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The regulation of bacterial two-partner secretion systems

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Abstract

Two-partner secretion (TPS) systems, also known as Type Vb secretion systems, allow the translocation of effector proteins across the outer membrane of Gram-negative bacteria. By secreting different classes of effectors, including cytolytins and adhesins, TPS systems play important roles in bacterial pathogenesis and host interactions. Here, we review the current knowledge on TPS systems regulation and highlight specific and common regulatory mechanisms across TPS functional classes. We discuss in detail the specific regulatory networks identified in various bacterial species and emphasize the importance of understanding the context-dependent regulation of TPS systems. Several regulatory cues reflecting host environment during infection, such as temperature and iron availability, are common determinants of expression for TPS systems, even across relatively distant species. These common regulatory pathways often affect TPS systems across subfamilies with different effector functions, representing conserved global infection-related regulatory mechanisms.

1 | INTRODUCTION

The coordinated expression of membrane components is crucial for bacterial niche-specific survival and adaptation. Bacterial secretion systems have evolved to constitute a wide family of molecular machineries allowing the export of proteins or DNA (Green & Mecsas, 2016). In Gram-negative bacteria, several types of secretion systems allow export across the two membranes, each exhibiting a unique architecture, mechanism of secretion, and nature of secreted effector(s) (for a review, see Costa et al., 2015). Some bacterial species, such as *Pseudomonas aeruginosa*, potentially synthesize more than a dozen different types of secretion systems (Bleves et al., 2010; Filloux, 2011), most of them being tightly regulated and assembled only in response to specific external signals.

Two-partner secretion (TPS) systems are simple export apparatus also referred to as subtype b of type V secretion systems (for a review, see Guerin et al., 2017). As opposed to all other subtypes of type V secretion systems where the two partners are fused into a single polypeptide (Clarke et al., 2022; Meuskens et al., 2019), members of this family include an exported effector, TpsA, and a cognate outer membrane transporter, TpsB. The genes encoding TpsA and TpsB are usually organized in an operon as a single transcriptional unit, ensuring their co-regulation. Some TpsB proteins export more than one effector proteins that usually play distinct roles (Garnett et al., 2015; Julio & Cotter, 2005). TpsA proteins are generally large and fold in elongated structures attached to the bacterial surface by their N-terminal domain (Guerin et al., 2017). Different functional classes of TpsA exist

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based on their C-terminal domain, including adhesins, cytolysins/hemolysins, and proteases.

TPS systems are particularly important in pathogens as they drive phenotypes such as cytotoxicity, proteolysis, host-cell adhesion, biofilm formation, and microbial competition. The two TPS systems ShlBA from *Serratia marcescens* and FhaBC from *Bordetella pertussis* were the first studied and are critical actors of pathogenesis through toxin secretion (Domenighini et al., 1990; Poole et al., 1988). More recently, contact-dependent growth inhibition (CDI) systems were identified as a subtype of TPS systems mediating direct cell-cell physical interactions, involved in bacterial competition, biofilm formation (Ruhe et al., 2013), and virulence toward mammalian hosts (Allen et al., 2020).

TPS systems, much like most secretion systems, are usually prone to be exchanged by horizontal gene transfer (Rojas et al., 2002; Trouillon et al., 2020; van Ulsen et al., 2008; Willett et al., 2015), which in turn brings diversification of both function and regulation. As of today, more than 1000 TPS systems have been identified in Gram-negative bacteria, especially in *Proteobacteria*, *Cyanobacteria*, and *Negativicutes* where they are abundant, representing the third most common family of secretion systems behind autotransporters and type I secretion systems (Abby et al., 2016). While the mechanisms of secretion and the effector functions of TPS systems have been thoroughly reviewed (Fan et al., 2016; Guerin et al., 2017; Jacob-Dubuisson et al., 2013; Leo et al., 2012; Meuskens et al., 2019), similar up-to date analyses are lacking concerning their regulation.

In this review, we assess the existence of common and specific regulatory mechanisms driving transcription of the most characterized TPS systems. Host or environmental signals such as temperature, iron, or inorganic phosphate availability, sensed through different mechanisms, as well as bacterial signaling molecules—cAMP, c-di-GMP, (p)ppGpp—are contributing to the regulation of TPS systems. In pathogenic bacteria, TPS systems are usually controlled by regulatory pathways that also drive the expression of other virulence

factors, as part of complex orchestrated responses. In addition, some TPS systems have evolved specific regulatory mechanisms to adapt their synthesis to the physiology of the different hosts.

2 | THE REGULATION OF CYTOLYSINS

A large family of TPS effector proteins includes cytolysins/hemolysins, which have the ability to disrupt membranes of host cells (Skals & Praetorius, 2013). Accordingly, conditions encountered within the host are common stimuli for their expression (Table 1). Iron is indispensable for bacterial growth and hosts often limit iron availability as part of their innate defense against invading bacteria (Weinberg, 2009). Iron-limiting conditions are thus perceived by bacterial pathogens as a signal for host invasion, which then respond by upregulating iron acquisition systems and virulence factors, such as cytolysins. Temperature is also a common host-associated cue that modulates virulence factor expression (Konkel & Tilly, 2000). Different temperature changes can be sensed and indicate on the nature of the host, that is, cold- or warm-blooded host, which in turn drives different regulatory responses. Finally, a regulatory interplay between flagellar and TPS gene expression exists in many bacterial pathogens, which relies on the master regulator FlhDC standing at the top of the flagellar gene regulatory cascade; this leads to coordinate synthesis of both cytotoxic factors and flagellum, which also contributes to virulence through motility, chemotaxis, adhesion to and invasion of host surfaces.

The ShlAB TPS system is a major virulence factor of *Serratia marcescens*. ShlA is a hemolysin promoting the release of iron-containing hemoglobin from lysed red blood cells, which in turn facilitates iron acquisition by the pathogen. Iron limitation was the first environmental cue shown to stimulate ShlAB expression (Poole & Braun, 1988a). The regulation of ShlAB has since been well characterized and represents a complex interplay between multiple regulatory pathways

TABLE 1 Common signals and regulators controlling TPS synthesis and their underlying mechanisms.

Signal	Function	TPS	Organism	Regulator	Reference
Iron	Cytolysin/Hemolysin	ShlAB	<i>Serratia marcescens</i>	Fur (putative BSs)	Poole and Braun (1988a), Lin et al. (2016)
				RssAB TCS	
		PhlAB	<i>Photobacterium luminescens</i>	Fur (putative BSs)	Brillard et al. (2002)
		XhlAB	<i>Xenorhabdus nematophila</i>	FlhDC cascade	Cowles and Goodrich-Blair (2005), Jubelin et al. (2011)
		HpmAB	<i>Proteus mirabilis</i>	Fur (putative BS)	Uphoff and Welch (1990)
		EthBA	<i>Edwardsiella tarda</i>	Fur	Weinberg (2009)
Iron acquisition		HxuAB	<i>Haemophilus influenzae</i>	Fur	Harrison et al. (2013)
Temperature	Cytolysin	ShlAB	<i>Serratia marcescens</i>	RssAB TCS (via FlhDC)	Lin et al. (2016)
		EthAB	<i>Edwardsiella tarda</i>	nucleoid protein Hha _{Et}	Wang et al. (2010)
	Adhesin	FhaBC	<i>Bordetella pertussis</i>	BvgSA TCS	Cotter and Miller (1994)
		Iron acquisition	HxuAB	<i>Haemophilus parasuis</i>	?

Note: The question mark indicates that the regulators involved are not known or confirmed.

Abbreviations: BS(s), binding site(s); TCS, two-component system.

(Figure 1). As for other cytolysins, putative-binding sites for the ferric uptake regulator (Fur) transcription factor, which controls iron homeostasis in many bacteria (Fillat, 2014), are present in the *shlBA* promoter, inferring a direct negative control in the presence of iron (Poole & Braun, 1988a). Another iron-sensing mechanism relies on the RssAB two-component regulatory system (TCS) that negatively controls *shlBA* expression (Lin et al., 2010). TCSs are typically composed of two proteins, a signal-sensing histidine kinase which induces the activation of a cognate response regulator, often a transcription factor, by phosphorylation upon signal sensing (Stock et al., 2000). The RssAB system, which is directly stimulated by environmental Fe^{3+} ferric ions (Lin et al., 2016), was shown to repress *shlBA* expression and swarming motility at 37°C, favoring transition toward a biofilm lifestyle (Lin et al., 2010). The negative effect of temperature is in agreement with swarming occurring at 30°C and not 37°C (Lai et al., 2005) and a reduced hemolytic activity at 37°C (Poole & Braun, 1988b). *S. marcescens* can infect both vertebrate and invertebrate hosts (Grimont & Grimont, 1978) and is only an opportunistic pathogen for humans, which might explain why the optimal temperature for expression of some virulence factors is not that of the human body. Through their control of RssAB signaling, other factors such as saturated fatty acids (Lai et al., 2005)

and biofilm lifestyle (Tsai et al., 2011) might also influence ShlA synthesis. RssAB effect on *shlBA* expression is not direct but carried out through the master flagellar regulator FlhDC which activates the synthesis of the sigma factor FliA that in turn binds to the *shlBA* promoter and initiates transcription (Di Venanzio et al., 2014; Lin et al., 2010). Therefore, activation of RssB leads to reduced levels of *shlBA* by inhibition of *flhDC* expression and consequent *fliA* down-regulation. FlhDC synthesis is also regulated in response to envelope stresses through the Rcs phosphorelay (Di Venanzio et al., 2014), a complex signal transduction system comprising the inner membrane sensor proteins RcsC and RcsD, the response regulator RcsB, and the outer membrane lipoprotein RcsF, responsible for sensing cellular stresses (Wall et al., 2018; Wall et al., 2020). In *S. marcescens*, phosphorylated RcsB binds to *flhDC* and *shlBA* promoters, thus inhibiting *shlBA* expression both indirectly and directly (Di Venanzio et al., 2014). Furthermore, the inactivation of *gumB*, encoding an inner membrane IgaA-like protein that senses cell stresses, leads to strong downregulation of *shlBA* through an Rcs-dependent mechanism (Brothers et al., 2019; Romanowski et al., 2021). In agreement, GumB is required for survival and proliferation of *S. marcescens* in murine phagocytic cells and in *Galleria mellonella* larvae, potentially due to its positive effect on ShlA synthesis. The Rcs signaling is

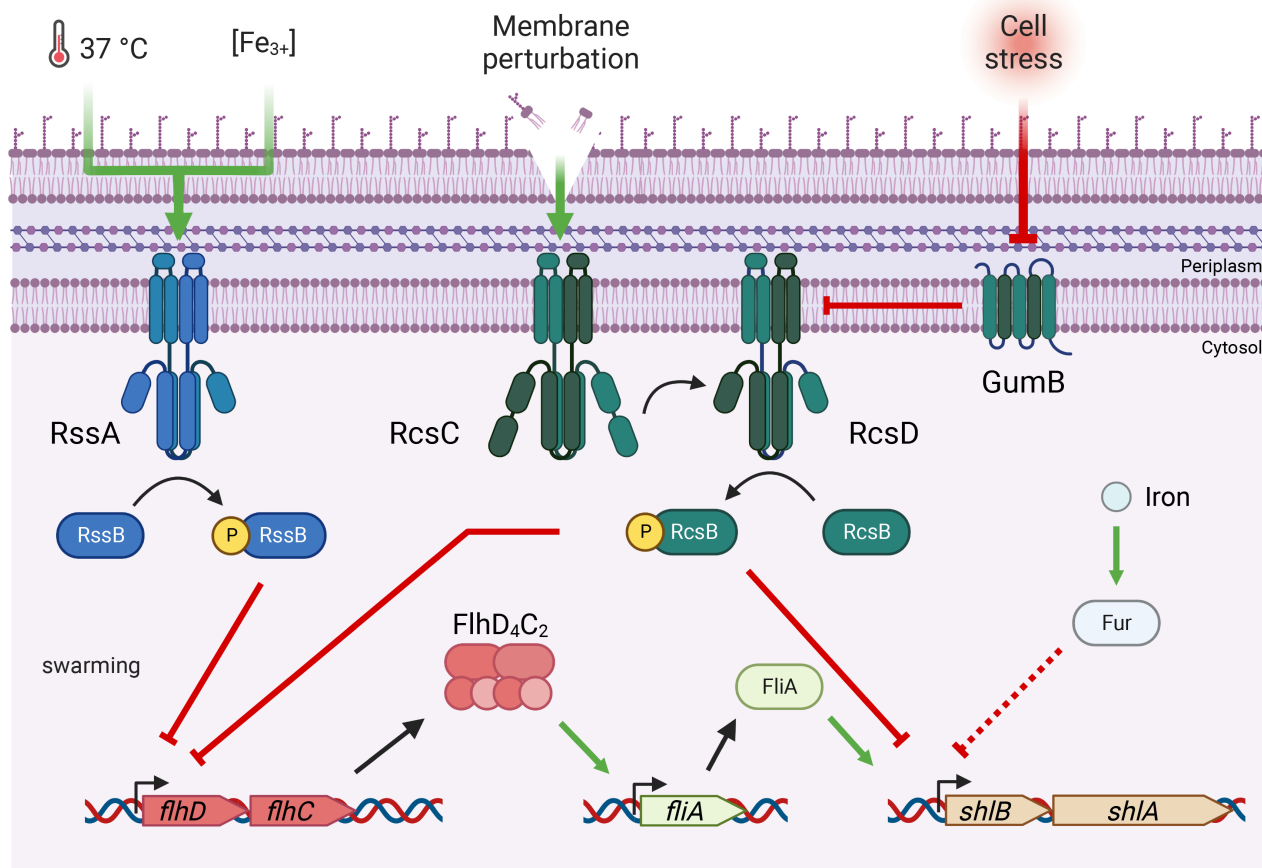


FIGURE 1 The regulation of *shlBA* in *Serratia marcescens*. The different proteins and genes involved in *shlBA* regulation are shown. Red and green lines indicate inhibition and activation, respectively. The dotted line represents a potential direct regulation identified by binding site sequence search.

also triggered by cell surface perturbations, such as impaired synthesis of different lipids in the outer membrane (LPS or the glycolipid Enterobacterial Common Antigen "ECA") or high osmolarity (Castelli & Vescovi, 2011). Accordingly, alteration in ECA synthesis inhibits *shlBA* expression as well as hemolytic activity (Di Venanzio et al., 2014).

Iron limitation has been shown to control the expression of two cytolysins in entomopathogenic bacteria, PhIA in *Photorhabdus luminescens* and XhIA in *Xenorhabdus nematophila*. *P. luminescens* lives symbiotically in the gut of nematodes and kills insects when released in their hemolymph. Although the role of PhIA in *P. luminescens* life-style is still unknown, the *phlBA* operon is upregulated upon iron restriction, in agreement with a higher hemolytic activity measured in this condition (Brillard et al., 2002). Several putative Fur-binding sites have been located in the *phlBA* promoter, suggesting its direct involvement in this regulation although it has not been experimentally tested. The second entomopathogen, *X. nematophila*, kills insects either directly from hemolymph or in cooperation with its symbiotic host nematode (for review Forst et al., 1997). Among the virulence factors required for overcoming the insect immune system are at least three hemolysins, including the TpsA-like protein XhIA. The XhIAB system is required for full virulence toward *Manduca sexta* larvae, providing evidence that the hemolysin is active during insect infection (Cowles & Goodrich-Blair, 2005). Most likely secreted by XhIB, XhIA exhibits a surface-associated hemolytic activity that can also target granulocytes and plasmacytes. Expression of the *xhlBA* operon is higher in stationary phase and modulated by iron availability in a Fur-independent manner (Jubelin et al., 2011). Strikingly, iron seems to exert two opposite effects on the operon depending on the growth phase; its limitation was first reported as upregulating the *xhlA* expression in exponentially growing cells but a repressive effect exerted through the flagellar gene regulatory cascade was observed in stationary phase (Cowles & Goodrich-Blair, 2005; Jubelin et al., 2011). At the top of this regulatory cascade lies the master flagellar regulator FlhDC which is required for full virulence by directly and indirectly controlling both flagellar genes and non-flagellar virulence genes (Jubelin et al., 2011). Its large regulon relies in part on its control over the *fliAZ* expression: This operon encodes the σ^{28} factor FliA and the regulator FliZ, which in turn directly activates *flhDC* (positive feedback loop) and *xhlAB*, among other genes (Givaudan & Lanois, 2017; Lanois et al., 2008). A link between iron availability and flagellar gene regulatory cascade was found when both *flhDC* and *fliAZ* operons were shown downregulated during iron limitation, although not targeted by the Fur regulator directly (Jubelin et al., 2011). Production of hemolysins and other secreted products triggered in iron-rich sites of infection, such as in larvae cadavers, would facilitate dissemination of the bacteria and degradation of the tissues (Jubelin et al., 2011). Besides integrating iron signals, FliZ is a global regulator for over 270 genes and plays a role in stochastic phenotypic variation in *X. nematophila* (Jubelin et al., 2013). Indeed, the expression of FliZ target genes, including *xhlA*, was shown to be bimodal, leading to phenotypic heterogeneity between genetically identical bacteria (Jubelin et al., 2013). Other transcriptional

regulators were found to control *xhlBA* expression, such as the global regulator Lrp that directly activates the operon expression (Cowles et al., 2007; Cowles & Goodrich-Blair, 2005). Lrp regulates various metabolic pathways in a coordinated manner as well as antibiotic production, protease activity, motility, and the *lysR*-like homolog A (*lrhA*) gene, which encodes a regulator controlling the expression of virulence traits (Cowles et al., 2007; Richards et al., 2008; Richards & Goodrich-Blair, 2009). It should be noted that both Lrp and LrhA have a positive effect on the transcription of the flagellar regulator FlhDC (Richards et al., 2008), thus also indirectly affecting *xhlBA* expression.

P. mirabilis is a common pathogen of the urinary tract and provokes infections in individuals with urinary tract abnormalities or with vesicular catheters. Among its different virulence determinants, HpmA is a potent cytolysin able to mediate lysis of a wide range of cell types (for a review, see Armbruster et al., 2018). The *hpmBA* expression is upregulated during swarming (Fraser et al., 2002) and several endonucleolytic cleavages occur along the unstable *hpmBA* mRNA transcript leading to an excess of stable *hpmA* mRNAs. This unbalanced mRNA quantities potentially leads to the production of more secreted effectors than transporters (Fraser et al., 2002). Control of HpmAB synthesis by iron limitation is suggested by the presence of a putative Fur-binding site in the *hpmBA* promoter region (Uphoff & Welch, 1990). Both synthesis and secretion of HpmA are increased in hyper-flagellated swarming cells compared to vegetative cells, accompanied by a 20-fold higher hemolytic activity during swarming, which is coordinately regulated with flagellum synthesis in swarming cells (Allison et al., 1992; Gygi et al., 1995). FlhDC, Lrp, and UmoB control positively *hpmBA* expression and flagellar biosynthesis during swarming, even if only Lrp directly controls *hpmBA* by binding to the operon promoter, probably at multiple sites (Fraser et al., 2002). Similarly to Gumb in *S. marcescens*, UmoB is an IgaA-like membrane-bound positive regulator of FlhDC; it inhibits the Rcs phosphorelay that controls negatively *flhDC* expression upon surface contact (Figure 1). However, UmoB cannot functionally complement Gumb, unlike other IgaA proteins (Brothers et al., 2019; Morgenstein & Rather, 2012). Initially, FlhA, the inner membrane component of the flagellum export machine, was shown to positively regulate the flagellin-encoding genes *fliC* and *hpmA* in swarming cells (Gygi et al., 1995). However, the reduced levels of *fliC* and *hpmA* transcripts in the *flhA* mutant may result from a negative feedback loop on *flhDC* expression due to the induced defect in the membrane assembly of the flagellum (Dufour et al., 1998).

The Gram-negative opportunistic pathogen *Edwardsiella tarda* has a broad host range, causing hemorrhagic septicemia in fish and both gastro- and extra-intestinal infections in humans. Its virulence mainly relies on the EthA hemolysin, as well as Type III (T3SS) and Type VI (T6SS) secretion systems. The regulation of the *ethBA* operon is complex and involves at least six different regulatory pathways (Figure 2). The expression of *ethB* was found upregulated during infection of Japanese flounder (in liver and kidney) compared to in vitro growth conditions (Wang et al., 2009). Similar to many hemolysin-encoding genes, *ethA* is directly regulated by iron

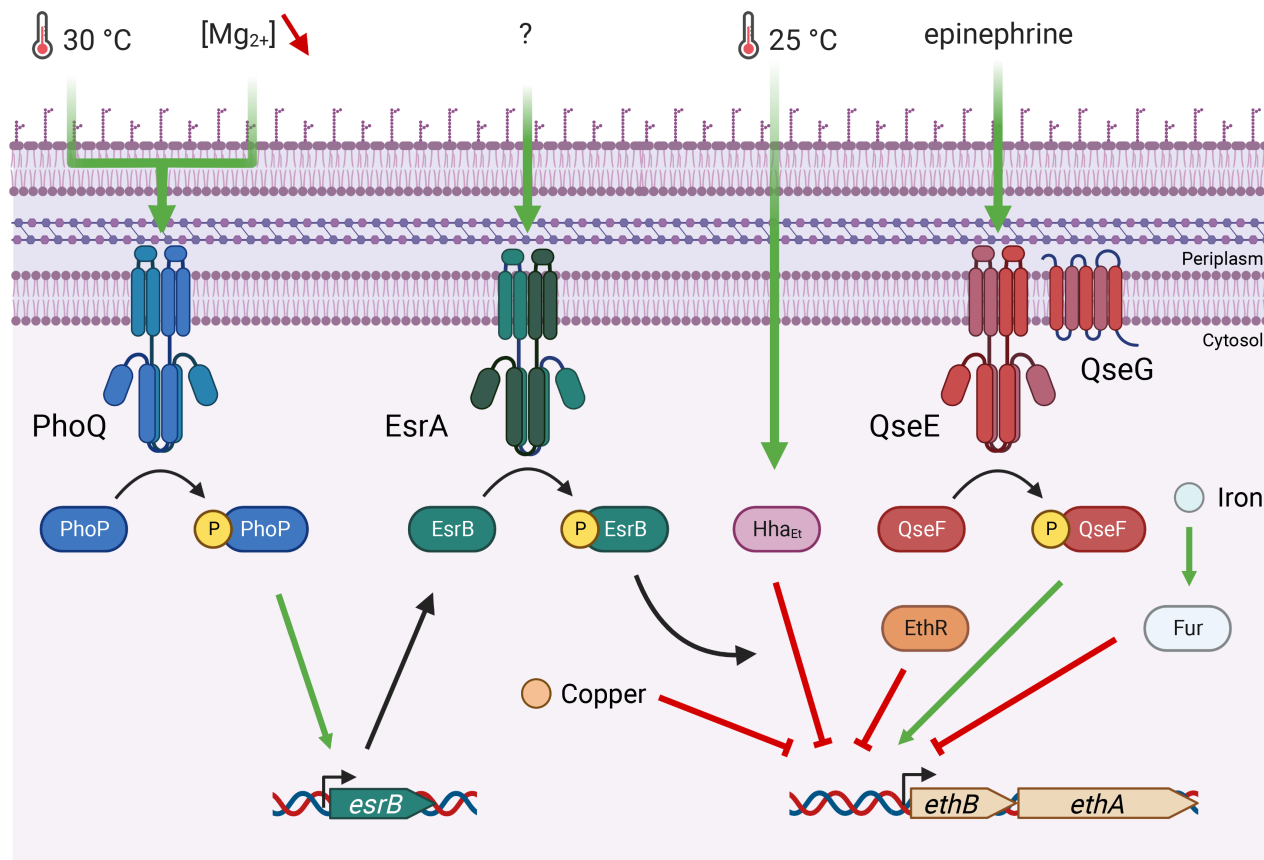


FIGURE 2 The regulation of *ethBA* in *Edwardsiella tarda*. The different proteins and genes involved in *ethBA* regulation are shown. Red and green lines indicate inhibition and activation, respectively.

availability in a Fur-dependent manner (Hirono et al., 1997; Wang et al., 2009). A genetic screen for *ethBA* regulators identified a direct repressor belonging to the GntR family called EthR (*ethB* Regulator) (Wang et al., 2009). This regulator is also a direct activator of the *luxS* gene, modulating the LuxS/AI-2 quorum sensing system also involved in bacterial pathogenicity (Wang et al., 2009). To note, both *ethBA* and *luxS* expressions are highly reduced by copper, a potent antimicrobial metal (Hu et al., 2010). In addition, the regulation of *ethBA* involves multiple TCSs which directly or indirectly modulate its expression in response to different signals. For instance, the EsrAB TCS negatively regulates EthA synthesis but activates both T3SS and T6SS (Wang et al., 2010; Zheng et al., 2005). Deletion of the *esrB* gene permits a temperature-dependent regulation of *ethA* with a higher EthA hemolytic activity observed at 37°C than at 25°C (Wang et al., 2010). While hemolytic activity is reduced at this temperature (Wang et al., 2010), the EsrB-dependent upregulation of T3SS and T6SS gene clusters is higher at 25°C (Rao et al., 2004). The induction observed in an *esrB* mutant relies on the activity of the nucleoid protein Hha_{Et} that can bind upstream of *ethA* and directly modulates its expression, probably by alteration of DNA supercoiling (Wang et al., 2010). *esrB* transcription is also affected by an interplay between the temperature-responsive PhoQP TCS and EsrAB where PhoP directly upregulates *esrB* at 30°C (Guijarro et al., 2015). In addition to temperature changes, PhoQ also responds to low Mg²⁺

concentration, the two signals having an additive effect (Chakraborty et al., 2010). Magnesium availability controls the pathogenicity of different bacteria and its low concentration could be a signal encountered during human intestinal infection (Liu et al., 2020). Finally, the QseEF TCS also regulates *ethA* expression, along with the membrane protein QseG, and is required for full virulence and survival in macrophages (Xiao et al., 2012).

The Gram-negative bacterium *P. aeruginosa* is able to infect various hosts such as plants, insects, and humans, and is one of the leading causes of nosocomial infections (Diggle & Whiteley, 2020). While its major virulence factor is the well-known T3SS (Klockgether & Tummeler, 2017), a taxonomic outlier group contains the PA7-like strains that do not possess the T3SS-encoding genes (Freschi et al., 2019). Instead, they express ExlAB, a TPS secreting the cytotoxin ExlA (Elsen et al., 2014; Huber et al., 2016). Interestingly, these two secretion systems appear to be functionally incompatible, as no strain possessing both T3SS and ExlAB has ever been identified. To note, PA7-like strains were recently reclassified as part of the new species *Pseudomonas paraeruginosa* due to their high phylogenetic distances to classical *P. aeruginosa* strains (Rudra et al., 2022). ExlA is translocated through the outer membrane by ExlB and induces formation of pores in eukaryotic cell membranes, leading to membrane disruption and eventually cell death (Basso, Ragno, et al., 2017; Elsen et al., 2014), making ExlAB the major virulence factor of the PA7-like

strains. Two main transcription factors are known to regulate the expression of *exlBA* (Figure 3). This operon is under the direct regulation of the virulence factor regulator Vfr (Berry et al., 2018). Vfr is a homolog of the global regulator CRP in *Escherichia coli* and is known to regulate hundreds of genes, many of them encoding virulence factors (West et al., 1994; Wolfgang et al., 2003). Vfr is activated upon sensing of its cognate allosteric activator, the second messenger cAMP, and is thus involved in the cAMP- and c-di-GMP-dependent planktonic–biofilm lifestyle switch of *P. aeruginosa* (Moradali et al., 2017). cAMP levels are tightly regulated by two adenylate cyclases and are known to increase in response to calcium depletion or host cell contact (Lory et al., 2004). The activation of *exlBA* by Vfr illustrates the key role of the regulator in virulence across the entire species. Indeed, it has been thoroughly characterized in T3SS⁺ strains where it activates the T3SS. Vfr regulates its own expression through a positive feedback loop (Fuchs et al., 2010), and is under post-transcriptional regulation by the RNA-binding proteins RsmA and Hfq (Irie et al., 2020). Hfq is a regulatory protein that mainly facilitates regulation of translation via interaction with small non-coding RNAs and their mRNA targets (Vogel & Luisi, 2011). Vfr was additionally shown to be post-transcriptionally activated by the RhIS small RNA in a Hfq-dependent manner in an *exlBA*⁺ strain (Trouillon et al., 2022). The second messenger c-di-GMP is also affecting the

TPS at the post-translational level. c-di-GMP controls several important cellular traits associated with the biofilm lifestyle, with a high intracellular concentration stimulating the expression of extracellular matrix components and adhesion factors (for reviews, see Romling et al., 2013; Valentini & Filloux, 2019). The ExlB-dependent secretion of ExlA is enhanced when c-di-GMP levels are reduced (Deruelle et al., 2021), through an unknown mechanism that differs from that of CdrA (see below). In addition to Vfr, a second regulator of *exlBA* has recently been identified, the exolysin regulatory factor A (ErfA) transcription factor, which strongly inhibits the expression of the operon, thus counteracting the positive effect of cAMP-activated Vfr (Trouillon et al., 2020). This might explain the absence of increased cytotoxicity or virulence on animal models when Vfr or CyaB is overexpressed in an *exlBA*⁺ strain (García-Reyes et al., 2021). It was demonstrated that ErfA and Vfr bind to the *exlBA* promoter at two different locations, allowing independent binding of both regulators. However, while the activating signal of Vfr is known, the existence and nature of a potential signal detected by ErfA are still to be elucidated in order to comprehensively apprehend virulence regulation in the PA7-like lineage. Moreover, it was recently shown that as many as 13 response regulators were able to bind to the *exlBA* promoter in vitro (Trouillon et al., 2021), suggesting a much more complex regulatory mechanism for this TPS. The ExlAB secretion

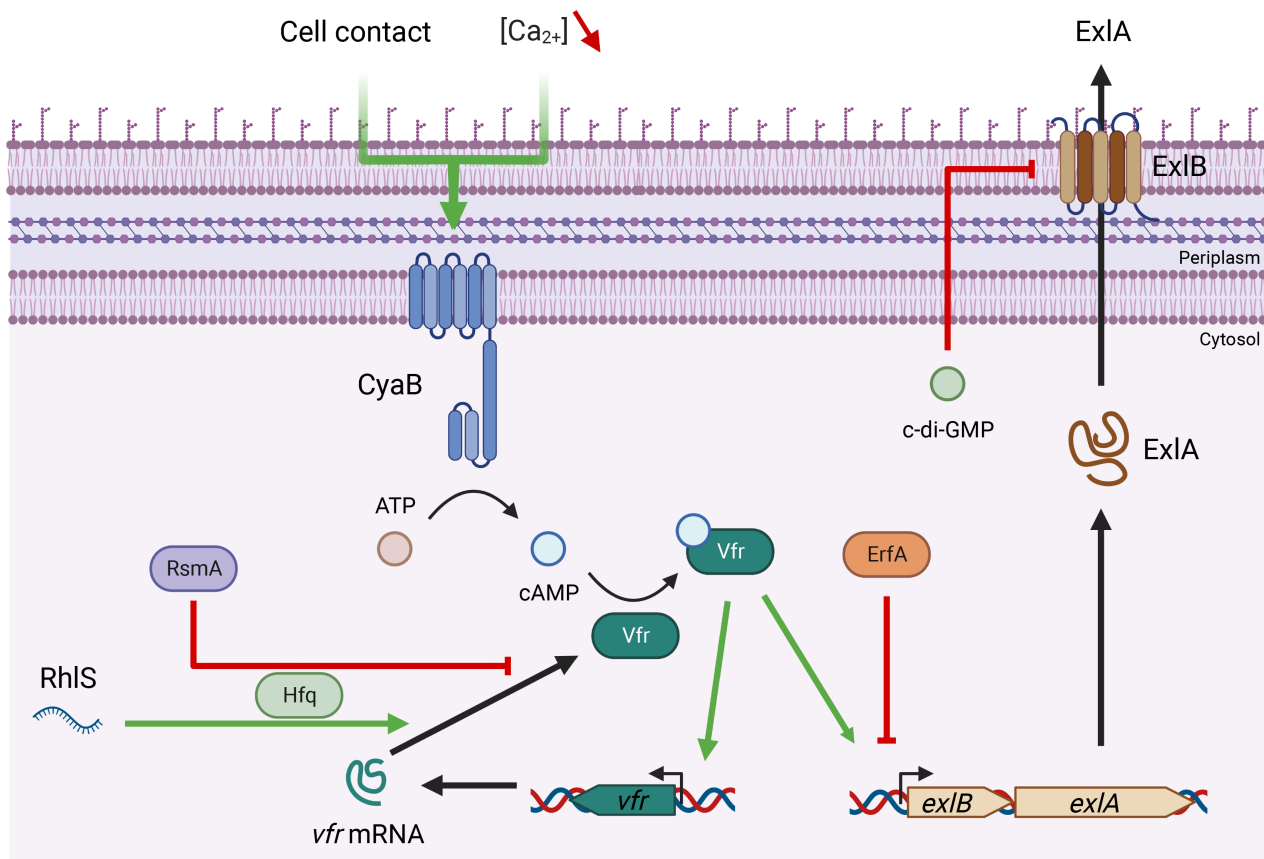


FIGURE 3 The regulation of *exlBA* in *Pseudomonas (par)aeruginosa*. The different proteins, RNAs, and genes involved in *exlBA* regulation are shown. Red and green lines indicate inhibition and activation, respectively.

system is also found in several other *Pseudomonas* species, including *P. chlororaphis*, *P. protegens*, *P. putida*, and *P. entomophila*, in which the toxin activity toward host cell membranes differs mainly due to differences in their C-terminal domains (Basso, Wallet, et al., 2017; Job et al., 2022). In-depth comparative genomic and phylogenetic analyses revealed the complex evolutionary history of the *exlBA* operon, which was acquired several times within the genus (Job et al., 2022). All of these *Pseudomonas* species thrive in different environments and range from plant- and soil-dwelling biocontrol agents to human pathogens. Looking for an underlying explanation behind the conservation of such potent virulence factor in this wide array of different species, the *exlBA* promoter sequence was recently found to exhibit a high diversity across the *Pseudomonas* genus, with the presence of both ErfA- and Vfr-binding sites being restricted to *P. paraeruginosa* (Trouillon et al., 2020). After acquisition of *exlBA* by horizontal gene transfer, the operon was wired to the ErfA and Vfr regulons by evolution of their respective binding sites in the *P. paraeruginosa exlBA* promoter. In other species, ErfA and Vfr are not involved and different putative binding sites are found on the *exlBA* promoter, reflecting an evolutionary mechanism allowing the regulatory adaptation of newly acquired genes to better fit each individual niche.

3 | THE REGULATION OF ADHESINS

Many TpsA proteins exhibit adhesive properties. Adhesion is an essential step in pathogenesis, as it is required during host colonization; therefore, as observed with cytolytins, conditions encountered within the human host often stimulate their expression, such as temperature and iron availability. The best studied example is the filamentous hemagglutinin FHA of *Bordetella pertussis* (Figure 4), the causative agent of the whooping cough. FHA is a 220kDa protein whose precursor, FhaB (367kDa), is translocated by its cognate transporter FhaC and processed during secretion by cleavage of its C-terminal prodomain. FHA folds into a rigid β -helix on bacterial surface and is ultimately released. In addition to adhesion to epithelial cells, the protein promotes biofilm formation, modulates immune responses (reviewed in Scheller & Cotter, 2015), and contributes to bacterial persistence in the lower respiratory tract of infected mice (Melvin et al., 2015). Expression of FHA, as of other virulence factors including the pertussis toxins PTX and Cya, is modulated by DNA gyrase-inhibiting drugs, indicating a role of DNA supercoiling (Graeff-Wohlleben et al., 1995). The TPS-encoding genes are organized in a complex locus—*bvgSA-fhaB-fimA-fimBCDfhaC*—with *fhaC* being transcribed from the *fimB* promoter (Scheller & Cotter, 2015;

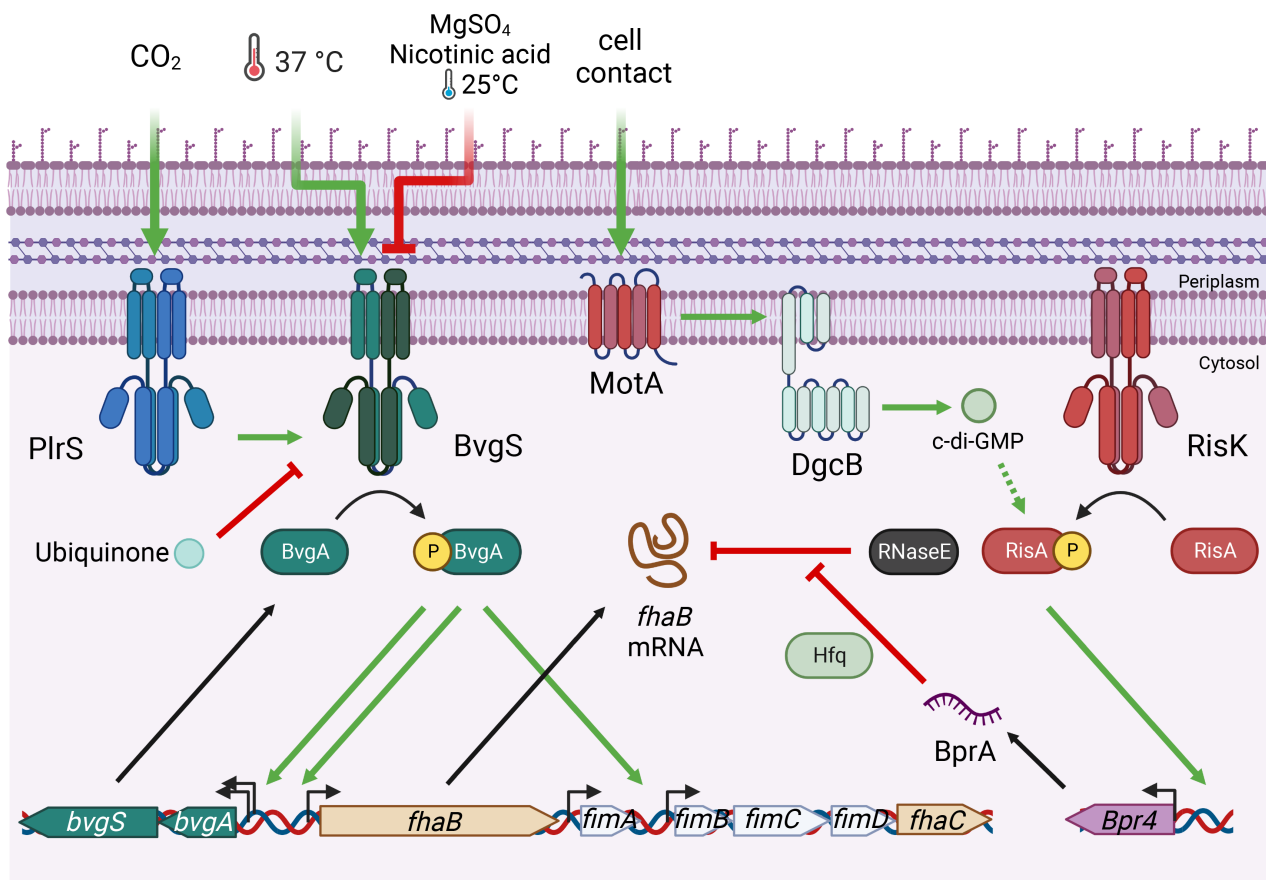


FIGURE 4 The regulation of *fhaB-fhaC* in *Bordetella pertussis*. The different proteins, RNAs, and genes involved in IspBA2-IspA1 regulation are shown. Red and green lines indicate inhibition and activation, respectively. Dotted lines indicate regulatory interactions with unclear molecular mechanisms.

Willems et al., 1992). The BvgSA TCS, a master regulatory system in numerous *Bordetella* species (Chen & Stibitz, 2019), regulates hundreds of genes, including virulence factors such as fimbriae FIM and FHA, therefore controlling the transition between avirulent, intermediate, and virulent phenotypic phases (Moon et al., 2017). Virulence occurs when the BvgS kinase activates BvgA, which in turn binds to multiple high- and low-affinity binding sites within *fhaB* and *fimB* promoters as well as in the *bvgAS* promoter, activating their transcription (Boucher et al., 1997; Boucher et al., 2003; Cotter & Jones, 2003). The Bvg system is active in vivo at 37°C during respiratory tract infection (Cotter & Miller, 1994). At 25°C, as well as in the presence of the so-called in vitro “modulating signals” MgSO₄ and nicotinic acid, BvgS kinase activity is turned off and BvgA is unphosphorylated, presumably due to the reversal of the BvgSA phosphorelay (Boulangier et al., 2013; Scarlato et al., 1991). BvgS is composed of several linkers and sensory domains which each reflects a different potential regulatory role, suggesting a complex set of signals potentially sensed and integrated by this TCS (Dupre et al., 2015; Lesne et al., 2018; Sobran & Cotter, 2019). Notably, oxidized ubiquinone is able to inhibit BvgS activity through its cytoplasmic PAS domain that harbors a probable quinone binding site (Bock & Gross, 2002), indicating a role of this protein in sensing the redox state of ubiquinone. The BvgSA TCS can also integrate signals indirectly as its activity is controlled by the PrISR TCS (Bone et al., 2017). The histidine kinase PlrS is indeed able to increase BvgSA-dependent virulence-associated phenotypes, such as hemolysis and adhesion, in response to elevated CO₂ in vitro and is required for full Bvg system activity in the murine lower respiratory tract. The PlrSR system also controls important BvgSA-independent functions required for persistence in this environment and might respond to some of its specific cues, such as low O₂ and high CO₂ levels (Bone et al., 2017). Recently, the small regulatory RNA Bpr4 was found to post-transcriptionally upregulate *fhaB* by direct interaction mediated by Hfq, leading to the protection of *fhaB* mRNA from degradation by RNaseE (Hiramatsu et al., 2022). Bpr4 itself is upregulated upon cell contact due to interference with flagellar rotation through the flagellar stator MotA, which activates c-di-GMP production by DgcB, which in turn activates Bpr4 expression through the TCS RisKA. Altogether this pathway allows a flagellum-triggered regulatory response that contributes to *B. pertussis* adhesion and infection. To note, the FhaBC system in *Bordetella bronchiseptica* also possesses a second effector, the FhaS protein (FhaS_{Bb}), that is also transported by FhaC (Julio & Cotter, 2005). FhaS_{Bb} and FhaB are very similar but the two proteins have different functions, as FhaS_{Bb} is not involved in adherence. Importantly, even if the *fhaS_{Bb}* gene is also activated by BvgSA, probably indirectly, its expression pattern is different from all other Bvg-regulated genes (Julio & Cotter, 2005).

P. aeruginosa encodes one filamentous β -helical protein belonging to the FHA-like subfamily of adhesins. Its name, CdrA, derives from cyclic diguanylate-regulated TPS partner A, as both its synthesis and outer membrane addressing are c-di-GMP-dependent (Borlee et al., 2010; Rybtke et al., 2015). CdrA attaches to the bacterial cell surface through two cysteines linked by disulfide bond

located at its Cterminus, which keeps the adhesin anchored to the transporter, CdrB (Cooley et al., 2016). This location ensures its function in bacterial auto-aggregation and contributes to biofilm structural integrity by binding and maintaining the exopolysaccharide Psl (Borlee et al., 2010). CdrA also prevents biofilm dispersion by tethering cell–cell interactions (Cherny & Sauer, 2020). The *cdrAB* operon is under the direct control of FleQ, a transcription factor whose activity is controlled by direct interaction with c-di-GMP and with the anti-activator ATPase FleN. The promoter of *cdrAB* contains three FleQ boxes centered at positions –109 (for FleQ-mediated *cdrAB* repression in the absence of c-di-GMP), +45 and +67 (for FleQ-mediated *cdrAB* activation in the presence of c-di-GMP) relative to its transcription start site. The binding of c-di-GMP to FleQ triggers conformational modifications, leading to FleQ switching from a repressor to an activator on the *cdrAB* promoter (Baraquet et al., 2012; Baraquet & Harwood, 2016). In addition to the impact on transcription, intracellular c-di-GMP levels regulate the CdrA release through the membrane-bound LapD/G system. LapG is a periplasmic protease sequestered by the membrane-anchored LapD when its cytosolic EAL domain is loaded with available c-di-GMP. Upon the drop of c-di-GMP levels, LapD releases LapG, which in turn cleaves the periplasmic cysteine-hook in CdrA, thus releasing the bacterium from the adhered surface, leading to biofilm disintegration (Cooley et al., 2016). The LapD/G system is widely conserved in many bacterial species, including important pathogens, and post-transcriptionally controls the location of other adhesins such as LapA and MapA of *Pseudomonas fluorescens* and BrtA of *B. bronchiseptica* (Ambrosio et al., 2016; Collins et al., 2020; Navarro et al., 2011; Newell et al., 2011). Overall, c-di-GMP ensures both the synthesis and the activity of the CdrA adhesin by stimulating the expression of its operon and preventing its proteolysis, allowing its retention within the outer membrane.

The synthesis of many bacterial adhesins is under the control of the so-called “phase variation” mechanism, a reversible transcriptional ON/OFF switch (Ahmad et al., 2017). Phase variation is a potent adaptive mechanism increasing pathogenicity of bacteria by creating a phenotypic heterogeneity within a clonal population. In the case of adhesins, this generates an adhesion gradient and a balance between adhesion and immune evasion (Dawid et al., 1999). Other extracellular protein structures can exhibit bimodal expression, as mentioned above for the XhIA hemolysin and the flagellum of *X. nematophila* (Jubelin et al., 2013). Two surface-exposed high-molecular-weight (HMW) proteins, and potential vaccine candidates (Winter & Barenkamp, 2016), are regulated by phase variation in *Haemophilus influenzae* NTHi, a worldwide leading cause of respiratory tract infections (Davis et al., 2014). These adhesins confer an advantage for the attachment step but are also a target of the immune system (Davis et al., 2014). Even if otherwise very similar, HMWA1 and HMWA2 exhibit different cellular binding specificities (Barenkamp & Leininger, 1992; Buscher et al., 2004; St Geme et al., 1993). The two proteins are encoded within two loci, *hmw1ABC* and *hmw2ABC*, which potentially originated from gene duplication. Around 75% of *H. influenzae* strains possess the two loci, but the HMW adhesion genes

display a large genetic diversity in their binding domain-encoding portions (Buscher et al., 2004; Giufre et al., 2006). The TpsB-like HmwB proteins (St Geme & Grass, 1998) and the glycosyltransferases HmwC (McCann & St Geme, 2014) are required for adhesin processing and secretion, and the two homologs are conserved and interchangeable. Both transcription and post-translational modifications of the two HMWA are under phase variation control. One mechanism controlling their expression relies on 7-bp simple sequence repeats (SSRs) located in the promoter regions (Barenkamp & Leininger, 1992; Dawid et al., 1999). The heptameric repeats are present between the two promoters (P1 and P2) identified upstream from *hmwA*, affecting negatively the activity of P2, the principal promoter driving the operon expression. The repeat location suggests a DNA polymerase slippage mechanism during DNA replication. Additionally, the numbers of SSRs (9–28 copies) vary spontaneously both in vitro and in vivo (animal model and human infections) in a Rec-independent manner (Dawid et al., 1999; Fernández-Calvet et al., 2021; Giufre et al., 2008). The number of SSRs increases during persistent infection, contributing to a within-patient switch to biofilm growth (Fernández-Calvet et al., 2021). These repeat numbers correlate with the mRNA abundance and thus adhesin synthesis; an increase of SSRs leads to a reduction of mRNAs quantities, either due to an effect on mRNA stability or on transcription (Dawid et al., 1999). The *hmw* gene expression relies also on the N⁶-adenine DNA-methyl transferase ModA (Atack et al., 2015). The *modA* expression itself is regulated by phase variation and 20 distinct *modA* alleles encode as many proteins which methylate different sequences, controlling different targets, including the *hmw* genes and genes involved in iron acquisition. This phase variation mechanism was shown to be important in bacterial virulence and niche adaptation (Atack et al., 2015). Finally, the SSRs are also present in promoters controlling the synthesis of HmwC1 and HmwC2, the two enzymes affecting HmwA glycosylation, implying a distribution of heterogeneous proteoforms on the bacterial surface potentially important for efficient immune evasion (Elango & Schulz, 2020).

PfhB1 and PfhB2 (also named FhaB1 and FhaB2, respectively) are putative filamentous hemagglutinins of *Pasteurella multocida*, the causative agent of fowl cholera and several animal diseases (Wilson & Ho, 2013). They are both required for in vivo virulence (Fuller et al., 2000; Guo et al., 2014; Tatum et al., 2005). PfhB1/B2 are encoded in the locus *lspB-pfhB1-lspB-pfhB2*, along with genes encoding two TpsB proteins (May et al., 2001). Hemagglutinin biosynthesis is coordinated with that of the capsule by Fis and Hfq. The global regulator Fis is a growth phase-dependent nucleoid-associated transcriptional factor that controls positively the expression of *lspB-fhaB2* and capsular genes (Steen et al., 2010). Hfq plays a crucial role in regulating virulence factors and its inactivation reduces *P. multocida* virulence and fitness in mice (Megroz et al., 2016), as observed with other bacteria. PfhB2 and LspB2 are among the numerous virulence factors it controls, as their abundances were reduced in a *hfq* mutant, reflecting a potential effect on mRNA stability (Megroz et al., 2016). Of note, Fis seems to also positively regulate Hfq expression, adding further complexity to the involved regulation (Megroz et al., 2016).

In *P. aeruginosa*, the TpsA-like CupB5 protein is a putative surface-exposed adhesin encoded within the *cupB* operon (Ruer et al., 2008) which plays a regulatory role in alginate production and mucoid conversion (de Regt et al., 2014). In addition to *cupB5*, the six-gene *cupB* operon codes for a chaperone-usher pathway (*cup*) assembling fimbriae at the bacterial surface (Ruer et al., 2008). CupB5 belongs to a multi-effector TPS system, being the second effector of LepB, the transporter of the LepAB system (Garnett et al., 2015). The large extracellular protease LepA protein contains a trypsin-like serine protease motif and is endowed with proteolytic activity (Kida et al., 2008). To the best of our knowledge, it is the only TpsA protease reported in the literature. By activating the NF- κ B pathway through protease-activated receptors (PARS), LepA induces inflammatory responses in human cells and modulates host response against infection (Kida et al., 2008). LepA also degrades hemoglobin, a source of heme iron and peptides for a pathogen (Kida et al., 2011). Interestingly, the expression of both *lepA* and the *cupB* operon is controlled by RocS1, a membrane-bound histidine kinase, pointing to the coordinate synthesis of the two TPS effectors (Garnett et al., 2015). RocS1 also promotes biofilm maturation, reduces antibiotic resistance and expression of T3SS-encoding genes by modulating the phosphorylation status of several response regulators, including RocA1 and RocA2 (Francis et al., 2017; Mikkelsen et al., 2011; Sivaneson et al., 2011). The molecular mechanism used by RocS1 to control *cupB* and *lep* genes has not been clarified yet; however, recent DAP-seq analyses revealed both RocA1 and RocA2 binding on the *lepBA* promoter in vitro, suggesting their potential direct control in vivo (Trouillon et al., 2021). To note, the RocS1A1R phosphorelay system of *P. aeruginosa* is homologous to the BvgSAR system that regulates many virulence traits in *Bordetella* species (Mikkelsen et al., 2011), including the ShIA adhesin of *B. pertussis*.

4 | THE REGULATION OF HEMOPEXIN-BINDING PROTEINS

Some bacteria have evolved TPS systems to capture molecules required for their survival. An example is *H. influenzae* that is unable to synthesize iron-containing heme (Evans et al., 1974), an essential cofactor required for the function of many cellular proteins. This obligate human commensal/pathogen responsible for a wide range of infections possesses several heme acquisition systems; one of them comprises the HxuAB TPS and the HxuC protein that permits the use of host hemopexin as an important source of both heme and iron (Cope et al., 1995; Cope et al., 1998; Fournier et al., 2011). The cell surface-exposed HxuA binds hemopexin and allows heme uptake by HxuC, a TonB-dependent receptor. Expression of the *hxuCBA* operon is upregulated in iron-heme restricted medium in a Fur-dependent manner (Harrison et al., 2013; Whitby et al., 2006). In *H. parasuis* serovar 5, the *hxuCBA* operon is upregulated in vitro in conditions mimicking infection with iron limitation and higher temperature (Alvarez-Estrada et al., 2018). Recently, as mentioned above for the Hmw adhesins and other outer membrane proteins,

the expression of the *hxcCBA* operon was found to be biphasic due to ModA (Atack et al., 2015).

5 | THE REGULATION OF CONTACT-DEPENDENT GROWTH INHIBITION SYSTEMS

Contact-dependent growth inhibition (CDI) systems are contact-mediated competition systems of the TPS family. Akin to T6SSs, CDI systems allow the direct injection of antimicrobial effectors into bacterial competitor cells (Aoki et al., 2010). To that aim, CdiA integrates its C-terminal toxin domain into a neighboring competitor cell leading to growth inhibition or cell death (Aoki et al., 2005; Morse et al., 2012). CdiA toxins activity includes nucleases, adenosine deaminases, ADP-ribosyl cyclases, and metallopeptidases. In order to protect themselves and their sibling cells, bacteria that possess CDI systems also encode an immunity protein (CdiI) which neutralizes CdiA toxin domain activity. CDI systems are generally encoded as a single transcriptional unit of three genes following a *cdiBAI* organization (Guerin et al., 2017). In addition to their role in bacterial competition, CDI systems have been shown to mediate community-associated behaviors, called contact-dependent signaling, through the regulation of gene expression upon delivery of the toxin into immune bacteria (Danka et al., 2017; Garcia et al., 2016). By doing so, CDI systems are involved in cell-cell communication, notably driving biofilm formation and high-cell density phenotypes (Garcia et al., 2013; Guilhabert & Kirkpatrick, 2005).

There are two different CDI systems in *P. aeruginosa*, CdiABI1 and CdiABI2, which exhibit C-terminal domain variability between strains and lineages (Allen & Hauser, 2019). Major domain families encompass deaminases, nucleases, tRNases, or have unknown functions. Both CDIs are post-transcriptionally inhibited by the RNA-binding regulator RsmA (Mercy et al., 2016). This regulation is condition dependent as it is abrogated during static growth, and is thought to be direct, by preventing the binding of the 30S ribosomal subunit to *cdi* mRNAs by RsmA, promoting mRNA degradation. RsmA is controlled by the complex GacSA/LadS/RetS regulatory pathway that governs the switch between planktonic lifestyle and biofilm formation (Brencic & Lory, 2009), and it has been shown in *P. aeruginosa* and other species that CDI systems play key roles in biofilm formation and community structure (Anderson et al., 2014; Mercy et al., 2016). This link suggests a function-related regulation of *P. aeruginosa* CDI systems and raises the need for further characterization of these competition systems in regard to the well-described lifestyle switch.

The BcpABI CDI system is present in several *Burkholderia* species, including the opportunistic human pathogen *B. pseudomallei* that possesses up to 10 CDI systems (Nikolakakis et al., 2012). BcpABI has been first identified in *B. thailandensis*, where it mediates biofilm formation independently of its CDI activity (Garcia et al., 2013). Within biofilms, BcpABI is stochastically regulated, as only a small subset of cells expresses the system. The action of BcpA onto sibling cells induces

transcriptional and phenotypic changes, including a downregulation of the *bcpAIOB* operon (Garcia et al., 2016; Majerczyk, Brittnacher, Jacobs, Armour, Radey, Schneider, et al., 2014). Furthermore, *bcpAIOB* is upregulated through quorum sensing by acyl-homoserine lactones (AHLs) and the associated transcriptional regulator BtaR1, highlighting the role of this CDI system in high-cell density (Majerczyk, Brittnacher, Jacobs, Armour, Radey, Schneider, et al., 2014). However, quorum sensing does not regulate BcpABI in *B. pseudomallei* and *B. mallei*, revealing regulatory differences between different *Burkholderia* species (Majerczyk, Brittnacher, Jacobs, Armour, Radey, Bunt, et al., 2014). This illustrates the regulatory diversity that can be found within a genus: These species are phylogenetically close but live in very different environments, with one saprophyte, one opportunistic pathogen, and one host-restricted pathogen, potentially explaining the need for different regulations.

In the plant pathogen *Dickeya dadantii*, the HecAB CDI system contributes to pathogenesis during infection of *Nicotiana clevelandii* seedlings (Rojas et al., 2002). Fis was found to activate *hecBA* expression under high DNA supercoiling conditions (Jiang et al., 2015), a condition that has already been associated with virulence in this pathogen (Lautier & Nasser, 2007; Ouafa et al., 2012). Recently, another nucleoid-associated protein was identified in the aggressive phytopathogen *Dickeya zeae* as controlling the expression of both *fis* and *hecB*, along with that of other virulence-related genes. The nucleoid-associated protein IHF is indeed able to bind to their promoter sequence in vitro and to upregulate their mRNA levels in vivo, integrating these two genes into the complex IHF regulatory network (Chen et al., 2022).

In *Xylella fastidiosa*, the HxfAB CDI system is important for colonization and cell-cell aggregation during plant infection (Guilhabert & Kirkpatrick, 2005). *X. fastidiosa* is a major plant pathogen causing diseases with important economic outcomes, with several past outbreaks decimating crops of olives or grapevines (Kyrkou et al., 2018; Saponari et al., 2019). The *hxfA* mutant exhibits higher virulence but lower colonization and biofilm formation, attributing this CDI system to milder infection phenotypes, but again to phenotypes related to high-cell density environments. RpfF, an enzyme involved in the synthesis of a fatty acid diffusible signaling factor (DSF), was shown to upregulate *hxfA* and *hxfB* expression (Wang et al., 2012). DSF mediates cell-cell communication in biofilm and decreased DSF production has been linked to lower expression of *hxfA* and *hxfB*, which are consequently both regulated by and regulating biofilm-associated cell-cell communication. This regulation was also linked to c-di-GMP levels, highlighting again the role of HfxAB in biofilm formation and signaling (Chatterjee et al., 2010).

6 | THE REGULATION OF TPSA PROTEINS WITH UNKNOWN FUNCTIONS

Although genes encoding TpsAs are usually easy to identify from their genetic organization and specific domains, the prediction of their exact molecular function is less obvious. Nevertheless, some

insights on regulation are available for several TpsAs with no attributed function.

In *P. aeruginosa*, the putative HMW-like adhesin PdtA is processed during secretion and is anchored and exposed to the cell surface (Faure et al., 2014). PdtA is involved in the pathogenesis of *P. aeruginosa* during infection of *Caenorhabditis elegans* (Faure et al., 2014) and it is synthesized during acute and chronic human infections, as some patient sera contained antibodies directed against PdtA (Llamas et al., 2009). Unexpectedly, PdtA and its transporter PdtB are coded by two genes that are not contiguous, but separated by the *phdA* gene involved in a toxin-antitoxin system controlling bacteriophage-mediated lysis during biofilm formation (Petrova et al., 2011). In addition, they belong to two different transcriptional units, one consisting of the single gene *pdtA* and the other comprising 11 genes and starting with *phdA* (Faure et al., 2013; Quesada et al., 2016). However, the two units are co-regulated and show higher expression in condition of inorganic phosphate (Pi) limitation, giving rise to the name "Phosphate depletion-regulated TPS," or Pdt (Figure 5) (Faure et al., 2013; Faure et al., 2014). Pi limitation is a common signal detected by bacteria, often leading to increased virulence in pathogens as recently illustrated in *P. aeruginosa* (Matilla et al., 2022). It is sensed by the Pst phosphate transport system and transmitted to the PhoRB TCS through the coupling protein PhoU. Among the *P. aeruginosa* Pho regulon is the operon encoding the PUMA3 system, a cell surface signaling system comprising the extra-cytoplasmic function sigma factor Vrel, the anti-sigma factor VreR, and

the receptor VreA (Faure et al., 2013; Llamas et al., 2009). In condition of Pi limitation, the phosphorylated response regulator PhoB activates the expression of the *vreAIR* operon and, consequently, σ^{Vrel} activates, among others, the expression of the two transcriptional units containing *pdtA* and *pdtB* (Faure et al., 2013; Llamas et al., 2009). In addition, PhoB also directly controls the expression of *pdtA* and *phdA* by binding to their promoters, which is required for the recruitment of σ^{Vrel} (Quesada et al., 2016). Of note, the importance of σ^{Vrel} in virulence has recently been highlighted in a zebrafish model of infection, where Pi starvation increases *P. aeruginosa* virulence in embryo in a Vrel- and VreR-dependent manner (Otero-Asman et al., 2020). A second TCS system, BfmSR, is involved in the PdtAB regulation. The response regulator BfmR is a key regulator of biofilm formation and other virulence phenotype (Fan et al., 2021). BfmR binds to the *phdA* promoter in vitro and activates its expression, which limits bacterial lysis and DNA release for proper biofilm development (Petrova et al., 2011). BfmR binding was observed on both *pdtA* and *phoBR* promoters in vivo (Fan et al., 2021), suggesting a direct and indirect control on the PdtAB synthesis. To note, some features of PdtA regulation are shared with CDI systems from *Acinetobacter baumannii*, including the regulation of the CDI₂ system by PhoB (Roussin et al., 2019) and of the CDI₁ system by the BfmRS TCS (Krasauskas et al., 2019).

The Lsp system of *Haemophilus ducreyi*, a pathogen responsible for the sexually transmitted chancroid infection, is involved in phagocytosis inhibition (Dodd et al., 2014; Vakevainen et al., 2003). This

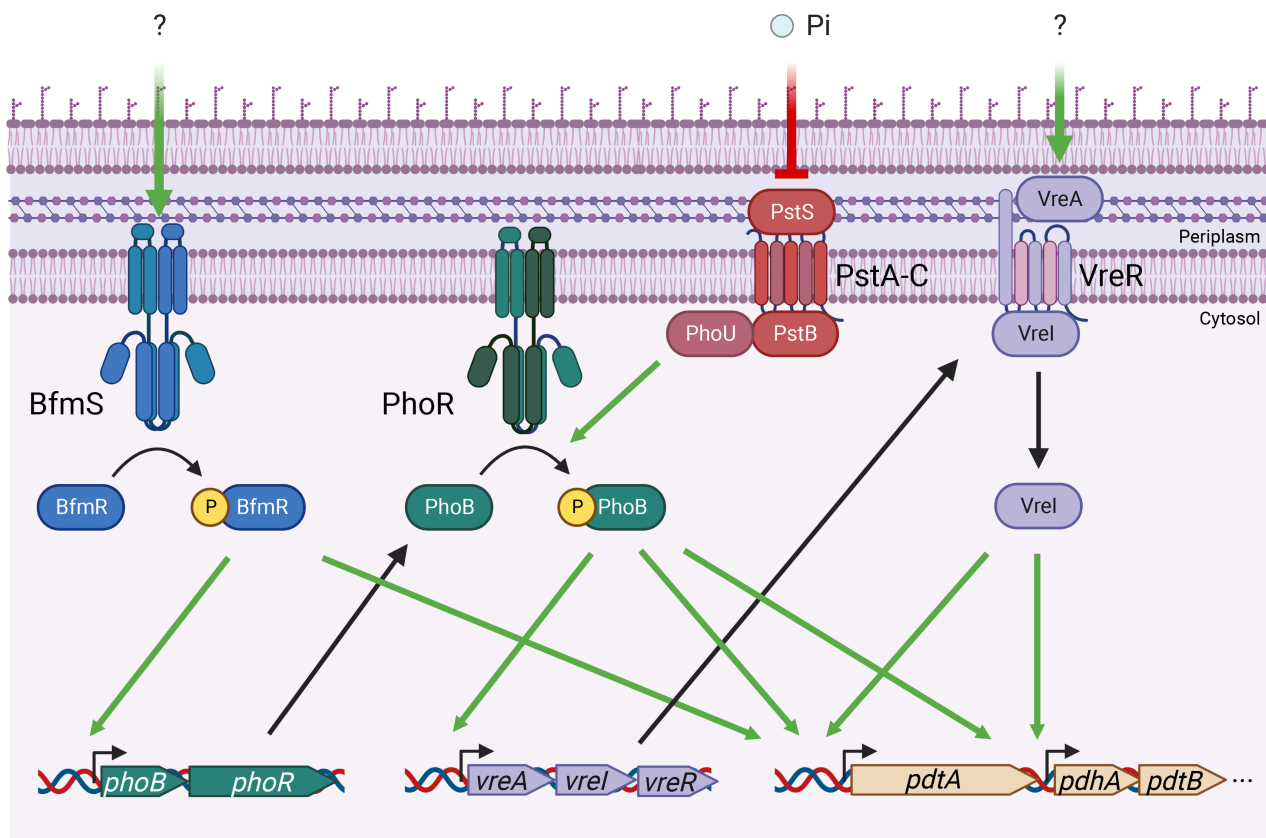


FIGURE 5 The regulation of *pdtA-pdtB* in *Pseudomonas aeruginosa*. The different proteins and genes involved in *pdtA-pdtB* regulation are shown. Red and green lines indicate inhibition and activation, respectively.

system contains two effectors, LspA1 and LspA2 (Ward et al., 2004), which share 86% of identity. LspB and LspA2 are encoded in a bicistronic operon whereas *lspA1* is distantly located, each transcriptional unit having different regulation (Figure 6). Only the *lspBA2* operon is upregulated during infection in pustules compared to in vitro growth (Gangaiah et al., 2016). While the *lspA1* gene is constitutively expressed in vitro, *lspA2* expression is growth phase dependent, with higher expression in stationary phase. Hfq positively affects the transcript levels of several virulence factors, including *lspBA2*, likely by mRNA stabilization (Gangaiah et al., 2014). Hfq is a major contributor of stationary phase gene regulation in *H. ducreyi* (Gangaiah et al., 2014), which is devoid of RpoS homolog. The presence of fetal calf serum was found to induce the expression of *lspA2* and the LspB-dependent release of LspA1 (Labandeira-Rey et al., 2009). The *lspBA2* locus is also negatively regulated by the cell envelope stress response TCS, CpxAR, as are many other key virulence factors involved in chancroid formation (Gangaiah et al., 2013). CpxR was shown to bind directly to the *lspBA2* promoter in vitro (Labandeira-Rey et al., 2009). Another regulatory element is the nucleoid-associated protein Fis, which deletion decreases the expression of the *lspBA2* operon but does not abolish phagocytosis inhibition, probably due to the unaffected *lspA1* (Labandeira-Rey et al., 2013). The last involved pathway,

intertwined with the ones already described, concerns the alarmone (p)ppGpp and DksA, known to regulate *H. ducreyi* virulence in humans (Holley et al., 2014; Holley et al., 2015). Both (p)ppGpp and DksA have a positive effect on *lspB* expression and consequently on phagocytosis inhibition (Holley et al., 2015).

7 | CONCLUSION

While the molecular functions of many TPS systems and their effectors have been thoroughly studied, there is less knowledge on their regulation. However, clear common triggering signals and regulatory pathways are shared between many bacterial species. Indeed, we could identify at least two major signals that are common regulators of TPS systems expression (Table 1) and several recurring involved regulators. First, iron availability seems to be an important cue, with the expression of seven TPS systems from seven different phyla being under its influence. One of them, HxuAB of *H. influenzae*, is a TPS involved in iron acquisition, which gives to its iron-dependent expression an intuitive physiological logic. All six others, however, are not directly functionally related to iron and are considered virulence factors, with five secreting cytolysins and one adhesin. In these

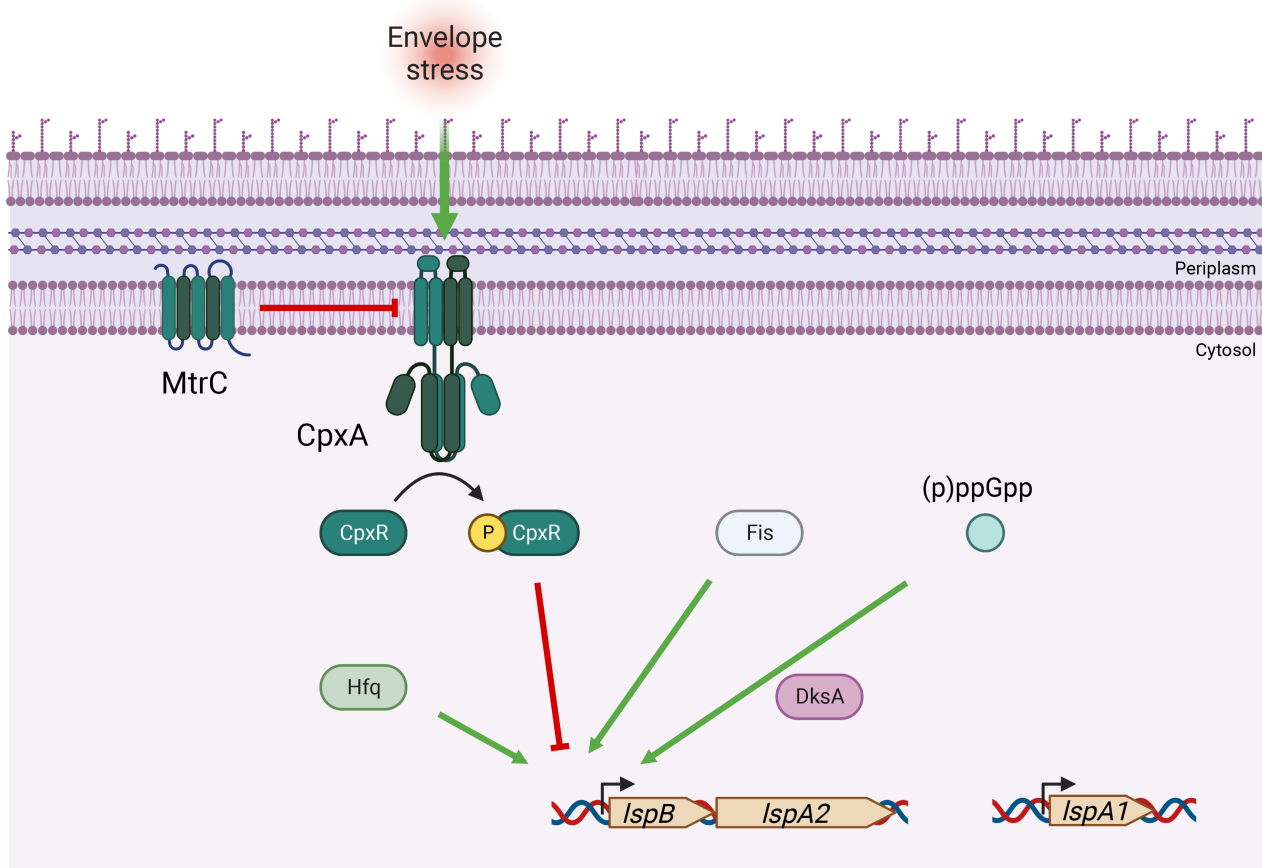


FIGURE 6 The regulation of *lspBA2-lspA1* in *Haemophilus ducreyi*. The different proteins and genes involved in *lspBA2-lspA1* regulation are shown. Red and green lines indicate inhibition and activation, respectively.

cases, iron availability is rather sensed as signal reflecting conditions encountered within an infected host, where these toxins are needed. In most cases, the iron-dependent regulation seems to be driven by the Fur transcription factor, which was initially described as regulating iron homeostasis. It appears that genes encoding cytolysins were added to its regulon, thus allowing this host-sensing mechanism. The second major triggering signal identified is temperature, which was shown to drive the expression of four TPS systems. Here again, this signal reflects the arrival into a host to infect and regulates several functional types of TPS systems. In some cases, different temperatures even inform on the nature of the host, allowing an appropriate response. Several TPS systems regulated by temperature are also regulated by systems sensing membrane perturbations. Membrane composition is known to change in response to changes in temperature (Siliakus et al., 2017), which could represent an additional layer of temperature-sensing mechanisms. Among recurring involved TFs is the FlhDC regulator, which is also involved in the regulation of other virulence factors and motility apparatus needed for infection, such as the flagellum. Altogether, several environmental cues and TFs seem to be primordial for TPS systems regulation and infection. Many TPS systems have no identified regulators but could also be under the control of these common mechanisms. Additionally, since they seem to be frequently involved, other host signals, such as host-specific molecules, should be investigated as potential drivers of TPS systems expression. Lastly, bacterial physiological cues representing the fitness of the cell, sensed through intracellular signals such as cAMP, c-di-GMP, and (p)ppGpp, appear to be an additional common signal for TPS systems regulation.

AUTHOR CONTRIBUTIONS

Julian Trouillon: Conceptualization; investigation; writing – original draft. **Ina Attrée:** Conceptualization; investigation; writing – original draft. **Sylvie Elsen:** Conceptualization; investigation; writing – original draft.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ETHICS STATEMENT

Authors declare that no human or animal subjects were used in this study.

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