



HAL
open science

Regulation of σ factors by conserved partner switches controlled by divergent signalling systems

Sophie Bouillet, Dallel Arabet, Cecile Jourlin-Castelli, Vincent Méjean,
Chantal Iobbi-Nivol

► To cite this version:

Sophie Bouillet, Dallel Arabet, Cecile Jourlin-Castelli, Vincent Méjean, Chantal Iobbi-Nivol. Regulation of σ factors by conserved partner switches controlled by divergent signalling systems. *Environmental Microbiology Reports*, 2018, 10 (2), pp.127 - 139. 10.1111/1758-2229.12620 . hal-01890037

HAL Id: hal-01890037

<https://amu.hal.science/hal-01890037v1>

Submitted on 20 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Regulation of σ factors by conserved partner switches controlled by divergent signaling**
2 **systems**

3
4 **Sophie Bouillet***, Dallel Arabet, Cécile Jourlin-Castelli, Vincent Méjean and Chantal Iobbi-
5 **Nivol**

6

7 From Aix-Marseille Univ, CNRS, BIP UMR7281, 13402 Marseille, France.

8

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

10

11 *To whom correspondence should be addressed: Sophie Bouillet, BIP-IMM, CNRS, 31, Chemin
12 Joseph Aiguier, 13402 Marseille Cedex 20, France, E-mail: bouillet.sophie@outlook.fr

13

14 **Keywords:** Environmental signal and stress responses, gene regulation, anti-sigma factor,
15 chemosensory systems, phylogeny.

16

17 **Abstract**

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

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

29 **Introduction**

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

39 Except for *Escherichia coli* σ^{54} and homologs, all σ factors belong to the σ^{70} family, divided itself
40 into four subfamilies based on phylogenetic and structural properties (Paget and Helmann,
41 2003; Helmann, 2011). The group 1 of σ^{70} family is composed of the whole housekeeping σ
42 factors, σ^{70} also called σ^A in Gram-positive bacteria. The group 2 gathers σ factors that resemble
43 to that of group 1 except that they are not essential in laboratory conditions, as σ^S factor
44 involved in the General Stress Response (GSR) of proteobacteria (Helmann, 2011; Battesti *et al.*,
45 2011). The group 3 contains more diverse σ factors, which have roles in various cellular
46 processes as σ^{32} that regulates genes involved in response to heat shock, σ^{28} in flagella synthesis
47 and also σ^E , σ^F , σ^G , σ^K , of *Bacillus subtilis* that control different stages of sporulation. The group 4
48 contains very diverse σ factors called σ^{ECF} (for ExtraCyttoplasmic Function) that have been
49 classified into at least 56 subgroups (Staroń *et al.*, 2009; Huang *et al.*, 2015; Sineva *et al.*, 2017).

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

54 Battesti *et al.*, 2011); the cleavage of the σ factor from an inactive to an active form, including
55 pro- σ^E and pro- σ^K in *B. subtilis* (Hilbert and Piggot, 2004; Higgins and Dworkin, 2012; Fimlaid
56 and Shen, 2015) and the inactivation of the sigma factor activity by an anti- σ factor (Hughes and
57 Mathee, 1998; Helmann, 1999; Österberg *et al.*, 2011; Feklístov *et al.*, 2014; Paget, 2015). The
58 anti- σ factors sequester their target σ factor, disabling it to interact with the core RNAP and thus
59 to transcribe genes of its regulon (Campbell *et al.*, 2002; Sorenson *et al.*, 2004; Feklístov *et al.*,
60 2014). In doing so, anti- σ factors could protect their σ factor partner from proteolysis
61 (Barembuch and Hengge, 2007; Mao *et al.*, 2013, 2014; Bouillet *et al.*, 2017). Thus, anti- σ factors
62 enable the cell to maintain a pool of σ factor molecules that can be rapidly released to act
63 without *de novo* synthesis when suddenly required. Most of the σ^{ECF} are regulated by anti- σ
64 factors and are usually co-transcribed into the same transcriptional unit to keep a 1:1
65 stoichiometry of the two proteins (Brooks and Buchanan, 2008; Campagne *et al.*, 2015). The
66 release of the σ factor is also precisely driven to ensure the transcription of its regulon in
67 response to specific signals. Different strategies are thus employed including cell-surface
68 signaling (regulation of σ^{24} also called σ^E in *E. coli*) (Brooks and Buchanan, 2008; Ho and
69 Ellermeier, 2012), the secretion of the anti- σ factors (FliA/FlgM in *E. coli*) (Hughes *et al.*, 1993;
70 Kutsukake, 1994; Smith and Hoover, 2009), the direct sensing of redox state by cysteine
71 residues of the anti- σ factors (RsrA/ σ^R in *Streptomyces coelicolor*) (Ilbert *et al.*, 2006; Jung *et al.*,
72 2011) as well as the involvement of an additional protein called anti- σ factor antagonist in a
73 mechanism known as the partner-switching system (PSS).

74 **Partner-switching systems**

75 The term “partner-switch” has been defined by Alper and colleagues in 1994 to describe the
76 mechanism that regulates σ^F , crucial in the sporulation process of *B. subtilis* (Alper *et al.*, 1994).
77 The nature of the PSS components as well as their function is a common feature among typical
78 PSS. Indeed, PSS are made up with an anti- σ factor having a serine kinase activity (HATPase
79 domain), a phosphorylatable STAS anti- σ antagonist, a PP2C serine phosphatase and a target σ
80 factor (Alper *et al.*, 1994; Mittenhuber, 2002). Anti- σ factors are constituted of a dimerization

81 interface and of a HATPase domain. The latter harbors conserved motifs for phosphorylation
82 and is found in many kinase families (Dutta and Inouye, 2000). STAS stands for Sulfate
83 Transporter and Anti-Sigma antagonist and proteins containing this domain play a role in either
84 of these two processes and are phosphorylatable on a specific serine residue (Sharma *et al.*,
85 2011). The PP2C domain characterizes a family of serine/threonine phosphatases that need
86 metallic ion for their activity (Shi, 2009; Pereira *et al.*, 2011; Bradshaw *et al.*, 2017).

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

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

103 Numerous PSS have been discovered in many bacteria including Gram-negative bacteria
104 whereas they were initially thought to be restricted to Gram-positive bacteria (Shi *et al.*, 1999;
105 Mittenhuber, 2002; Kozak *et al.*, 2005; Morris and Visick, 2010; Houot *et al.*, 2012; Morris and
106 Visick, 2013b; Eshghi *et al.*, 2014; Mercer and Lang, 2014; Lambert *et al.*, 2015; Thompson *et al.*,
107 2015; Bouillet *et al.*, 2016; Gebhardt and Shuman, 2017). Although PSS comprises four

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

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

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

124 However, anti- σ factor antagonist and phosphatase homologs have been found in Gram-positive
125 bacteria, as well as cyanobacteria, *Deinococcus* species and proteobacteria including
126 *Pseudomonas*, *Vibrio* and *Shewanella* species (Mittenhuber, 2002). For example, *B. subtilis* and *B.*
127 *cereus* possess 16 σ factors and two known PSS targeting σ^F and σ^B , *M. tuberculosis* harbors 13 σ
128 factors, one of which is subjected to two PSS (σ^F is submitted to UsfX/RsfAB and to Rv1364c
129 regulation). In *S. oneidensis*, 10 σ factors are present with a known and a putative PSS.
130 Surprisingly, some species including *E. coli* do not seem to possess PSS. In contrast, the Gram-
131 positive bacterium *S. coelicolor* is one of the organisms that contain the highest number of σ
132 factors (60 to 65) and its chromosome has been predicted to encode many PSS partners with 45
133 anti- σ factors, 18 anti-anti- σ factors and 44 PP2C proteins (Bentley *et al.*, 2002; Mittenhuber,
134 2002; Martínez *et al.*, 2009). Among them, few have been identified but their study appears

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

140 **Role of the Partner-switching systems**

141 The role of PSS is to control the availability of specific σ factors. However, various PSS have been
142 discovered in many bacteria but the targeted σ factor has not been found in some cases (Figure
143 1).

144 PSS signal transduction pathways seem to be implicated in various major cellular processes
145 including the control of type III secretion system synthesis, virulence, chemotaxis, biofilm
146 formation, exoprotein production, stress responses and also metabolism (Shi *et al.*, 1999; Mattoo
147 *et al.*, 2004; Kozak *et al.*, 2005; Bordi *et al.*, 2010; Bhuwan *et al.*, 2012; Houot *et al.*, 2012; Morris
148 and Visick, 2013b, 2013a; Eshghi *et al.*, 2014; Mercer and Lang, 2014; Lambert *et al.*, 2015;
149 Bouillet *et al.*, 2016; Gebhardt and Shuman, 2017). For instance, the spore formation of *B. subtilis*
150 is a complex multi-step mechanism under the control of many regulators (Higgins and Dworkin,
151 2012; Fimlaid and Shen, 2015). Notably, four main σ factors act successively during the
152 sporulation process. Each of them is thus tightly regulated but has to be also quickly freed to
153 make sure that all genes are correctly expressed in time. σ^F is active during the first stage of
154 sporulation only in the forespore compartment. The anti- σ factor SpoIIAB interacts with σ^F
155 disabling it to recruit the core RNAP. The release of σ^F is permitted by the anti- σ factor
156 antagonist SpoIIAA. When no signal is transduced to the regulatory system, SpoIIAB binds to σ^F
157 and also phosphorylates SpoIIAA. When a signal is launched, the PP2C-type phosphatase SpoIIE,
158 which is a membrane-anchored protein that perceives the signal dephosphorylates specifically
159 SpoIIAA. The anti- σ antagonist becomes thus efficient to interact with SpoIIAB, leading to the
160 release of σ^F (Diederich *et al.*, 1994; Duncan *et al.*, 1996; Magnin *et al.*, 1997; Campbell *et al.*,
161 2002; Masuda *et al.*, 2004; Levnikov *et al.*, 2012).

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

167 Beside Gram-positive bacteria PSS models, it has been recently shown in *Shewanella oneidensis*,
168 an aquatic bacterium from the γ -proteobacteria class, σ^S , the GSR- σ factor, is post-translationally
169 regulated by a PSS (Figures 1 and 2E). This system is composed of the two main proteins CrsA
170 and CrsR, an anti- σ factor antagonist and a three-domain response regulator, respectively. CrsR
171 contains a N-terminal receiver, a central PP2C and a C-terminal HATPase domains. The latter is
172 able to phosphorylate efficiently CrsA whereas the phosphatase domain dephosphorylates it. In
173 addition, direct interactions of CrsR-CrsA and of CrsR- σ^S combined with *in vivo* data have
174 revealed that σ^S is the target of the PSS. The CrsRA- σ^S is the first system that links GSR regulation
175 to PSS in a γ -proteobacterium as it is the case in many other bacterial species (Bouillet *et al.*,
176 2016, 2017).

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

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

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

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

197 **Atypical partner-switching modules**

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

204 In Gram-negative α -proteobacterial species, the σ factor controlling the GSR called σ^{ECFG} , RpoE or
205 SigT depending on the bacterium, is controlled by an atypical PSS (Figure 2F) (Francez-Charlot
206 *et al.*, 2009; Staroń *et al.*, 2009; Campagne *et al.*, 2012; Kaczmarczyk *et al.*, 2014; Kim *et al.*, 2014;
207 Francez-Charlot *et al.*, 2015; Fiebig *et al.*, 2015; Herrou *et al.*, 2015; Francez-Charlot *et al.*, 2016).

208 Indeed, the PSS partners are not homologous to canonical PSS proteins. Nevertheless, they use a
209 similar mechanism of sequestration and release of the σ factor as typical PSS. The PSS module of
210 GSR regulation is mostly shared by α -proteobacterial species with little divergences. In all cases,
211 two proteins are involved: the anti- σ factor NepR and the two-domain PhyR. The latter acts as an
212 anti- σ factor antagonist and contains a N-terminal σ -like factor and a C-terminal receiver
213 domains (Figure 2F). NepR possesses homologies with σ^{ECF} factors. Phosphorylation of the
214 receiver domain increases the affinity between the σ -like domain of PhyR and NepR so that the
215 σ^{ECF} is freed whereas unphosphorylated PhyR has almost no affinity for NepR that in turn binds

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

220 **Activation of partner-switching systems**

221 Diversified sensory and transducing modules appear to converge to PSS, although the
222 mechanisms of transduction have not been unraveled in most cases. Input modules could be
223 two-component systems, chemosensory systems or might be directly integrated in PSS modules
224 via the addition of sensing domain(s) in PSS proteins (Figures 1 and 2) (Hsu *et al.*, 2008; Morris
225 and Visick, 2013a; Lambert *et al.*, 2015; Norsworthy and Visick, 2015; Chambonnier *et al.*, 2016).

226 In the Gram-positive bacteria *Bacillus*, *Listeria* and *Staphylococcus*, the stress responsive σ
227 factor σ^{B} is submitted to a PSS. The PSS module formed by the anti- σ factor RsbW and the anti- σ
228 factor antagonist RsbV is conserved but the RsbV phosphatases as well as the mechanisms of
229 signal transduction to the PSS module diverge from one species to another (Figure 2A and 2B).

230 In *B. subtilis*, two sensing modules linked to two independent PP2C-containing phosphatases
231 (RsbU and RsbP) converge to the PSS module. Environmental stresses including blue light, heat
232 shock and osmolytes are detected by a protein complex called the stressosome that
233 subsequently transduces signals to the PP2C phosphatase RsbU. The energetic level of the cell is
234 perceived by the PAS domain containing phosphatase RsbP associated with the hydrolase RsbQ
235 (Figure 2A) (Benson and Haldenwang, 1993; Boylan *et al.*, 1993; Voelker *et al.*, 1996; Kim *et al.*,
236 2004; Marles-Wright *et al.*, 2008; Marles-Wright and Lewis, 2010; Price, 2011; Gaidenko and
237 Price, 2014; Guldemann *et al.*, 2016). RsbU, RsbP and a stressosome are absent from other
238 *Bacillales* as *B. cereus*. They are replaced by the RsbY protein composed of a receiver fused to a
239 PP2C domain and the complex histidine kinase RsbK, which is able to detect internal and
240 environmental stresses and thus transduces the signal to RsbY (Figure 2B) (van Schaik *et al.*,
241 2005; de Been *et al.*, 2010, 2011). Interestingly, genes coding for a stressosome-like complex are
242 also found in many species in particular among the proteobacteria, cyanobacteria and

243 actinobacteria phyla (Pané-Farré *et al.*, 2005; Jia *et al.*, 2016). Likewise, homologs of the histidine
244 kinase RsbK have been found in many species including the proteobacterial genus *Vibrio*,
245 *Pseudomonas*, *Magnetococcus* or *Myxococcus* but their roles are still unknown (de Been *et al.*,
246 2011).

247 *Streptomyces coelicolor* PSS regulation of σ^B resembles that of *B. subtilis* but possesses an
248 additional PSS absent from other species (Lee *et al.*, 2004): the Osa system that regulates σ^B
249 under “back to normal” conditions after an osmotic shock. OsaA is a RsbK homolog that may
250 detect signals from a GAF domain, OsaB is a two-domain protein with a N-terminal receiver and
251 an unknown C-terminal domain, and OsaC contains an anti- σ factor, a PAS, two GAF and a PP2C-
252 type phosphatase domains (Figure 2C) (Martínez *et al.*, 2009; Price, 2011). The regulatory
253 cascade that regulates σ^B availability is still unknown but the domain composition of OsaC
254 suggests a direct additional signal sensing by the phosphatase protein. In the Actinomycetales
255 *Mycobacterium tuberculosis*, the GSR σ factor called σ^F is also mediated by two PSS (DeMaio *et al.*,
256 1997). Notably, the protein Rv1364c is a PSS module organized in four domains corresponding
257 to a PAS, a phosphatase, an anti- σ factor and an anti- σ factor antagonist domain (Parida *et al.*,
258 2005; Sachdeva *et al.*, 2008; Greenstein *et al.*, 2009; Malik *et al.*, 2009; Jaiswal *et al.*, 2010; King-
259 Scott *et al.*, 2011). It has been shown that its anti- σ factor domain can bind to σ^F whereas its anti-
260 σ factor antagonist domain antagonizes the action of the anti- σ domain. However, the complex
261 network of σ^F post-translational regulation has not been completely unraveled yet, but Rv1364c
262 seems to detect signals itself without upstream sensing module.

263 The PSS composed of HsbR and HsbA from *P. aeruginosa* is activated by a complex
264 phosphocascade. The histidine phosphotransfer protein HptB constitutes the module activating
265 HsbR. HptB acts in the GacA-GacS two-component pathway regulating sRNA involved in *P.*
266 *aeruginosa* biofilm formation and pathogenesis (Lin *et al.*, 2006; Hsu *et al.*, 2008; Bordi *et al.*,
267 2010; Bhuwan *et al.*, 2012; Houot *et al.*, 2012; Chambonnier *et al.*, 2016). Moreover, other
268 histidine kinases have been shown to detect specific signals and transduce them by

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

271 **Activation of partner-switching modules in various proteobacteria: a role for** 272 **chemosensory systems**

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

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

286 Interestingly, it appears that chemosensory machinery adapted to regulate a partner-switch is a
287 common feature of aquatic proteobacteria (Figure 3). Indeed, *crsR* gene homologs are
288 widespread among those bacteria and an analysis of the genes surrounding *crsR* in 59 bacterial
289 genomes was carried out for this review (Bouillet *et al.*, 2017). This phylogenetic analysis clearly
290 shows that the CrsR-CrsA PSS is most of the time genetically related to a *che* locus. As shown in
291 Figure 3, the *crsR-crsA* genes are located in the vicinity of a central histidine kinase *cheA* gene in
292 71% of these bacteria (32 out of 45), or of two-component histidine kinase(s). This indicates
293 that the sensing modules of the CrsR-CrsA partner-switch could predominantly be a
294 chemosensory system. Interestingly, two genes coding for detectors MCP (Methyl-accepting
295 Chemotaxis Proteins) are comprised in the *che1* locus of *S. oneidensis*. One of them is predicted

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

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

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

317 **The case of GSR regulation in bacteria**

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

323 1993; Battesti *et al.*, 2011; Hengge, 2011; Price, 2011; Fiebig *et al.*, 2015; Francez-Charlot *et al.*,
324 2015). Despite the fact that all these σ factors are not homologous and present large differences
325 in terms of sequence and structure, they control analogous processes in the cells (Alvarez-
326 Martinez *et al.*, 2006; Sauviac *et al.*, 2007; Gourion *et al.*, 2009; Martínez-Salazar *et al.*, 2009;
327 Britos *et al.*, 2011; Hengge, 2011; Foreman *et al.*, 2012; Jans *et al.*, 2013; Kim *et al.*, 2013; Landini
328 *et al.*, 2014; Guldemann *et al.*, 2016). Moreover, although their global regulation is highly
329 divergent, the presence of a PSS is a relatively common feature for their post-translational
330 regulation (Figure 2).

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

338 **Concluding Remarks**

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

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

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

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

359

360 **Acknowledgements**

361 We would like to thank the anonymous reviewers for helpful suggestions, members of the group,
362 in particular Olivier Lemaire for fruitful discussions and Olivier Genest for discussions and
363 reviewing the manuscript. We also thank David Redelberger for assistance with drawings. This
364 work was supported by the Centre National de la Recherche Scientifique and Aix-Marseille
365 Université (AMU). S.B. was supported by a Ministère de l'Enseignement Supérieur et de la
366 Recherche fellowship and AMU.

367 Conflict of interest: The authors declare that they have no conflicts of interest with the contents
368 of this article.

369 **References**

- 370 Alper, S., Duncan, L., and Losick, R. (1994) An adenosine nucleotide switch controlling the
371 activity of a cell type-specific transcription factor in *B. subtilis*. *Cell* **77**: 195–205.
- 372 Alvarez-Martinez, C.E., Baldini, R.L., and Gomes, S.L. (2006) A *Caulobacter crescentus*
373 Extracytoplasmic Function Sigma Factor Mediating the Response to Oxidative Stress in
374 Stationary Phase. *J. Bacteriol.* **188**: 1835–1846.
- 375 Armitano, J., Méjean, V., and Jourlin-Castelli, C. (2013) Aerotaxis governs floating biofilm
376 formation in *Shewanella oneidensis*. *Environ. Microbiol.* **15**: 3108–3118.
- 377 Barembruch, C. and Hengge, R. (2007) Cellular levels and activity of the flagellar sigma factor
378 FliA of *Escherichia coli* are controlled by FlgM-modulated proteolysis. *Mol. Microbiol.* **65**:
379 76–89.
- 380 Battesti, A., Majdalani, N., and Gottesman, S. (2011) The RpoS-mediated general stress response
381 in *Escherichia coli*. *Annu. Rev. Microbiol.* **65**: 189–213.

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

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

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

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

393 Bentley, S.D., Chater, K.F., Cerdeño-Tárraga, A.-M., Challis, G.L., Thomson, N.R., James, K.D., et al.
394 (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor*
395 A3(2). *Nature* **417**: 141–147.

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

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

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

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

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

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

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

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

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

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

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

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

428 the GacS/GacA Two-Component System in *Pseudomonas aeruginosa*. *PLoS Genet.* **12**:
429 e1006032.

430 Chen, J.-C., Liu, J.-H., Hsu, D.-W., Shu, J.-C., Chen, C.-Y., and Chen, C.-C. (2015) Methylatable
431 Signaling Helix Coordinated Inhibitory Receiver Domain in Sensor Kinase Modulates
432 Environmental Stress Response in *Bacillus Cereus*. *PLoS ONE* **10**:.

433 Chen, L.-C., Chen, J.-C., Shu, J.-C., Chen, C.-Y., Chen, S.-C., Chen, S.-H., et al. (2012) Interplay of RsbM
434 and RsbK controls the σ B activity of *Bacillus cereus*. *Environ. Microbiol.* **14**: 2788–2799.

435 DeMaio, J., Zhang, Y., Ko, C., and Bishai, W.R. (1997) *Mycobacterium tuberculosis* sigF is part of a
436 gene cluster with similarities to the *Bacillus subtilis* sigF and sigB operons. *Tuber. Lung*
437 *Dis. Off. J. Int. Union Tuberc. Lung Dis.* **78**: 3–12.

438 Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., et al. (2008) Phylogeny.fr:
439 robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **36**: W465–469.

440 Diederich, B., Wilkinson, J.F., Magnin, T., Najafi, M., Errington, J., and Yudkin, M.D. (1994) Role of
441 interactions between SpoIIAA and SpoIIAB in regulating cell-specific transcription factor
442 sigma F of *Bacillus subtilis*. *Genes Dev.* **8**: 2653–2663.

443 Duncan, L., Alper, S., and Losick, R. (1996) SpoIIAA governs the release of the cell-type specific
444 transcription factor sigma F from its anti-sigma factor SpoIIAB. *J. Mol. Biol.* **260**: 147–
445 164.

446 Dutta, R. and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem.*
447 *Sci.* **25**: 24–28.

448 Eshghi, A., Becam, J., Lambert, A., Sismeiro, O., Dillies, M.-A., Jagla, B., et al. (2014) A putative
449 regulatory genetic locus modulates virulence in the pathogen *Leptospira interrogans*.
450 *Infect. Immun.* **82**: 2542–2552.

451 Feklístov, A., Sharon, B.D., Darst, S.A., and Gross, C.A. (2014) Bacterial sigma factors: a historical,
452 structural, and genomic perspective. *Annu. Rev. Microbiol.* **68**: 357–376.

453 Fiebig, A., Herrou, J., Willett, J., and Crosson, S. (2015) General Stress Signaling in the
454 Alphaproteobacteria. *Annu. Rev. Genet.* **49**: null.

455 Fimlaid, K.A. and Shen, A. (2015) Diverse mechanisms regulate sporulation sigma factor activity
456 in the Firmicutes. *Curr. Opin. Microbiol.* **24**: 88–95.

457 Foreman, R., Fiebig, A., and Crosson, S. (2012) The LovK-LovR Two-Component System Is a
458 Regulator of the General Stress Pathway in *Caulobacter crescentus*. *J. Bacteriol.* **194**:
459 3038–3049.

460 Francez-Charlot, A., Frunzke, J., Reichen, C., Ebnetter, J.Z., Gourion, B., and Vorholt, J.A. (2009)
461 Sigma factor mimicry involved in regulation of general stress response. *Proc. Natl. Acad.*
462 *Sci. U. S. A.* **106**: 3467–3472.

463 Francez-Charlot, A., Frunzke, J., Zingg, J., Kaczmarczyk, A., and Vorholt, J.A. (2016) Multiple σ EcfG
464 and NepR Proteins Are Involved in the General Stress Response in *Methylobacterium*
465 *extorquens*. *PLoS ONE* **11**:.

466 Francez-Charlot, A., Kaczmarczyk, A., Fischer, H.-M., and Vorholt, J.A. (2015) The general stress
467 response in Alphaproteobacteria. *Trends Microbiol.* **23**: 164–171.

468 Gaidenko, T.A. and Price, C.W. (2014) Genetic evidence for a phosphorylation-independent
469 signal transduction mechanism within the *Bacillus subtilis* stressosome. *PloS One* **9**:
470 e90741.

471 Galperin, M.Y. (2006) Structural Classification of Bacterial Response Regulators: Diversity of
472 Output Domains and Domain Combinations. *J. Bacteriol.* **188**: 4169–4182.

473 Gebhardt, M.J. and Shuman, H.A. (2017) GigA and GigB are Master Regulators of Antibiotic
474 Resistance, Stress Responses and Virulence in *Acinetobacter baumannii*. *J. Bacteriol.*

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

478 Greenstein, A.E., Hammel, M., Cavazos, A., and Alber, T. (2009) Interdomain communication in
479 the *Mycobacterium tuberculosis* environmental phosphatase Rv1364c. *J. Biol. Chem.* **284**:
480 29828–29835.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

512 Huang, X., Pinto, D., Fritz, G., and Mascher, T. (2015) Environmental Sensing in Actinobacteria: a
513 Comprehensive Survey on the Signaling Capacity of This Phylum. *J. Bacteriol.* **197**: 2517–
514 2535.

515 Hughes, K.T., Gillen, K.L., Semon, M.J., and Karlinsey, J.E. (1993) Sensing structural intermediates
516 in bacterial flagellar assembly by export of a negative regulator. *Science* **262**: 1277–
517 1280.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

571 Magnin, T., Lord, M., and Yudkin, M.D. (1997) Contribution of partner switching and SpoIIAA
572 cycling to regulation of sigmaF activity in sporulating *Bacillus subtilis*. *J. Bacteriol.* **179**:
573 3922–3927.

574 Malik, S.S., Luthra, A., and Ramachandran, R. (2009) Interactions of the *M. tuberculosis* UsfX with
575 the cognate sigma factor SigF and the anti-anti sigma factor RsfA. *Biochim. Biophys. Acta*
576 **1794**: 541–553.

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

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

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

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

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

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

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

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

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

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

605 Mittenhuber, G. (2002) A phylogenomic study of the general stress response sigma factor
606 sigmaB of *Bacillus subtilis* and its regulatory proteins. *J. Mol. Microbiol. Biotechnol.* **4**:
607 427–452.

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

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

612 Morris, A.R. and Visick, K.L. (2013b) The response regulator SypE controls biofilm formation and
613 colonization through phosphorylation of the syp-encoded regulator SypA in *Vibrio*
614 *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- σ 70 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 σ B 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.

- 661 Sorenson, M.K., Ray, S.S., and Darst, S.A. (2004) Crystal structure of the flagellar sigma/anti-
662 sigma complex sigma(28)/FlgM reveals an intact sigma factor in an inactive
663 conformation. *Mol. Cell* **14**: 127–138.
- 664 Staroń, A., Sofia, H.J., Dietrich, S., Ulrich, L.E., Liesegang, H., and Mascher, T. (2009) The third
665 pillar of bacterial signal transduction: classification of the extracytoplasmic function
666 (ECF) sigma factor protein family. *Mol. Microbiol.* **74**: 557–581.
- 667 Thompson, C.C., Griffiths, C., Nicod, S.S., Lowden, N.M., Wigneshweraraj, S., Fisher, D.J., and
668 McClure, M.O. (2015) The Rsb Phosphoregulatory Network Controls Availability of the
669 Primary Sigma Factor in *Chlamydia trachomatis* and Influences the Kinetics of Growth
670 and Development. *PLoS Pathog* **11**: e1005125.
- 671 Valentini, M., Laventie, B.-J., Moscoso, J., Jenal, U., and Filloux, A. (2016) The Diguanylate Cyclase
672 HsbD Intersects with the HptB Regulatory Cascade to Control *Pseudomonas aeruginosa*
673 Biofilm and Motility. *PLoS Genet.* **12**: e1006354.
- 674 Voelker, U., Voelker, A., and Haldenwang, W.G. (1996) Reactivation of the *Bacillus subtilis* anti-
675 sigma B antagonist, RsbV, by stress- or starvation-induced phosphatase activities. *J.*
676 *Bacteriol.* **178**: 5456–5463.
- 677 Wuichet, K. and Zhulin, I.B. (2010) Origins and Diversification of a Complex Signal Transduction
678 System in Prokaryotes. *Sci. Signal.* **3**: ra50.
- 679 Yuan, A.H., Gregory, B.D., Sharp, J.S., McCleary, K.D., Dove, S.L., and Hochschild, A. (2008) Rsd
680 family proteins make simultaneous interactions with regions 2 and 4 of the primary
681 sigma factor. *Mol. Microbiol.* **70**: 1136–1151.

682
683
684
685
686

685 **Figures legends**

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

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

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

700 The signal transduction pathways are divided in three major steps:

701 1(blue) - Signal sensing. Modules involved in the signal detection present a large range of
702 mechanisms with various level of complexity. This step is the most diversified and bacteria have
703 been very creative to detect signals and transduce them to the PSS modules.

704 2 (green) - Intermediate signal transduction from the input (signal sensing) to the output (σ
705 factors). The partner-switching mechanism is highly conserved. The rule is that according to the
706 environmental conditions, the σ factor should be sequestered or released to hamper or allow its
707 regulatory activity, respectively.

708 3 (red) - The σ factor involved in the GSR. The release of the σ factor leads to the expression of
709 the genes belonging to the σ factor regulon.

710 Question marks (?) indicate that the steps have not been experimentally demonstrated. *

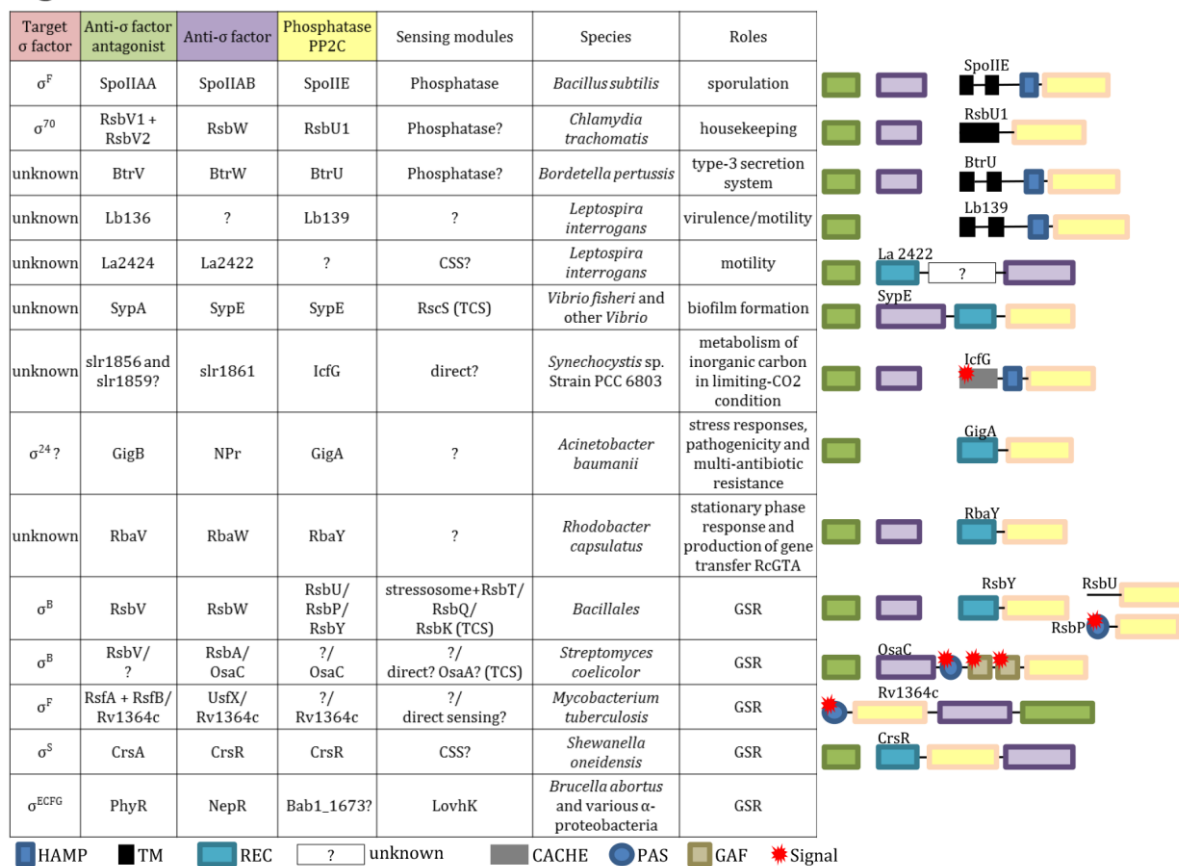
711 means that the components are not conserved in all alpha-proteobacterial species. A green
712 arrow represents a phosphorylation and a red arrow a dephosphorylation event. "HK-CA"
713 corresponds to the domains HisKA and HATPase involved in receiver (REC) phosphorylation.
714 Histidine kinases from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

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

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

725 *Oceanospirillales*, *Pseudomonadales*, *Thiotrichales*, *Vibrionales*. Symbol “*” indicate a genus.
726 Among the genus *Pseudomonas*, the species *P. aeruginosa*, *P. putida*, *P. chlororaphis*, *P.*
727 *fluorescens*, *P. syringae*, *P. stutzeri* were selected. The symbol “**” indicates that the synteny is
728 conserved in all *Pseudomonas* species except for *P. aeruginosa*. The genus *Vibrio* includes *V.*
729 *mimicus*, *V. cholerae*, *V. vulnificus*, and the genus *Shewanella* includes *S. xiamenensis*, *S.*
730 *decolorationis*, *S. sp.* HN-41, *S. baltica* OS185, *S. sp.* ANA-3, *S. sp.* MR-7, *S. putrefaciens* and *S.*
731 *oneidensis* MR-1. Genes surrounding *crsR* homologs were examined by hand using the BioCyc
732 database (Romero and Karp, 2004; Krummenacker *et al.*, 2005). The question mark (?) means
733 that the genes surrounding *crsR* homologs are not exhaustive because of the incomplete
734 database. “HK” stands for Histidine Kinase, “RR” for Response Regulator and “RR-GGDEF” for a
735 receiver domain fused to a GGDEF domain. GGDEF domains have an enzymatic activity
736 producing c-di-GMP necessary for biofilm formation. The corresponding proteins and the color
737 code of the bacterial species are summarized in the bottom of the figure. Inside gene drawings, 3
738 and 5 indicate the number of copies.
739
740

Figure 1



741

742

743

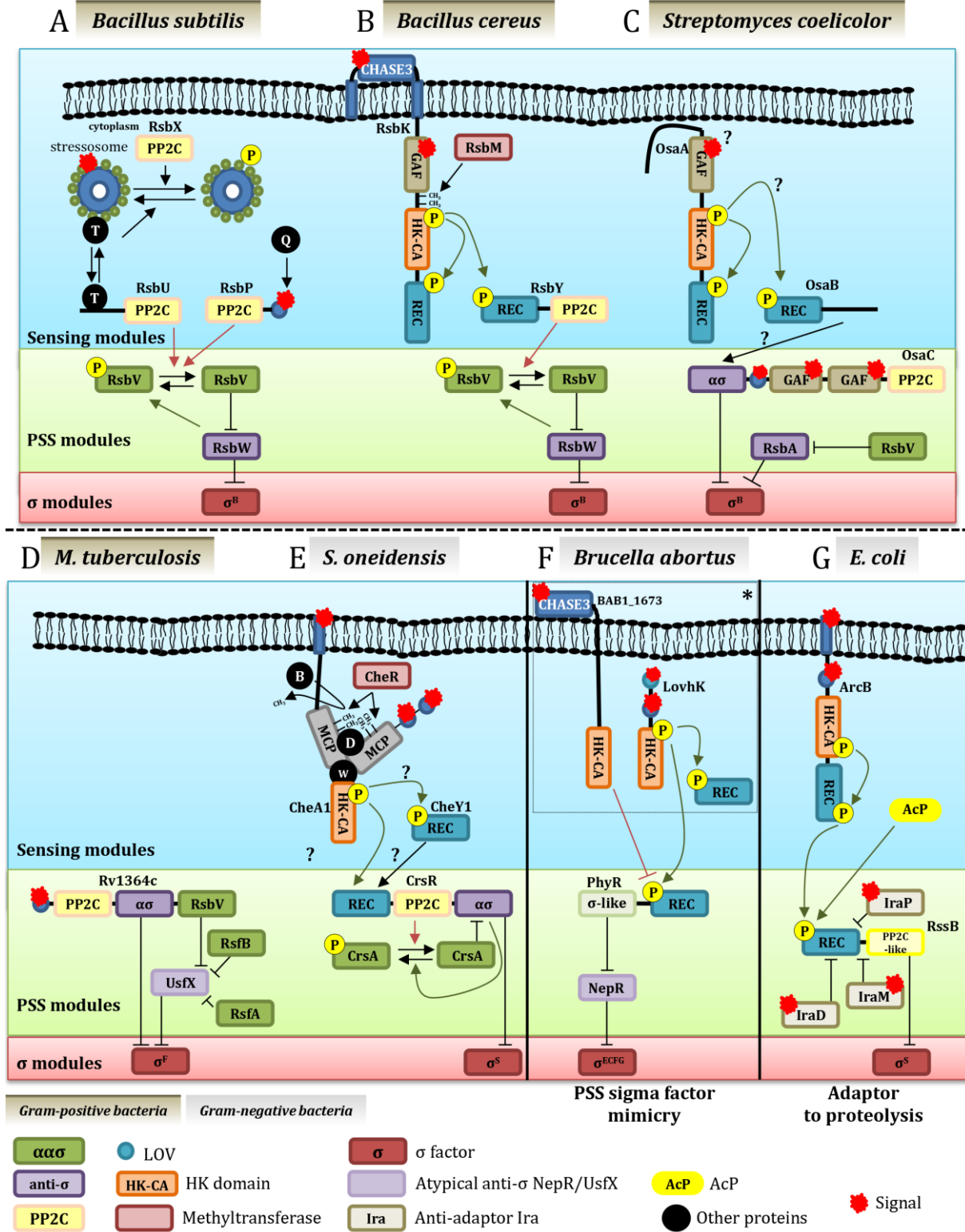
744

745

746

747

Figure 2



748

749

750

Figure 3

