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1 **Regulation of gene expression by non-phosphorylated response regulators**

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Abstract

Two-component systems (TCSs) are the main sensory system in bacteria. A prototypical TCS comprises a membrane-bound sensor histidine kinase (HK) responsible for sensing the signal and a cytoplasmic response regulator (RR) that controls target gene expression. Signal binding activates a phosphotransfer cascade from the HK to the RR. As a result, the phosphorylated RR undertakes a conformational change that results in activation of the response. Growing experimental evidence indicates that unphosphorylated RRs may also have regulatory functions and thus, the classical view that the RR is only active when it is phosphorylated needs to be revisited. In this review, we highlight the most recent findings showing that RRs in the nonphosphorylated state control critical bacterial processes that range from secretion of factors to the host, antibiotic resistance, iron transport, stress response and cell-wall metabolism to biofilm development.

32 **Introduction**

33 One of the specific features that defines a living organism is the capacity to sense and respond to
34 external stimuli. For that, sensory receptors have been developed that detect the presence of
35 environmental signals and transmit this information to an intermediary regulator that modifies its
36 activity to induce the required changes in terms of movement, secretion or enzyme production. In
37 bacteria, the major sensory system is comprised of two-component signaling systems (TCSs) (Stock
38 et al., 2000; West and Stock, 2001). A TCS was firstly described by Ninfa and Magasanik as a signal
39 transduction system involved in the response of *Escherichia coli* to nitrogen availability (Ninfa and
40 Magasanik, 1986). The system was composed of a kinase protein (NRII) that regulated the
41 phosphorylation of a second protein (NRI) responsible for activating the expression of genes
42 involved in nitrogen cycle regulation. Later on, proteins homologous to NRII/NRI involved in sensing
43 environmental stimuli were identified in different bacteria (Nixon et al., 1986). Based on the
44 homology among these proteins, the existence of a new signal transduction system composed of a
45 sensor protein with kinase activity and an efector protein that regulated biological processes once
46 it was phosphorylated by the sensor protein was proposed. It only took two more years to discover
47 that the sensor protein was phosphorylated in a histidine residue whereas the efector protein was
48 phosphorylated in an aspartic residue (Weiss and Magasanik, 1988). The phosphorylation cascade
49 between histidine and aspartic residues contrasted with the phosphorylation of serine, threonine
50 or tyrosine residues characteristic of signal transduction systems in eukaryots and it was decisive to
51 support that TCSs represent a new signal transduction system (West and Stock, 2001). Over the
52 years, the organization and function of TCSs in bacterial biology have been deeply studied, and
53 nowadays, it is well established that a prototypical TCS contains a histidine kinase (HK), which
54 autophosphorylates on a conserved histidine residue in response to extracellular stimuli, and
55 transfers the phosphoryl group to a conserved aspartate residue present in the N-terminal domain

56 of the response regulator (RR). The RRs are often DNA-binding transcriptional activators and/or
57 repressors, and phosphorylation frequently drives dimerization and increases the affinity for their
58 DNA targets. Thus, the primary consequence of the activation of a TCS is the expression of a specific
59 set of genes controlled by the corresponding RR. In fewer cases, the RR lacks the DNA-binding
60 domain and exerts its regulatory effect by establishing direct interactions with protein or RNA
61 targets and adaptation does not necessarily imply modifications in gene expression (Laub and
62 Goulian, 2007; Gao and Stock, 2009; Buschiazzo and Trajtenberg, 2019).
63 In the current understanding of the TC signal transduction, it is assumed that the regulation of gene
64 expression or modification of protein activity mainly occurs when the RR is phosphorylated upon
65 detection of the environmental signal, whereas the non-phosphorylated RR does not have
66 regulatory functions. However, increasing amount of evidence suggest that the non-phosphorylated
67 form of the RR can also be involved in regulatory functions. A comprehensive review by the group
68 of L. Kenney gathered examples of TCSs and orphan RRs with regulatory activity in their
69 unphosphorylated state (Desai and Kenney, 2017). Since then, new insights into the activity of these
70 unphosphorylated RRs and novel examples of gene regulation by other unphosphorylated RRs have
71 been described. The purpose of this microreview is to highlight the new evidence that strengthen
72 the idea that regulatory activity by non-phosphorylated RRs is a general principle in TC signalling
73 systems.
74
75 **AdeR**
76 *Acinetobacter baumannii* is an important opportunistic pathogen particularly in patients who are
77 critically ill or requiring mechanical ventilation. Many *A. baumannii* clinical isolates are resistant to
78 a wide range of antimicrobials, which makes the infections caused by this bacterium very difficult
79 to treat. The two-component regulatory system AdeRS controls the expression of the *adeABC* efflux

80 pump in *A. baumannii* (Marchand et al., 2004). Efflux pumps actively transport a broad range of
81 substrates, including antimicrobials, out of the cell. Functional mutations in conserved domains of
82 AdeRS results in overexpression of the AdeABC efflux pump and a decreased susceptibility to
83 aminoglycosides, tetracyclines, fluoroquinolones, chloramphenicol, erythromycin, trimethoprim
84 and tigecycline (Yoon et al., 2013). AdeRS has also been related with the capacity of the bacteria to
85 produce biofilms and with virulence, but these two phenotypes are strain-specific (Richmond et al.,
86 2016). Experiments with a purified AdeR protein and a target DNA containing the AdeR recognition
87 sequence showed that unphosphorylated AdeR exhibits a dissociation constant of 20nM, which is
88 much higher than that of other PhoP-like response regulators in an activated state (Wen et al.,
89 2017). To investigate the consequences of the activation of AdeR, BeF3 was used as a phosphate
90 analog that mimics the aspartate phosphorylation state and also, aspartate of position 63 was
91 mutated to glutamic acid (AdeR D63E). Interestingly, neither the presence of BeF3 nor the D63E
92 mutation had any influence on the AdeR binding affinity to its target DNA. A biological consequence
93 of this finding is that the expression of the efflux pump and transport of the antibiotics outside the
94 cell can also occur in the absence of the environmental signal that activates the TCS. The study did
95 not evaluate how differences in the activation of the AdeABC efflux pump by phosphorylated and
96 unphosphorylated AdeR forms affect the resistance of *A. baumanii* to antibiotics. Another important
97 aspect that needs further analysis is the contribution of unphosphorylated AdeR to *A. baumanii*
98 antibiotic resistance *in vivo*.
99
100 **AgrR**
101 *Cupriavidus metallidurans* is a Gram-negative bacteria (*Burkholderiaceae* family) characterized by
102 its high resistance to multiple metal ions. Genome analysis software predicted the presence of at
103 least 64 HKs and 87 RRs, of which 11 TCSs appear to be associated with resistance to metals. The

104 two component system AgrRS plays an important role in the survival in the presence of metals (Ali
105 et al., 2020). Mutants in the HK *agrS* showed an increased resistance to silver, strongly suggesting
106 that the non-phosphorylated form of AgrR is responsible for the silver resistance phenotype.
107 However, these results could not exclude that AgrR was being phosphorylated by another HK (cross-
108 talk), a process that is favoured when the phosphatase activity of the HK is absent. Binding assays
109 of AgrR and its phosphomimetic (D51E) and phosphoablative (D51A) derivatives to their target DNA
110 region revealed that AgrR D51A showed the strongest binding affinity, whereas AgrR and the
111 phosphomimetic derivative AgrRD51E bound much less efficiently. These results strongly suggested
112 that unphosphorylated AgrR is the most active DNA-binding form of AgrR. The non-phosphorylated
113 AgrR binds to an imperfect inverted repeat of 6 bp separated by a variable sequence of 4bp and
114 activates the expression of genes (*prsQ2, copA2, czcR2, czcL* or *czcl2*) encoding for proteins
115 functionally related with resistance to metals. This binding site is usually located 43 nt upstream the
116 transcription start site, close to the RNA polymerase binding site, strongly suggesting that the
117 binding of the RR itself can alter the accessibility of the RNA-polymerase to the promoter. In other
118 cases, the binding site is located much further upstream the promoter region suggesting that AgrR-
119 mediated regulation may require the help of another DNA binding protein with capacity to bend the
120 DNA to modify gene expression.
121
122 **AlgR**
123 *Pseudomonas aeruginosa* is one of the most prominent causes of nosocomial infections, particularly
124 in burn wounds and immunocompromised individuals. This is due, at least in part, to the nutritional
125 and metabolic versatility which allows this bacterium to colonize a wide variety of environmental
126 niches ranging from soil to plants and animals. In addition, *P. aeruginosa* has the capacity to produce
127 a large arsenal of virulence factors, and shows a high ability to form biofilms and elevated resistance

128 to a wide range of antibiotics (Gellatly and Hancock, 2013). To sense the environment and adjust
129 the optimal expression of the genes in each environmental condition, *P. aeruginosa* depends on at
130 least 64 HKs and 73 RRs (Rodrigue et al., 2000). The AlgZR two-component regulatory system
131 regulates the production of several iron- or heme related-genes, alginate, rhamnolipids, cyanide and
132 twitching motility (Mohr et al., 1991; Whitchurch et al., 1996; Lizewski et al., 2004). Analysis of gene
133 expression using microarray technology of *P. aeruginosa* PA01 producing a phosphomimetic (AlgR
134 D54E) or a phosphoablative (AlgR D54N) form of AlgR revealed that only 5 genes were regulated in
135 common between both AlgR forms. The mutant producing the unphosphorylated form of AlgR
136 produced lower levels of pyoverdine through a direct activation of expression of the small RNA *prrf2*
137 and repression of the *pvdS* transcriptional regulator. On the contrary, phosphorylated AlgR activated
138 the expression of *czcR* without modifying the expression of *prrf2* or *pvdS*, leading to an increased
139 production of pyoverdine and repression of pyocyanin (Little et al., 2018). The same study also
140 examined whether the phosphorylation status of AlgR affects *P. aeruginosa* pathogenicity by using
141 *Drosophila melanogaster* feeding and murine acute pneumonia and acute wound infection models.
142 The results revealed that *P. aeruginosa* producing the unphosphorylated AlgR protein was
143 attenuated probably due to a deregulation of siderophore production that impacts the capacity of
144 bacteria to establish and maintain an infection.
145
146 **CpxR**
147 *Legionella pneumophila* is a facultative intracellular pathogen which replicates in different amoebae
148 and in human alveolar macrophages. The TCS CpxAR regulates the expression of effectors secreted
149 through the Icm/Dot type-IV secretion system into host cells during infection (Altman and Segal,
150 2008). The first indication that phosphorylation of CpxR was not strictly necessary for CpxR
151 regulatory function came from the analysis of mutants in the cognate HK, CpxA, as well as in the

152 enzymes, AckA and Pta, responsible for the production of acetyl phosphate (Feldheim et al., 2016).
153 Deletion of the CpxA phosphodonor caused only a minor effect on the levels of expression of CpxR
154 regulated genes. However, these results could not exclude that CpxR was being phosphorylated by
155 another HK (cross-talk), a process that is favoured when the phosphatase activity of the HK is absent.
156 To further explore the possibility that CpxR might regulate gene expression in the
157 nonphosphorylated state, two different genetic approaches were next used. First, a *L. pneumophila*
158 strain was complemented with a mutated version of CpxR (CpxR^{D53A}) expressed under the Ptac
159 promoter (activated by IPTG). Second, the aspartic acid residue 53 was replaced by alanine in the
160 chromosomal copy of *cpxR*. Analysis of the expression of selected known target genes in the strains
161 producing CpxR^{D53A} revealed that the unphosphorylated CpxR protein repressed gene expression
162 similarly to the wild-type CpxR. In contrast, CpxR^{D53A} protein was able to activate gene expression to
163 a lesser extent than the wild-type CpxR. As this study focused on the effect of CpxR^{D53A} protein on
164 selected known targets of the CpxR regulon, it did not allow the identification of genes that would
165 only be regulated by the unphosphorylated CpxR protein.
166
167 **OmpR**
168 Enterobacteria have to face the challenge of the acidic barrier of the stomach and also, many
169 intracellular pathogens reside within a membrane-bound acidic compartment inside macrophages.
170 Therefore, the ability to survive exposure to strong acidic conditions is, in many instances, relevant
171 for pathogenicity. *Escherichia coli* and *Salmonella* Typhimurium are able to undertake large changes
172 in intracellular osmolality and tolerate cytoplasmic acidification to at least pH_i 5.6 (Chakraborty et
173 al., 2015). It was long believed that the cytoplasm of both bacteria shows only transient acidification
174 before rapidly returning to the normal pH of ∼7.4. However, groundbreaking studies by the group
175 of L. Kenney challenged this long held view and demonstrated prolonged cytoplasm acidification as

176 a consequence of both acid and osmotic stress (Chakraborty et al., 2015, 2017). To respond to these
177 stresses, *Escherichia coli* and *Salmonella* make use of the TCS EnvZ/OmpR (Stincone et al., 2011;
178 Quinn et al., 2014). It was first described in *Salmonella* that cytoplasmic acidification under acid
179 stress is completely dependent on the OmpR response regulator, but does not require well known
180 OmpR-regulated genes such as *ompC*, *ompF*, or *ssaC*. Instead, OmpR represses the *cadC*/*BA* operon
181 by directly binding to upstream DNA at *cadC* and *cadB*. CadC/BA drive amino acid decarboxylation,
182 a process that consumes intracellular protons, resulting in restoration of cytoplasmic pH. Thus,
183 *cadC*/*BA* repression by OmpR eliminates proton consumption, leading to acidification. This response
184 was shown to be essential for *Salmonella* replication and survival within macrophages (Chakraborty
185 et al., 2015). In 2017, OmpR mediated repression of *cadC*/*BA* was also confirmed in *E. coli* and a
186 detailed analysis of the acid stress response in both *E. coli* and *Salmonella* was carried out in single
187 bacterial cells (Chakraborty et al., 2017). Results showed that EnvZ phosphorylation was very slow
188 at acid pH and that cytoplasmic acidification occurred in an OmpR D55A mutant, incapable of
189 phosphorylation. Intriguingly, this response was completely dependent on the interaction of OmpR
190 with the EnvZ HK. Gel filtration chromatography and atomic force microscopy analysis to examine
191 OmpR dimerization and binding to DNA led to the following model: EnvZ senses acidic pH in the
192 cytoplasm which increases EnvZ helicity and promotes interaction with OmpR, inducing an OmpR
193 conformational change that drives unphosphorylated OmpR dimer formation and favors OmpR
194 binding to DNA. Importantly, authors underlined that this pathway constitutes a therapeutic target,
195 since preventing intracellular acidification of *Salmonella* renders it avirulent.

196

197 **PilR**

198 *Geobacter sulfurreducens* has the capacity to transfer electrons to extracellular acceptors such as
199 Fe(III) and generate bioelectricity. Type IV pili formed by a single protein encoded by the *pilA* gene

200 are necessary for the electron transfer to the extracellular oxides. The expression of *pilA* is under
201 the control of the TCS PilRS. By using different promoter fragments of *pilA* fused to *luxCDABE*
202 (*pilA*::*luxCDABE*), the group of K. Juarez was able to demonstrate that the non-phosphorylated form
203 of PilR is more efficient than its phosphorylated counterpart to activate the expression of *pilA*
204 (Hernández-Eligio et al., 2017). The PilA dependent activation by non-phosphorylated PilR required
205 a region between -191 and -130 upstream the start codon of *pilA* that contains a binding site for
206 PilR. Interestingly, another binding site is located between nucleotides -207 to -192 upstream the
207 *pilA* start codon (Juárez et al., 2009). Although each of these binging sites alone are able to promote
208 PilR binding *in vitro*, it is believed that PilR regulates *pilA* expression by binding to both sites. In
209 addition to *pilA*, PilR also controls the expression of genes encoding for proteins necessary for the
210 electron transport process and adaptation to the presence of Fe (III). Whether the regulation of
211 these genes depends on the phosphorylated or the non-phosphorylated form of PilR remains to be
212 determined.
213
214 **RcsB**
215 Rcs is a complex signal transduction system in enteric bacteria that detects cell envelope stress and
216 triggers an adaptive reponse that involves the outer membrane lipoprotein RcsF interacting with
217 IgaA, an inner membrane protein that, in basal conditions, inhibits the Rcs phosphorelay. This frees
218 the phosphorelay activity as follows: the HK RcsC phosphorylates itself and transfers the phosphoryl
219 group to RcsD, which in turn transfers it to a conserved aspartate residue (D56) of the RR RcsB.
220 Phosphorylated RcsB can homodimerize or heterodimerize with accessory regulator RcsA to bind
221 DNA and exert a positive or negative effect in transcription (Wall et al., 2018). RcsB and RcsA belong
222 to the FixJ/NarL family of DNA binding response regulators, with an N-terminal REC domain and a
223 LuxR-like, DNA-binding helix-turn-helix motif at the C terminus.

224 To add a further level of complexity, RcsB can also regulate gene expression in the absence of
225 phosphorylation through the interaction as heterodimers with other auxiliary proteins, such as GadE
226 (Castanié-Cornet et al., 2010), BglJ (Venkatesh et al., 2010), MatA (Pannen et al., 2016) and RflM
227 (Kühne et al., 2016), all belonging to the FixJ/NarL family of transciptional regulators. In these cases,
228 it has been proposed that regulation of the targets of these heterodimers is likely to be primarily
229 dependent on the signals regulating availability and/or activity of the auxiliary protein (Wall et al.,
230 2018). Processes that are regulated through these interactions, independently of the Rcs
231 phosphorelay, include the glutamate-dependent acid resistance pathway, degradation of β-
232 glucosides, synthesis of mat fimbriae and biofilm formation in *E. coli*, and flagellar synthesis in
233 *Salmonella*. Moreover, unphosphorylated RcsB has been described to activate expression of *csgD*
234 in *Salmonella* Typhimurium . CsgD, a regulator of the LuxR family, is the master regulator of biofilm
235 formation since it activates the synthesis of essential elements of the biofilm matrix, namely curli
236 fimbriae (Römling et al., 2000), the surface protein BapA (Latasa et al., 2005)and cellulose (Zogaj et
237 al., 2001; Solano et al., 2002). It was shown that prevention of RcsB phosphorylation either by
238 impeding phosphorelay from RcsC or RcsD or by producing a nonphosphorylatable RcsB D56Q
239 variant enhanced biofilm development (Latasa et al., 2012). Although a significant increase in the
240 mRNA levels of *csgD* in the RcsB D56Q mutant compared to those in the wild type strain was
241 determined, gel retardation assays failed to detect an interaction of unphosphorylated RcsB to the
242 promoter region of *csgD*. A plausible explanation is that unphosphorylated RcsB might be acting
243 with auxiliary proteins to control *csgD* expression.

244 Very recently, structural and functional studies with different RcsB variants were carried out and
245 showed that DNA binding may stabilize an active conformation in unphosphorylated RcsB, similar
246 to that shown by phosphorylated RcsB. Also, transcriptional profiling in *Salmonella* Typhimurium
247 expressing RcsB variants including a D56A non-phosphorylatable mutant revealed differences in the

248 trascritional profile depending on the phosphorylation state in RcsB. A very important issue was
249 raised in this study, pointing out that just active forms of the RRs can be adscribed to DNA binding
250 rather than unphosphorylated or phosphorylated forms (Huesa et al., 2021).
251
252 **RegA**
253 *Acidithiobacillus ferrooxidans* is a strict acidophilic chemolithoautotrophic bacterium that obtains
254 its energy from the oxidation of iron. The expression of genes involved in iron and sulphur oxidation
255 pathways are controlled by the RegBA TCS (Ponce et al., 2012). The HK RegB is activated by redox
256 signals in the membrane and/or cytoplasm and activates RegA that binds to its target DNA . When
257 iron is present, RegA induces the expression of genes involved in Fe(II) oxidation, while repressing
258 those responsible for inorganic sulfur oxidation. The phosphorylation of RegA is not extrictly
259 necessary for binding to DNA and altering gene expression. A phosphoablative (D68A) derivative
260 form of RegA was able to bind with the same affinity than the phosphorylated wildtype protein to
261 the promoter of genes involved in the iron and sulphur oxidation pathway (Moinier et al., 2017).
262 According to electrophoretic mobility shift assays, RegA binds to regions located upstream the -35
263 and -10 sequences in the promoter of representative target genes, suggesting that RegA does not
264 compete with the RNA polymerase for the DNA-binding site. How does RegA control the expression
265 of the target genes? It is assumed that RegA would block binding of a repressor to the promoter(s)
266 of the genes involved in Fe(II) oxidation whereas it would prevent binding of an inducer to the
267 promoter(s) of the genes involved in sulfur oxidation. A similar situation has been described for a
268 RegA homolog in *Rhodococcus capsulatus*. In aerobic conditions, RegA induces the expression of the
269 respiratory cytochrome cbb_3 whereas in anaerobic conditions, where it is thought that RegA is non-
270 phosphorylated, it represses the expression of *cbb3* (Schindel and Bauer, 2016). The question that

271 remains open is whether the interaction of RegA with other transcription factors depends on the
272 phosphorylation state of the protein.
273
274 **WalR**
275 The two component system WalKR, also known as YycGF, is essential in many gram positive bacteria.
276 In *Streptococcus pneumoniae*, only the RR is essential whereas in the case of *Bacillus subtilis*,
277 *Staphylococcus aureus, Listeria monocytogenes and Enterococcus faecalis,* both the HK and the RR
278 are indispensable. Taking the advantage that only WalR is essential in *S. pneumoniae*, Mohedano et
279 al generated a mutant in WalK by introducing a Km cassette inside the gene (*walK*::kan) (Mohedano
280 et al., 2016). Complementation of this strain with a plasmid harbouring the entire *walKR* system was
281 assumed to represent the phosphorylated state of WalR, whereas complementation with a plasmid
282 harboring only the RR was assumed to represent the unphosphorylated counterpart. Transcriptome
283 and proteome analysis of these strains revealed that the non-phosphorylated form of WalR
284 regulates fatty acid biosynthesis by repressing the *fabT* repressor, which results in the activation of
285 the transcription of the *facKDGF* operon that encodes for proteins related with the fatty acid chain
286 elongation (Mohedano et al., 2005, 2016). Electrophoretic mobility shift assays with
287 nonphosphorylated WalR and the promoter region of *fabT* confirmed that nonphosphorylated WalR
288 binds specifically to the promoter of *fabT* and inhibits its transcription in a dose-dependent mode.
289 The ability of nonphosphorylated WalR to control membrane metabolism explains why the RR can
290 compensate for the absence of the HK. However, this regulatory process should be transient
291 because the accumulation of free fatty acids is deleterious for the cells. The phosphorylated WalR
292 does not regulate fatty acid metabolism while it does control the expression of genes involved in
293 cell wall metabolism and cell division (Ng et al., 2003, 2005; Mohedano et al., 2005).
294

295 **Concluding remarks and future prospects**

296 TCSs have been extensively studied since they were first described 35 years ago. The enduring
297 interest on TCSs is mainly due to the variety and relevance of the biological processes under their
298 control, the high amount of systems that are present in most bacteria, the easiness to unequivocally
299 identify the HK and RR proteins due to the high conservation of their domains, and the conservation
300 and apparent simpleness of the signal transduction procedure. Despite the extraordinary advances
301 in our understanding of the functioning of HKs and RRs, there are still questions that need further
302 analysis. First, which are the signals sensed by each HK? In many TCSs, the signals sensed by the HKs
303 are not well defined. Which is the regulon controlled by each TCS? The identification of genes that
304 are regulated by each TCS to orchestrate the appropriate adaptation to a particular signal is crucial
305 to understand bacterial biology. This task has been traditionally performed through the comparative
306 analysis of the transcription profiles between the wild type and isogenic mutants in the respective
307 HK or RR genes. Obviously, most of these studies were assuming that the observed changes in gene
308 expression were exclusively due to the lost of the phosphorylated RR. The results summarized in
309 this review suggest that this interpretation might be an oversimplification. Future characterization
310 of the regulon of any specific TCS should consider to distinguish between the regulon that
311 corresponds to the phosphorylated and non-phosphorylated RR.

312 **Table 1 Examples of response regulators with regulatory functions in the unphosphorylated state**

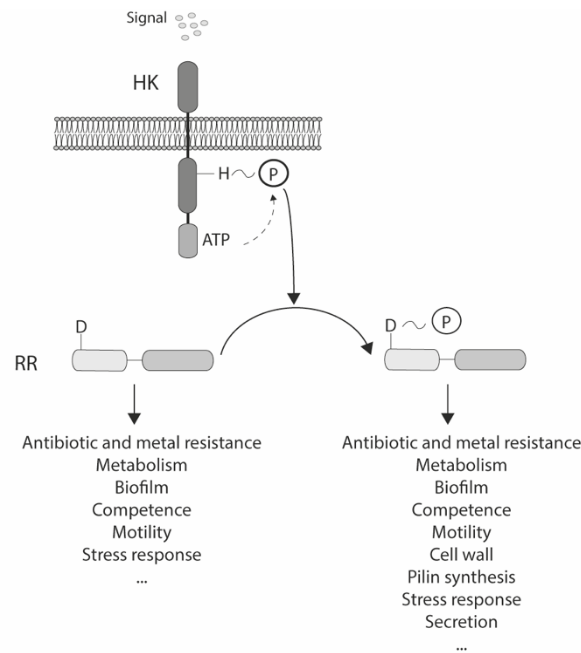
Organism	TCS	Regulated genes by non-phosphorylated RR	Reference
Acinetobacer baumanii	AdeRS	Efflux pump expression (*adeABC*)	(Wen et al., 2017)
Cupriavidus metallidurans	AgrRS	Silver resistance	(Ali et al., 2020)
Pseudomonas aeruginosa	AlgR	Capsular polysaccharide (*alg* genes)	(Little et al., 2018)
Legionella pneumophila	CpxAR	Effectors secreted by Icm/Dot type-IV secretion system	(Feldheim et al., 2016)
Bacillus subtilis	DegU	Competence (*comK*)	(Hamoen et al., 2000)
Salmonella enterica/Escherichia coli	OmpR/ EnvZ	Acidification in response to acid and osmotic stress (*cadC/BA*)	(Chakraborty et al., 2017)
Geobacter sulfurreducens	PilSR	Type IV pili	(Hernández-Eligio et al., 2017)
Salmonella enterica	RcsCB	Biofilm (*csgD*)	
Acidithiobacillus ferrooxidans	RegBA	Iron and inorganic sulphur compounds oxidation pathways	(Moinier et al., 2017)
Salmonella enterica	SrrA/B	Biofilm (*csgD*)	(Desai et al., 2016)
Streptococcus pneumoniae	WalKR	Fatty acid metabolism (*fabT*)	(Mohedano et al., 2016)

313
314

315

316 **Figure 1.** Representation of a prototypical two-component phosphorylation pathway and regulated
317 cellular processes. The histidine kinase binds ATP and autophosphorylates a conserved histidine in
318 the DHp domain. The phosphoryl group is then transferred to an aspartate in the response regulator.
319 Phosphorylation of the response regulator activates its output domain to effect changes in different
320 biological processes. In some cases, the non-phosphorylated response regulator is also able to
321 regulate cellular processes.

322



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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

Not applicable

COMPETING INTERESTS

Carmen Gomez-Arrebola, Cristina Solano and Iñigo Lasa declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHORS' CONTRIBUTIONS

Carmen Gomez-Arrebola: investigation, data curation, writing review&editing. Cristina Solano: investigation, writing-review&editing. Iñigo Lasa: conceptualization, investigation, writing-original draft.

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