* **5**: 1245–1265.
623 Paget, M.S.B. and Helmann, J.D. (2003) The sigma70 family of sigma factors. *Genome Biol.* **4**: 203.
624 Pané-Farré, J., Lewis, R.J., and Stülke, J. (2005) The RsbRST stress module in bacteria: a signalling
625 system that may interact with different output modules. *J. Mol. Microbiol. Biotechnol.* **9**:
626 65–76.
627 Parida, B.K., Douglas, T., Nino, C., and Dhandayuthapani, S. (2005) Interactions of anti-sigma
628 factor antagonists of *Mycobacterium tuberculosis* in the yeast two-hybrid system. *Tuberc.*
629 *Edinb. Scotl.* **85**: 347–355.
630 Park, Y.-H., Lee, C.-R., Choe, M., and Seok, Y.-J. (2013) HPr antagonizes the anti-σ70 activity of Rsd
631 in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **110**: 21142–21147.
632 Park, Y.H., Um, S.H., Song, S., Seok, Y.J., and Ha, N.C. (2015) Structural basis for the sequestration
633 of the anti-σ(70) factor Rsd from σ(70) by the histidine-containing phosphocarrier
634 protein HPr. *Acta Crystallogr. D Biol. Crystallogr.* **71**: 1998–2008.
635 Pereira, S.F.F., Goss, L., and Dworkin, J. (2011) Eukaryote-like serine/threonine kinases and
636 phosphatases in bacteria. *Microbiol. Mol. Biol. Rev. MMBR* **75**: 192–212.
637 Price, C.W. (2011) The general stress response in *Bacillus subtilis* and related Gram-positive
638 bacteria. In, *Bacterial Stress Responses, 2nd edition, ASM*. Hengge Regine, Storz Gisela, p.
639 301.
640 Romero, P.R. and Karp, P.D. (2004) Using functional and organizational information to improve
641 genome-wide computational prediction of transcription units on pathway-genome
642 databases. *Bioinforma. Oxf. Engl.* **20**: 709–717.
643 Sachdeva, P., Narayan, A., Misra, R., Brahmachari, V., and Singh, Y. (2008) Loss of kinase activity
644 in *Mycobacterium tuberculosis* multidomain protein Rv1364c. *FEBS J.* **275**: 6295–6308.
645 Sauviac, L., Philippe, H., Phok, K., and Bruand, C. (2007) An extracytoplasmic function sigma
646 factor acts as a general stress response regulator in *Sinorhizobium meliloti*. *J. Bacteriol.*
647 **189**: 4204–4216.
648 van Schaik, W., Tempelaars, M.H., Zwietering, M.H., de Vos, W.M., and Abee, T. (2005) Analysis of
649 the Role of RsbV, RsbW, and RsbY in Regulating σB Activity in *Bacillus cereus. J. Bacteriol.*
650 **187**: 5846–5851.
651 Sharma, A.K., Rigby, A.C., and Alper, S.L. (2011) STAS domain structure and function. *Cell. Physiol.*
652 *Biochem.* **28**: 407–422.
653 Shi, L., Bischoff, K.M., and Kennelly, P.J. (1999) The icfG Gene Cluster of *Synechocystis sp.* Strain
654 PCC 6803 Encodes an Rsb/Spo-Like Protein Kinase, Protein Phosphatase, and Two
655 Phosphoproteins. *J. Bacteriol.* **181**: 4761–4767.
656 Shi, Y. (2009) Serine/threonine phosphatases: mechanism through structure. *Cell* **139**: 468–484.
657 Sineva, E., Savkina, M., and Ades, S.E. (2017) Themes and variations in gene regulation by
658 extracytoplasmic function (ECF) sigma factors. *Curr. Opin. Microbiol.* **36**: 128–137.
659 Smith, T.G. and Hoover, T.R. (2009) Deciphering bacterial flagellar gene regulatory networks in
660 the genomic era. *Adv. Appl. Microbiol.* **67**: 257–295.

Sorenson, M.K., Ray, S.S., and Darst, S.A. (2004) Crystal structure of the flagellar sigma/anti-sigma complex sigma(28)/FlgM reveals an intact sigma factor in an inactive conformation. *Mol. Cell* **14**: 127–138.

Staroń, A., Sofia, H.J., Dietrich, S., Ulrich, L.E., Liesegang, H., and Mascher, T. (2009) The third pillar of bacterial signal transduction: classification of the extracytoplasmic function (ECF) sigma factor protein family. *Mol. Microbiol.* **74**: 557–581.

Thompson, C.C., Griffiths, C., Nicod, S.S., Lowden, N.M., Wigneshweraraj, S., Fisher, D.J., and McClure, M.O. (2015) The Rsb Phosphoregulatory Network Controls Availability of the Primary Sigma Factor in *Chlamydia trachomatis* and Influences the Kinetics of Growth and Development. *PLoS Pathog* **11**: e1005125.

Valentini, M., Laventie, B.-J., Moscoso, J., Jenal, U., and Filloux, A. (2016) The Diguanylate Cyclase HsbD Intersects with the HptB Regulatory Cascade to Control *Pseudomonas aeruginosa* Biofilm and Motility. *PLoS Genet.* **12**: e1006354.

Voelker, U., Voelker, A., and Haldenwang, W.G. (1996) Reactivation of the *Bacillus subtilis* anti-sigma B antagonist, RsbV, by stress- or starvation-induced phosphatase activities. *J. Bacteriol.* **178**: 5456–5463.

Wuichet, K. and Zhulin, I.B. (2010) Origins and Diversification of a Complex Signal Transduction System in Prokaryotes. *Sci. Signal.* **3**: ra50.

Yuan, A.H., Gregory, B.D., Sharp, J.S., McCleary, K.D., Dove, S.L., and Hochschild, A. (2008) Rsd family proteins make simultaneous interactions with regions 2 and 4 of the primary sigma factor. *Mol. Microbiol.* **70**: 1136–1151.

## Figures legends

**Figure 1: Domain organization of PSS modules of chosen bacterial species.**

The color code is: red for σ factors, purple for anti-σ factor domains, green for anti-σ factor antagonists (anti-anti-σ factors) and yellow for phosphatase domains. HAMP domains (present in Histidine kinases, Adenyl cyclases, Methyl-accepting proteins and Phosphatases) are linkers possessing a role in signal transduction. Transmembrane domains of membranous proteins are mentioned by "TM" (in black), other proteins are cytoplasmic. REC stands for Receiver domain. Sensing domains comprise: CACHE (CAlcium channels and CHEmotaxis receptors), PAS for Per (Period Circadian Protein), Arnt (Aryl hydrocarbon Receptor Nuclear Translocator protein), Sim (Single-Minded Protein) and GAF (for cGMP-specific phosphodiesterases, Adenylyl cyclases and FhlA). When known, the physiological role of PSS is indicated as well as its sensing modules. The domain organizations appear on the right part of the figure.

**Figure 2: Conserved PSS modules regulating GSR σ factors are controlled by various signal transduction systems.**

The signal transduction pathways are divided in three major steps:

1(blue) - Signal sensing. Modules involved in the signal detection present a large range of mechanisms with various level of complexity. This step is the most diversified and bacteria have been very creative to detect signals and transduce them to the PSS modules.

2 (green) - Intermediate signal transduction from the input (signal sensing) to the output (σ factors). The partner-switching mechanism is highly conserved. The rule is that according to the environmental conditions, the σ factor should be sequestered or released to hamper or allow its regulatory activity, respectively.

3 (red) - The σ factor involved in the GSR. The release of the σ factor leads to the expression of the genes belonging to the σ factor regulon.

Question marks (?) indicate that the steps have not been experimentally demonstrated. * means that the components are not conserved in all alpha-proteobacterial species. A green arrow represents a phosphorylation and a red arrow a dephosphorylation event. "HK-CA" corresponds to the domains HisKA and HATPase involved in receiver (REC) phosphorylation. Histidine kinases from two-component systems and the CheA1 kinase from the Che1 chemosensory system are represented. Protein names are indicated inside the drawing except if the protein harbors multiple domains, in this case the name is written above. The cytoplasmic membrane is symbolized in dark. The colors of the σ factors and the PSS components are those of figure 1. Protein hampering is indicated by a line ending by a small horizontal line. ασ and αασ stand for anti-sigma factor and anti-anti-sigma factor (or anti-sigma factor antagonist).

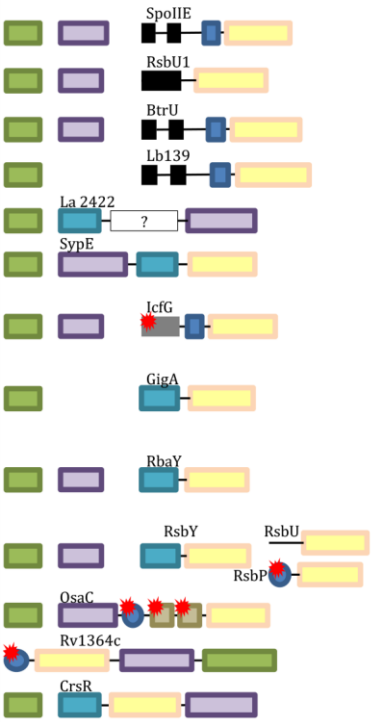
**Figure 3: Occurrence and synteny of CrsR homologs in bacteria**

Searches for homologous proteins to *S. oneidensis* CrsR were performed using the bioinformatics BLAST tool from the NCBI database (NCBI Resource Coordinators, 2016) and the sequences were assembled using the program "Phylogeny" (Dereeper *et al.*, 2008). Among the Gamma-proteobacteria, CrsR homologs are found in *Alteromonadales*, *Chromatiales*, *Methylococcales*,

*Oceanospirillales*, *Pseudomonadales*, *Thiotrichales*, *Vibrionales*. Symbol "*" indicate a genus. Among the genus *Pseudomonas*, the species *P. aeruginosa, P. putida, P. chlororaphis, P. fluorescens, P. syringae, P. stutzeri* were selected. The symbol "**" indicates that the synteny is conserved in all *Pseudomonas* species except for *P. aeruginosa*. The genus *Vibrio* includes *V. mimicus, V. cholerae, V. vulnificus,* and the genus *Shewanella* includes *S. xiamenensis, S. decolorationis, S. sp*. HN-41, *S. baltica* OS185, *S. sp.* ANA-3, *S. sp.* MR-7, *S. putrefaciens* and *S. oneidensis* MR-1. Genes surrounding *crsR* homologs were examined by hand using the BioCyc database (Romero and Karp, 2004; Krummenacker *et al.*, 2005). The question mark (?) means that the genes surrounding *crsR* homologs are not exhaustive because of the incomplete database. "HK" stands for Histidine Kinase, "RR" for Response Regulator and "RR-GGDEF" for a receiver domain fused to a GGDEF domain. GGDEF domains have an enzymatic activity producing c-di-GMP necessary for biofilm formation. The corresponding proteins and the color code of the bacterial species are summarized in the bottom of the figure. Inside gene drawings, 3 and 5 indicate the number of copies.

## Figure 1

| Target σ factor | Anti-σ factor antagonist | Anti-σ factor | Phosphatase PP2C | Sensing modules | Species | Roles |
|---|---|---|---|---|---|---|
| $\sigma^F$ | SpoIIAA | SpoIIAB | SpoIIE | Phosphatase | *Bacillus subtilis* | sporulation |
| $\sigma^{70}$ | RsbV1 + RsbV2 | RsbW | RsbU1 | Phosphatase? | *Chlamydia trachomatis* | housekeeping |
| unknown | BtrV | BtrW | BtrU | Phosphatase? | *Bordetella pertussis* | type-3 secretion system |
| unknown | Lb136 | ? | Lb139 | ? | *Leptospira interrogans* | virulence/motility |
| unknown | La2424 | La2422 | ? | CSS? | *Leptospira interrogans* | motility |
| unknown | SypA | SypE | SypE | RscS (TCS) | *Vibrio fisheri* and other *Vibrio* | biofilm formation |
| unknown | slr1856 and slr1859? | slr1861 | IcfG | direct? | *Synechocystis* sp. Strain PCC 6803 | metabolism of inorganic carbon in limiting-CO2 condition |
| $\sigma^{24}$? | GigB | NPr | GigA | ? | *Acinetobacter baumanii* | stress responses, pathogenicity and multi-antibiotic resistance |
| unknown | RbaV | RbaW | RbaY | ? | *Rhodobacter capsulatus* | stationary phase response and production of gene transfer RcGTA |
| $\sigma^B$ | RsbV | RsbW | RsbU/ RsbP/ RsbY | stressosome+RsbT/ RsbQ/ RsbK (TCS) | *Bacillales* | GSR |
| $\sigma^B$ | RsbV/ ? | RsbA/ OsaC | ?/ OsaC | ?/ direct? OsaA? (TCS) | *Streptomyces coelicolor* | GSR |
| $\sigma^F$ | RsfA + RsfB/ Rv1364c | UsfX/ Rv1364c | ?/ Rv1364c | ?/ direct sensing? | *Mycobacterium tuberculosis* | GSR |
| $\sigma^S$ | CrsA | CrsR | CrsR | CSS? | *Shewanella oneidensis* | GSR |
| $\sigma^{ECFG}$ | PhyR | NepR | Bab1_1673? | LovhK | *Brucella abortus* and various α-proteobacteria | GSR |




HAMP TM REC ? unknown CACHE PAS GAF Signal

Figure 2

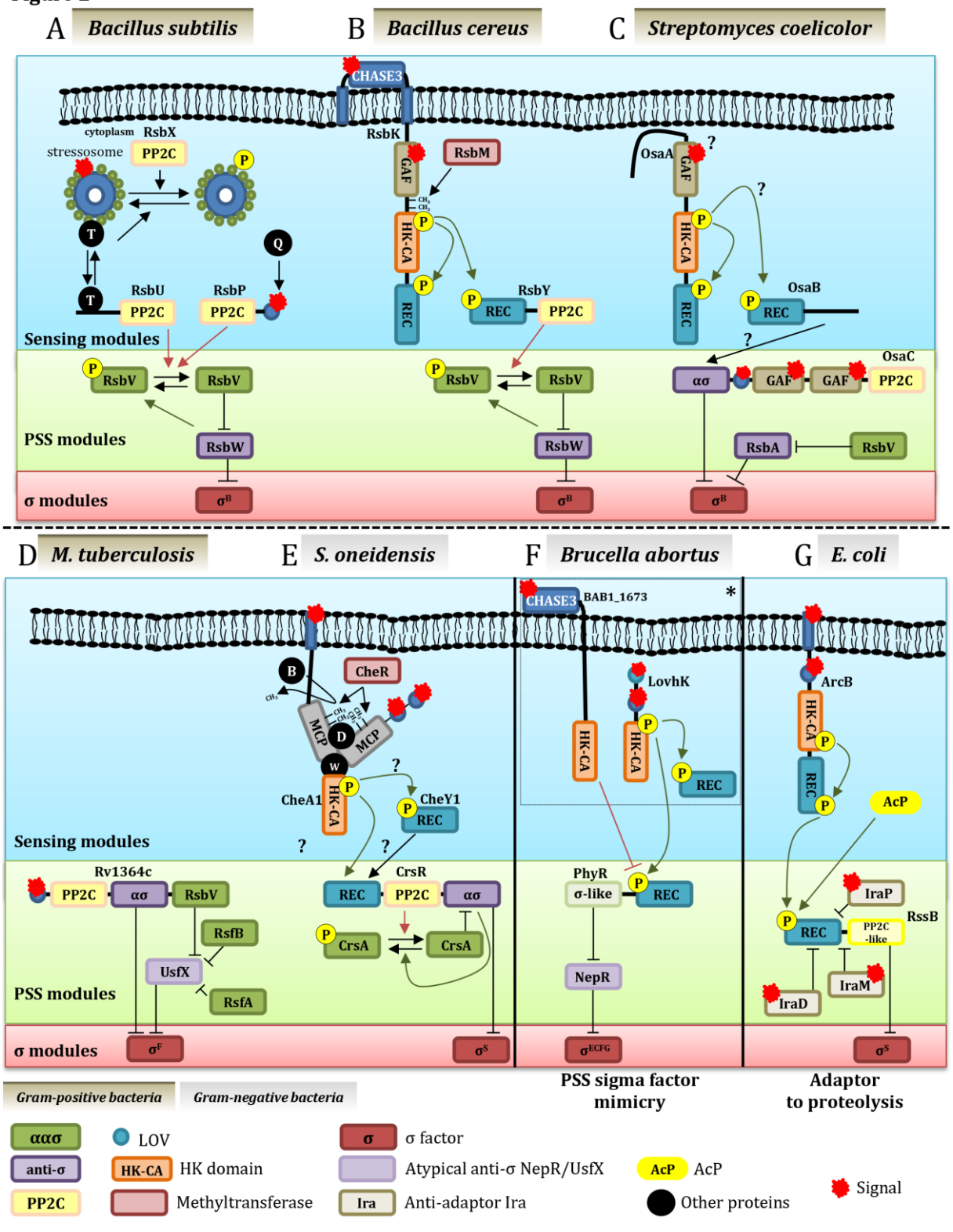
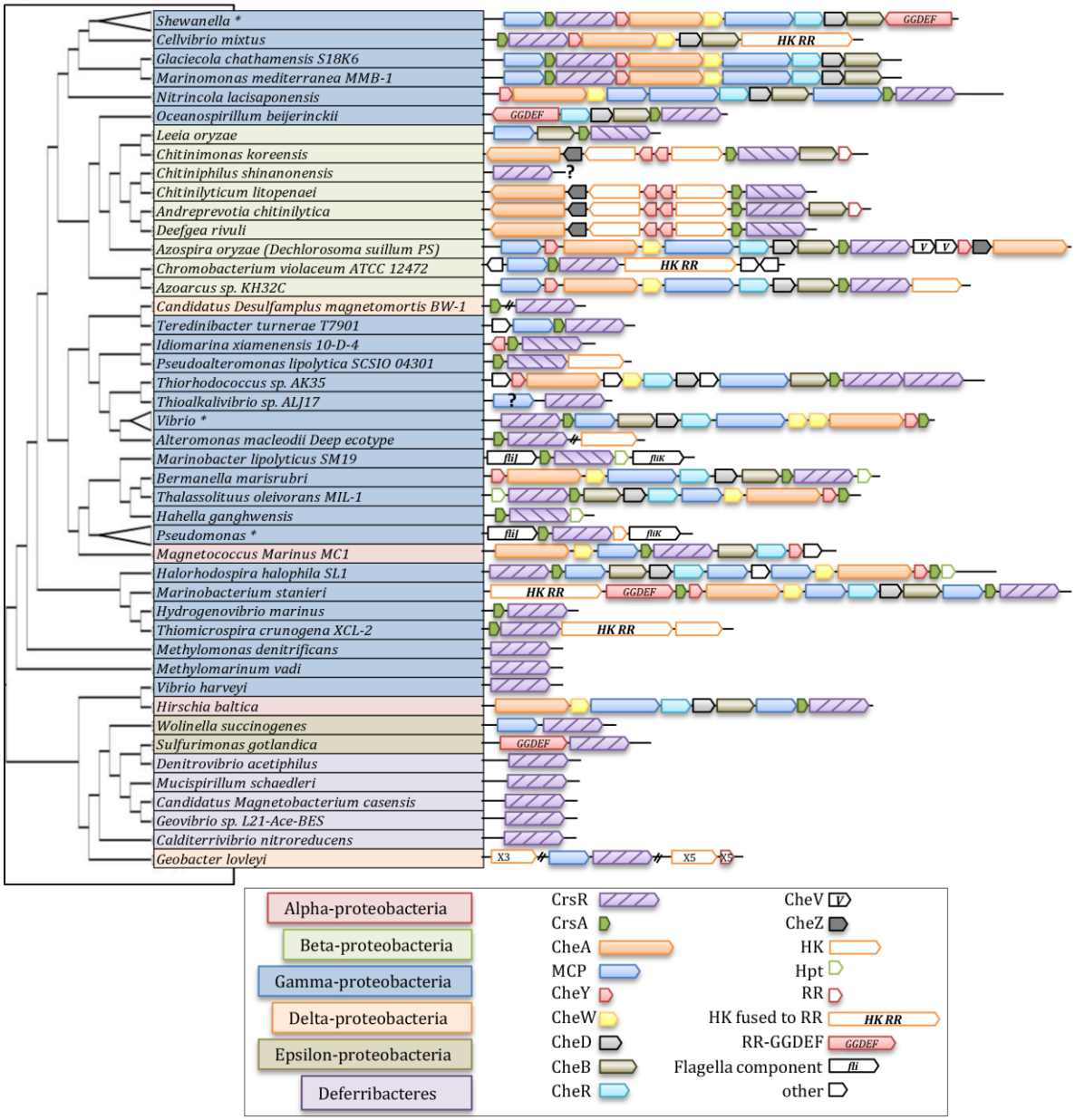

A Bacillus subtilis
B Bacillus cereus
C Streptomyces coelicolor
CHASE3
RsbK
cytoplasm
RsbX
stressosome
PP2C
RsbM
GAF
HK-CA
REC
RsbU
RsbP
RsbY
OsaA
OsaB
OsaC
Sensing modules
RsbV
RsbW
PSS modules
σ modules
RsbA
αα
D M. tuberculosis
E S. oneidensis
F Brucella abortus
G E. coli
BAB1_1673
CheR
CheA1
CheY1
MCP
Rv1364c
RsfB
UsfX
RsfA
CrsR
CrsA
LovhK
PhyR
σ-like
NepR
ArcB
AcP
IraP
RssB
PP2C -like
IraM
IraD
Gram-positive bacteria
Gram-negative bacteria
PSS sigma factor mimicry
Adaptor to proteolysis
ααα
anti-σ
PP2C
LOV
HK-CA
HK domain
Methyltransferase
σ
σ factor
Atypical anti-σ NepR/UsfX
Ira
Anti-adaptor Ira
AcP
Other proteins
Signal

**Figure 3**


Shewanella *
Cellvibrio mixtus
Glaciecola chathamensis S18K6
Marinomonas mediterranea MMB-1
Nitrincola lacisaponensis
Oceanospirillum beijerinckii
Leeia oryzae
Chitinimonas koreensis
Chitiniphilus shinanonensis
Chitinilyticum litopenaei
Andreprevotia chitinilytica
Deefgea rivuli
Azospira oryzae (Dechlorosoma suillum PS)
Chromobacterium violaceum ATCC 12472
Azoarcus sp. KH32C
Candidatus Desulfamplus magnetomortis BW-1
Teredinibacter turnerae T7901
Idiomarina xiamenensis 10-D-4
Pseudoalteromonas lipolytica SCSIO 04301
Thiorhodococcus sp. AK35
Thioalkalivibrio sp. ALJ17
Vibrio *
Alteromonas macleodii Deep ecotype
Marinobacter lipolyticus SM19
Bermanella marisrubri
Thalassolituus oleivorans MIL-1
Hahella ganghwensis
Pseudomonas *
Magnetococcus Marinus MC1
Halorhodospira halophila SL1
Marinobacterium stanieri
Hydrogenovibrio marinus
Thiomicrospira crunogena XCL-2
Methylomonas denitrificans
Methylomarinum vadi
Vibrio harveyi
Hirschia baltica
Wolinella succinogenes
Sulfurimonas gotlandica
Denitrovibrio acetiphilus
Mucispirillum schaedleri
Candidatus Magnetobacterium casensis
Geovibrio sp. L21-Ace-BES
Calditerrivibrio nitroreducens
Geobacter lovleyi
GGDEF
HK RR
V
?
fliJ
fliK
X3
X5
Alpha-proteobacteria
Beta-proteobacteria
Gamma-proteobacteria
Delta-proteobacteria
Epsilon-proteobacteria
Deferribacteres
CrsR
CrsA
CheA
MCP
CheY
CheW
CheD
CheB
CheR
CheV
CheZ
HK
Hpt
RR
HK fused to RR
RR-GGDEF
Flagella component
other

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