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Minireview

Inside the complex regulation of *Pseudomonas aeruginosa* chaperone usher systems

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Summary

Pseudomonas aeruginosa **assembles several cell surface-associated organelles, including those of the chaperone usher (CU) pathway. Five different CU loci have been identified and characterized in various strains of *P. aeruginosa*. However, their potential functional redundancy, particularly in biofilm formation, is supported by the control of their expression by a complex and specific regulatory network. Here, we review recent findings relating to this network. The control exerted by this network involves transcriptional repressors and activators, a phase-variable mechanism, a second intracellular messenger (c-di-GMP) and chemosensory and two-component systems.**

The CU pathway

Gram-negative bacteria have several types of cell surface-associated organelles, including fimbriae assembled through the well conserved chaperone usher (CU) pathway.

Fimbrial assembly by the CU pathway in Gram-negative bacteria (Saulino *et al*., 1998; Thanassi *et al*., 1998a; 2005; Soto and Hultgren, 1999) is based on a mechanism independent of ATP hydrolysis. This process involves an outer membrane (OM) protein, the usher, a periplasmic chaperone and at least one fimbrial subunit. Fimbrial subunits entering the periplasm via the Sec system are captured by the chaperone and assembled into a soluble complex in a donor strand complementation process. The resulting complex is initially targeted to the usher protein, a pore-forming OM protein (Dodson *et al*., 1993; Thanassi *et al*., 1998b). The chaperone – adhesin complex is responsible for opening the translocation pore (Saulino *et al*., 1998; Ng *et al*., 2004) by inducing a conformational change that unplugs the translocation channel (Remaut *et al*., 2008). The usher protein was thought to function in tandem: one usher molecule in the open state and the other in the closed state, to facilitate substrate recruitment for further assembly (Remaut *et al*., 2008) via the open channel. However, it has recently been demonstrated that fimbrial assembly can occur through a monomeric pore (Phan *et al*., 2011). Fimbrial subunits are then assembled into fibres by a donor strand exchange (DSE) mechanism (Choudhury *et al*., 1999), leading to the tip-to-base growth of fimbriae through the usher (Sauer *et al*., 2002). The inherent compatibility of each N-terminal unstructured extension with the groove of the next subunit has been shown to play an important role in determining the order of subunit assembly in this DSE process (Rose *et al*., 2008).

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CU pathway in *Pseudomonas aeruginosa*

In *Pseudomonas aeruginosa*, the CU pathway was first discovered in transposon mutants derived from a PAK strain with no type IVa pili displaying defective biofilm formation (Vallet *et al*., 2001). One of the disrupted genes belongs to a cluster of five CU genes: *cupA*. These genes encode two specialized periplasmic chaperones (CupA2 and CupA5), the OM assembly platform, referred to as the usher (CupA3), and assembled fimbrial subunits: the CupA1 subunit, which probably forms the pilus rod and is produced in large amounts, and the CupA4 subunit, which acts as the adhesin, with one molecule of this subunit probably assembled at the tip of the fimbriae. In addition to demonstrating the existence of this CU pathway through phenotypic screening, *P. aeruginosa* genome mining also identified several other CU clusters (Giraud *et al*., 2010) – the *cupB* and *cupC* (Vallet *et al*., 2001; Ruer *et al*., 2007; 2008), *cupF* and *cupE* (Filloux *et al*., 2004; Giraud *et al*., 2011) clusters in the PAO1 genome sequence – and the *cupD* cluster in the PAPI-I pathogenicity island of the PA14 genome sequence (He *et al*.,

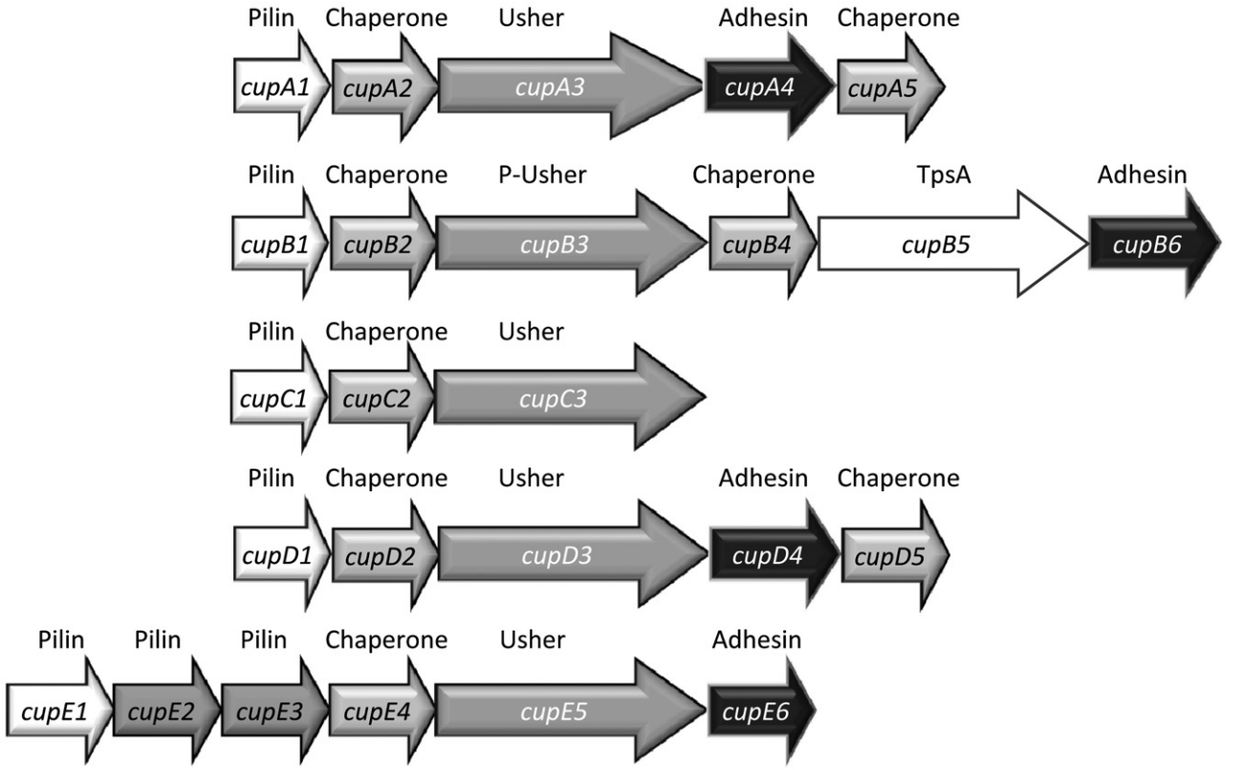

Fig. 1. Genetic organization of genes encoding the five complete identified CU systems (CupA–E). The *cupA, cupC* and *cupD* gene clusters encode an usher (CupA3, CupC3, CupD3), one or two chaperones (CupA2 and CupA5, CupC2, CupD2 and CupD5) and at least one classical fimbrial subunit (CupA1, CupC1, CupD1). The *cupB* gene cluster encodes an OM mosaic protein involved in both fimbrial subunit assembly and TpsA secretion from the two-partner secretion (TPS) family renamed P-Usher (CupB3), two chaperones (CupB2 and CupB4), one classical fimbrial subunit (CupB1), one adhesin (CupB6) and one TPS substrate (CupB5). The *cupE* gene cluster encodes an usher (CupE5), one major fimbrial subunit (CupE1), two minor fimbrial subunits (CupE2, CupE3), a unique chaperone of the FGS subfamily (CupE4) and one putative adhesin (CupE6).

2004; Mikkelsen *et al.*, 2009; Nicastro *et al.*, 2009) (Fig. 1). All these paralogues were identified on the basis of the detection of a gene encoding a chaperone and another gene encoding the OM usher, with the exception of the *cupF* cluster, for which no gene encoding an usher could be identified.

The five complete identified CU systems (CupA–E) correspond to FGS (F1–G1 short loop)-assembled organelles of several different phylogenetic clades (Nuccio and Bäumler, 2007). The CupA, CupB, CupC and CupD systems are found in the γ4-fimbrial clade of the FUP (fimbrial usher protein) family (Nuccio and Bäumler, 2007). The *cupA, cupC* and *cupD* gene clusters encode an usher (CupA3, CupC3, CupD3), one or two chaperones (CupA2 and CupA5, CupC2, CupD2 and CupD5) and at least one classical fimbrial subunit (CupA1, CupC1, CupD1) (Filloux *et al.*, 2004; Giraud *et al.*, 2010). The *cupB* gene cluster encodes an OM mosaic protein involved in both fimbrial subunit assembly and type Vb substrate secretion from the two-partner secretion (TPS) family renamed P-Usher (CupB3) (Ruer *et al.*, 2008), two chaperones (CupB2 and CupB4), one classical fimbrial subunit (CupB1), one adhesin (CupB6) and one TPS substrate (CupB5). The CupC system is a highly simplified system, with only three components (the fimbrial subunit, the chaperone and the usher). There seems to be some phylogenetic distance between the CU pili assembled by the CupE machine, which belong to the σ-fimbrial clade, and those assembled by the other machineries (Nuccio and Bäumler, 2007). Investigations of the genetic environment of the chaperone and usher genes showed these genes to belong to a 5.8 kb locus containing another four genes encoding proteins with none of the characteristics of fimbrial proteins reported for the other classical CU systems of *P. aeruginosa* (Giraud *et al.*, 2011). Instead, the flanking sequences display similarity to the conserved COG domain, COG5430, present in a bacterial family of spore coat proteins, the U or SCPU proteins, which are secreted at the surface of the *Myxococcus xanthus* spore (Gollop *et al.*, 1991) and are commonly referred to as SCPU domains. Thus, the *cupE* gene cluster encodes an usher (CupE5), one major fimbrial subunit (CupE1), two

minor fimbrial subunits (CupE2, CupE3), a unique chaperone of the FGS subfamily (CupE4) and one putative adhesin (CupE6). *Pseudomonas aeruginosa* therefore assembles panoply of different CU pili, although CupA and CupD are phylogenetically closely related. This suggests that the CupD CU system may have been generated by duplication of the CupA CU system.

Pseudomonas aeruginosa CupA, B, C and E CU pili have been visualized by transmission electron microscopy and found to be long fibrils, many up to 2 μm in length. Their contribution to adhesion and the structuring of the biofilm has been studied. The CupA system is thought to be required for the stable attachment of bacteria to the abiotic surface and therefore has an impact on biofilm structure independently of type IVa pili, and at an earlier stage (Vallet *et al*., 2001). The CupA system is involved in the establishment and physical structure of the Pel-dependent pellicle (Friedman and Kolter, 2004). The overproduction of this system contributes to the autoaggregative SCV (small colony variant, Häussler, 2004) morphotype observed in strains associated with cystic fibrosis (D'Argenio *et al*., 2002; Meissner *et al*., 2007). This morphotype displays particularly efficient biofilm formation. The CupB and CupC systems cooperate in cell – cell interactions and microcolony formation during the biofilm maturation process (Kulasekara *et al*., 2005; Ruer *et al*., 2007). The PvrR-dependent abolition of the autoaggregative rough SCV phenotype of the *P. aeruginosa* strain PA14 suggests possible effects on the CupD system (Drenkard and Ausubel, 2002; Häussler, 2004) in addition to effects on the CupA system. The CupE fimbriae play an important role in biofilm formation and structuration, at both early and late stages, and all of the subunits, with the exception of one minor pilin, are required for CupE-dependent biofilm formation (Giraud *et al*., 2011).

Regulation of CU gene clusters in *P. aeruginosa*

The regulation of the different CU gene clusters in *P. aeruginosa* shares some common rules, while individual gene cluster has its own regulation features. A cluster by cluster update of piece of regulation knowledge will be first detailed in the following paragraphs, while general trends in *cup* regulation, and identification of largest gaps of knowledge in the field will be summarized at the end of the paragraph.

cupA regulation (Fig. 2)

The control of *cupA* gene expression in *P. aeruginosa* is complex and involves numerous regulatory proteins acting in two pathways: in the first, anaerobiosis is sensed by the transcriptional regulator Anr, triggering *cgr* gene transcription, thereby activating *cupA* gene expression; the second involves the intracellular messenger c-di-GMP, high levels of which are associated with high levels of *cupA* gene expression (Fig. 2). The expression of *cupA* has been shown to be repressed in many laboratory conditions.

Search for activators in transposon-mediated mutagenesis experiments has led to the identification of the PA4315 gene as encoding a negative regulator of *cupA* expression (Vallet *et al*., 2004). This ORF, renamed *mvaT*, encodes a protein that is 82% similar to the P16 subunit of the *Pseudomonas mevalonii* heteromeric transcriptional regulator MvaT (Rosenthal and Rodwell, 1998). In the absence of MvaT, the *cupA* genes are expressed in a phase-variable manner (i.e. the gene cluster displays reversible on/off expression with switches from the ON to the OFF phase 20 times more frequent that switches from the OFF to the ON phase) (Vallet-Gely *et al*., 2005). A second MvaT-like regulator, MvaU (PA2667), a putative transcriptional regulator 51% identical and 68% similar to MvaT, has also been identified (Vallet-Gely *et al*., 2005). MvaT and MvaU, both of which belong to the histone-like nucleoid structuring (H-NS) family, can form both heteromeric and homomeric complexes. The formation of these complexes is mediated by the N-terminal regions of MvaT and MvaU, both of which are predicted to adopt a coiled-coil conformation. The N-terminal region of MvaT, spanning residues 35–62, and including the F36 and R41 residues in particular, appears to be involved in mediating the formation of higher-order oligomers (MvaT tetramers and oligomers of higher complexity) and to play a critical role in DNA binding and, therefore, in the repression of *cupA* fimbrial gene expression in *P. aeruginosa* (Castang and Dove, 2010). Like MvaT, MvaU can repress the phase-variable expression of the *cupA* gene cluster (Vallet-Gely *et al*., 2005). MvaT and MvaU regulate the expression of identical sets of target genes and associate with the same regions of the chromosome, with this pair of H-NS family members functioning coordinately (Castang *et al*., 2008). The reciprocity between MvaT and MvaU (i.e. deletion of the gene encoding one of these regulators leads to an increase in the expression of the other) suggests that they are redundant (Castang *et al*., 2008). However, MvaT seems to play a more important role than MvaU as a repressor of *cupA* expression, and the depletion of both MvaT and MvaU results in a strong synergistic effect on *cupA* expression (Li *et al*., 2009). In addition, MvaT and MvaU preferentially associate with AT-rich regions, suggesting that MvaT and MvaU are involved in silencing foreign DNA elements in *P. aeruginosa* (Castang *et al*., 2008). The mechanistic control over *cupA* expression exerted by MvaT has been demonstrated to involve both direct control over the expression of the *cgr* regulatory genes upregulating *cupA* gene expression (Vallet-

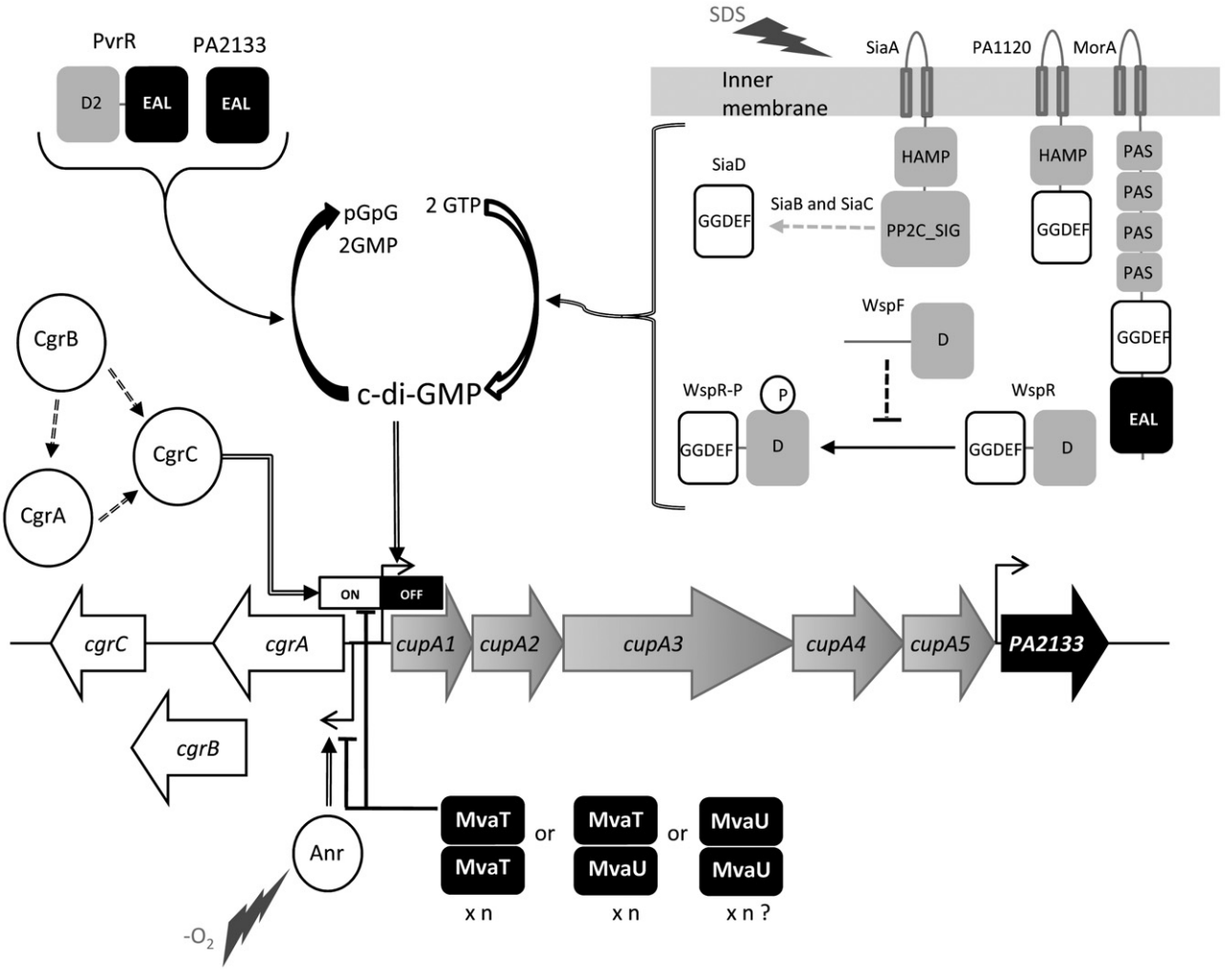

Fig. 2. Regulation of the *cupA* locus: the repression of *cupA* phase-variable expression by the H-NS-like MvaT and MvaU proteins is dependent on anaerobic conditions. Positive regulators of *cupA* expression include three local regulators encoded by the *cgrABC* genes and Anr, a global regulator of anaerobic gene expression. *cupA* expression is also modulated by the intracellular level of c-di-GMP, via proteins containing EAL and GGDEF domains, including the MorA, PA1120, PvrR, WspR, PA2133 and SiaD proteins, the last of these proteins being encoded by the *siaABCD* operon of SDS-induced aggregation genes.

Gely *et al*., 2007) and direct binding to the *cupA* promoter and *cupA1* coding region (Castang *et al*., 2008), potentially inhibiting the initiation of transcription, through the occupation of regions both upstream and downstream from the target promoter.

Positive regulators of *cupA* expression include three local regulators encoded by the *cgrABC* genes and Anr, a global regulator of anaerobic gene expression (Vallet-Gely *et al*., 2007). The *cupA* genes are expressed in a phase-variable manner under anaerobic conditions, and *cgrABC* genes (*cupA* gene regulator genes, located upstream from the *cupA* locus), are essential for this expression. The effects of Cgr proteins are essentially limited to the *cupA* genes (Vallet-Gely *et al*., 2007). Furthermore, *cgr* gene expression is positively controlled by Anr and anaerobiosis. Expression of the *cupA* genes therefore appears to involve a regulatory cascade in which anaerobiosis, signalled through Anr, stimulates expression of the *cgr* genes, resulting in an increase in *cupA* gene expression; this action on *cgr* genes is probably direct, because a putative Anr binding site has been identified in the *cgrA-cupA1* intergenic region (Vallet-Gely *et al*., 2007). CgrA is a member of the adenine nucleotide α-hydrolase superfamily and contains a putative phosphoadenosine phosphosulfate/adenosine phosphosulfate-binding domain, although it remains unclear whether this domain is important for the activity of the protein. CgrB is a putative member of the GNAT family of acetyltransferases and is thought to acetylate either CgrA or CgrC, to promote the activity of these proteins, or to target a small molecule, the acetylation of which is required for either CgrA or CgrC to function. CgrC is a

DNA-binding protein that directly binds to the *cupA* promoter region. CgrA and CgrC interact directly with each other and this interaction is required for the phase-variable expression of *cupA* genes. CgrC could exert its direct regulatory effect on the *cupA* promoter possibly by recruiting CgrA (McManus and Dove, 2011). Together with CgrA and CgrB, CgrC may remove a repressor from the promoter of *cupA* or activate transcription from the *cupA* promoter. If the Cgr proteins do remove a repressor from the *cupA* promoter region, that repressor is unlikely to be MvaT, because *cgr* genes are required for *cupA* gene expression independently of MvaT.

The expression of CupA fimbriae is involved in the development of an auto-aggregative small-colony variant (SCV) phenotype, as frequently observed in clinical situations, such as cystic fibrosis, and *cupA* expression is modulated by the intracellular level of c-di-GMP, via proteins with EAL and GGDEF domains (Meissner *et al*., 2007), which trigger c-di-GMP degradation and synthesis through phosphodiesterase (PE) and diguanylate cyclase (DGC) respectively. These proteins include MorA and PA1120, both of which are putative membrane proteins with a transmembrane domain and a C-terminal GGDEF domain. MorA also has an EAL domain, although its DGC activity appears to predominate. These two proteins upregulate *cupA* expression through their DGC activity, but they are not redundant. This upregulation is counteracted by the overproduction of PvrR, a protein with PE activity (Meissner *et al*., 2007). The expression of *cupA*, *cupA*-dependent autoaggregation and the biofilm-associated phenotype are also controlled by the GGDEF-containing response regulator WspR (D'Argenio *et al*., 2002; Kulasekara *et al*., 2006), the activity of which is linked to its level of phosphorylation, which is itself downregulated by WspF, from a Wsp system predicted to form a chemosensory pathway (Hickman *et al*., 2005). The effects of loss of the *wspF* gene, resulting in an increase in the intracellular levels of c-di-GMP, due to constant phosphorylation of the WspR response regulator and, therefore, to WspR-P-catalysed c-di-GMP synthesis, are reversed by overexpression of the PA2133 gene (located downstream from the *cupA* gene locus), which encodes a protein with an EAL domain and predicted to function as a c-di-GMP phosphodiesterase. The overexpression of this gene leads to c-di-GMP degradation, but its effects on *cupA* gene expression have not been evaluated directly.

The induction of cell aggregation in response to sodium dodecylsulfate (SDS) stress demonstrates the involvement of the *siaABCD* operon (SDS-induced aggregation genes) encoding a transduction module consisting of a transmembrane protein SiaA with a HAMP domain and a PP2C-like phosphatase domain, two proteins (SiaB and SiaC) of unknown function and a predicted cytoplasmic SiaD protein with putative DGC activity. The SDS-dependent aggregation mediated by the Sia transduction system leads to an increase in *cupA* transcription, probably through an increase in c-di-GMP levels (Klebensberger *et al*., 2009). Temperature seems to be an environmental determinant of importance for *cupA* expression and, thus, for the auto-aggregative *P. aeruginosa* phenotype and biofilm formation (Meissner *et al*., 2007); *cupA* expression is clearly enhanced by culturing the bacteria at 28°C rather than 37°C, particularly for the SCV morphotype.

cupD regulation (Fig. 3)

The *cupD* gene cluster is paralogous to the *cupA* gene locus present in the pathogenicity island PAPI-1 of the PA14 strain, on the accessory genome of *P. aeruginosa*. *cupD* is located between two direct repeat (DR) sequences, next to two sets of genes encoding the phosphorelay systems PvrSR and RcsCB, suggesting possible simultaneous acquisition (Fig. 3). Despite the genetic identity between the *cupA* and *cupD* genes (62.4–76.1%), these two gene loci are regulated very differently, although *cupD* transcription rates are also higher in cultures grown at 28°C (Nicastro *et al*., 2009). Despite the putative horizontal origin of this cluster, *cupD* expression is not controlled by the H-NS-like repressor protein MvaT (Nicastro *et al*., 2009).

The role in *cupD* expression of the two phosphorelay systems close to *cupD* genes was therefore investigated. The Rcs system comprises the unorthodox membrane histidine kinase RcsC, which has a cytoplasmic PAS domain, and the response regulator RcsB, which has an output domain containing a helix–turn–helix (HTH) motif. The Pvr system comprises the membrane hybrid sensor PvrS, which functions with an unidentified Hpt protein, and the response regulator PvrR, which has an EAL domain and thus displays PE activity. The expression of *cupD* expression is upregulated by the response regulator RcsB (Mikkelsen *et al*., 2009; Nicastro *et al*., 2009). Deletion of the gene encoding the cognate histine kinase RcsC also increases *cupD* expression, highlighting the probable role of RcsC as a phosphatase, as in enterobacteria in the absence of the activating signal (Majdalani and Gottesman, 2005). The promoter of the *cupD* operon has been characterized and a putative RcsB-binding box similar to that of *Escherichia coli* (Davalos-Garcia *et al*., 2001; Sturny *et al*., 2003; Boulanger *et al*., 2005) has also been identified close to the –35 box suggesting that the RcsB-dependent activation of *cupD* expression is direct. However, in *E. coli*, the RcsB regulator can interact either alone with the DNA via the RcsB-Box or in complex with RcsA via the RcsAB-Box, suggesting that in the absence of a corresponding *rcsA* gene in *P. aeruginosa* genome,

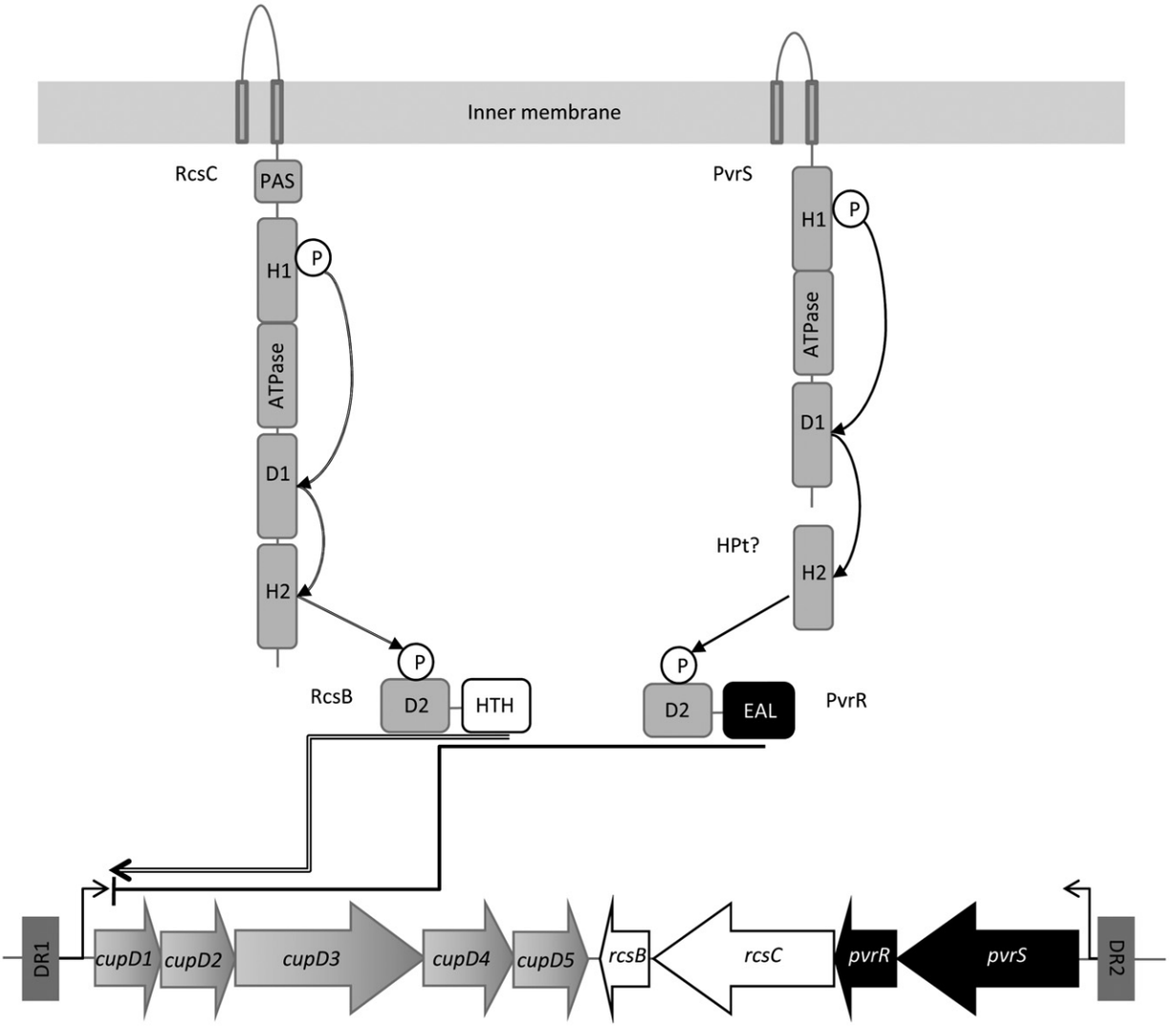

Fig. 3. Regulation of the *cupD* locus present in the PAPI-1 of the PA14 strain: the *cupD* cluster acquired together with two two-component systems (TCSs) is activated by the response regulator RcsB of the RcsCB TCS, the control of which is antagonized by the response regulator PvrR, which contains an EAL, from the PvrSR TCS.

RcsB could exert its regulatory function on *cupD* expression as a homodimer. The response regulator PvrR has an EAL domain and has been shown to antagonize the positive effect of the Rcs system on *cupD* expression (Mikkelsen *et al*., 2009).

cupB and *cupC* regulation (Fig. 4)

The *cupB* and *cupC* loci are organized as independent gene loci, but have regulatory features in common. The levels of the *cupB* and *cupC* transcripts are moderately higher in the *mvaT* mutant than in the wild type, independently of the second histone-like nucleoid protein MvaU (Vallet *et al*., 2004), suggesting that MvaT represses both these loci. However, no CupB1 or CupC1 production was observed in the absence of MvaT (S. Ruer and S. de Bentzmann, unpubl. data). Furthermore, the regulation of *cupB* and *cupC* is under the control of a complex phosphorelay network involving the Roc (regulator of *cup* genes) systems (Fig. 3). The Roc1 system consists of an unorthodox sensor, RocS1, which has a periplasmic domain similar to those found in solute-binding proteins (Sbp3) and a cytoplasmic PAS domain, and two response regulators, RocA1 and RocR. The output domain of RocA1 is an HTH motif, predicted to bind directly to DNA, whereas the output domain of RocR contains an EAL domain with putative PE activity. The Roc2 system consists of an unorthodox sensor, RocS2, which is paralo-

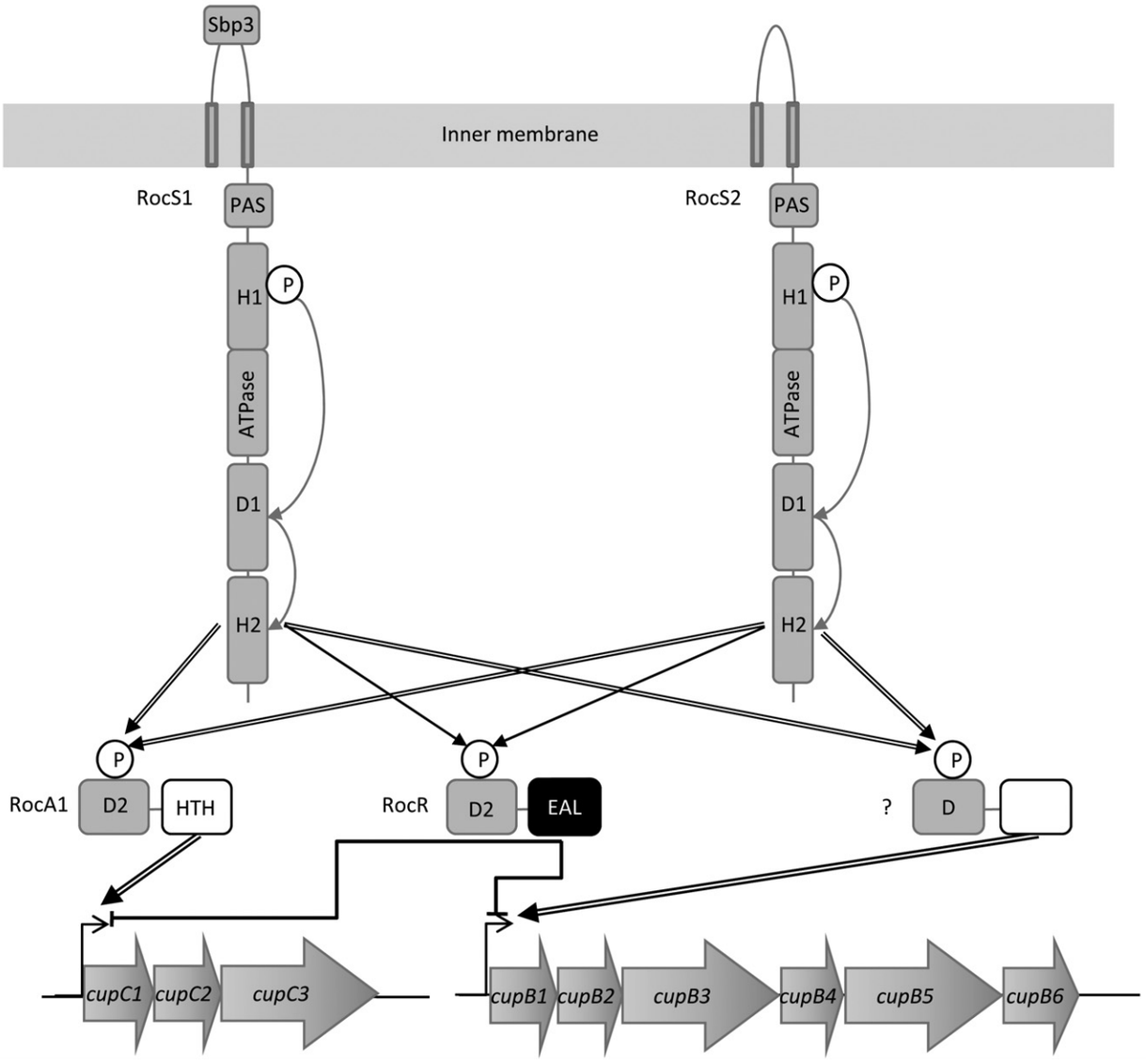

Fig. 4. Regulation of the *cupB* and *cupC* loci: the expression of *cupB* and *cupC* expression is under the control of a complex phosphorelay network involving the Roc (regulator of *cup* genes) systems. RocS1 and RocS2 both activate *cupC* gene expression through the same response regulator, RocA1, or repress *cupC* gene expression through a single response regulator, RocR, which contains an EAL domain. In addition, both sensors can also induce *cupB* gene expression, independently of RocA1 and RocA2.

gous to RocS1, no recognizable sensory domain in the periplasm, and a response regulator, RocA2, which has an HTH motif in its output domain (Kulasekara *et al*., 2005). The Roc3 system consists of an unorthodox sensor, RocS3, with no identified associated response regulator.

The RocS1 sensor signals through both RocA1 and RocR, which have opposite effects on *cupC* gene expression. The expression of *rocA1* activates *cupC* gene expression, leading to fimbrial production and an increase in attachment, whereas the expression of *rocR* reduces *cupC* gene expression by an unknown mechanism, presumably involving the degradation of c-di-GMP (Kulasekara *et al*., 2005). Like RocS1, the RocS2 sensor kinase also induces *cupC* gene expression. However, RocS2 induction is independent of the RocA2 response regulator, instead requiring RocA1, although the orphan RocS3 histidine kinase cannot activate *cupC* gene expression through RocA1 (Sivaneson *et al*., 2011). RocS1 and RocS2 thus both signal through the same response regulator (RocA1) to activate *cupC* gene expression. In addition, both sensors induce *cupB* gene expression, this process requiring neither RocA1 nor RocA2, but probably instead depending on an as yet

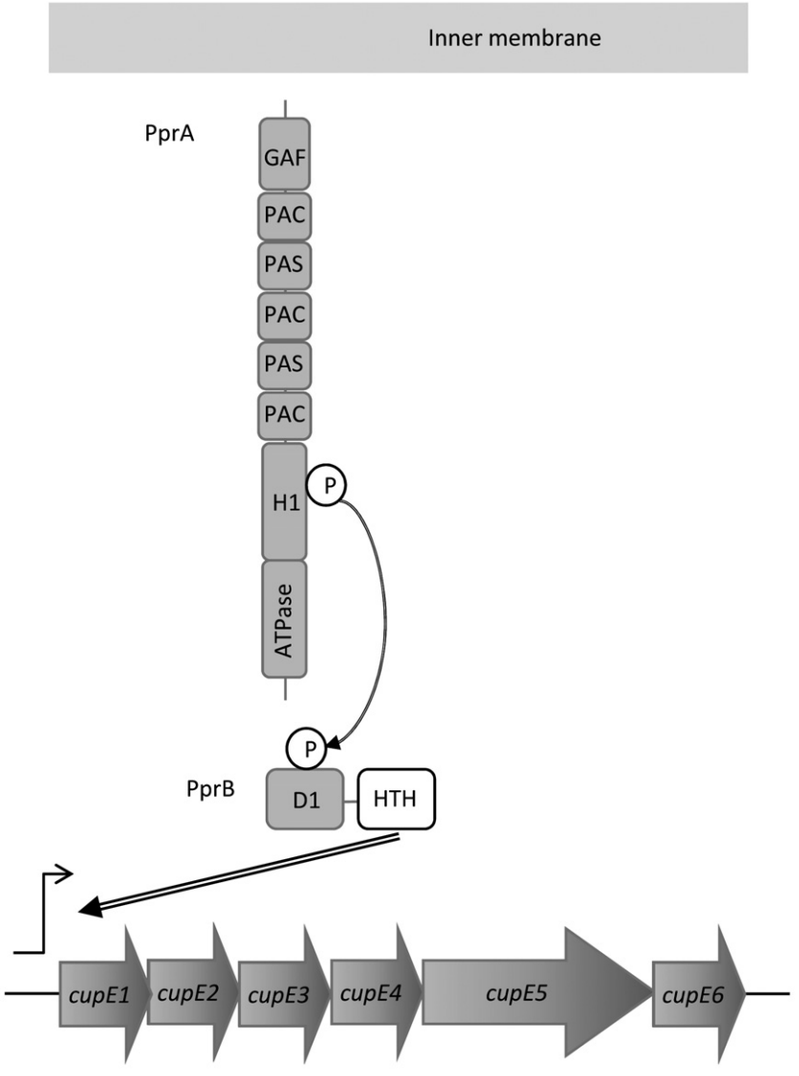

Fig. 5. Regulation of the *cupE* locus: *cupE* expression is directly upregulated by the classical PprAB TCS.

unidentified component, highlighting the extensive cross-regulation between the Roc1 and Roc2 systems (Sivaneson *et al*., 2011).

cupE regulation (Fig. 5)

The *cupE* genes are also controlled by MvaT, with levels of expression five times higher in an *mvaT* mutant than in a wild-type strain, whereas the *cupA* locus has a derepression factor of 15 (Vallet *et al*., 2004). In *mvaT* mutants, the absence of *cupE* expression (C. Giraud and S. de Bentzmann, pers. data) highlights an as yet undetermined level of regulation in addition to that involving H-NS-dependent repression. The observed production of CupE fimbriae after prolonged culture on solid medium or in static liquid culture conditions, their involvement in biofilm formation and the lack of detection of *cupE* expression in shaken liquid cultures, suggest that *cupE* expression is tightly regulated.

In transposon mutagenesis experiments, *cupE* gene regulation has been shown to be controlled by a phosphorelay mechanism involving the PprAB two-component system (Fig. 5). PprA is a classical cytoplasmic histidine kinase with one GAF, three PAC and two PAS domains, which has been demonstrated to function with PprB (Wang *et al*., 2003). The PprB response regulator is a member of the NarL family (Ventre *et al*., 2004), with a receiver domain (REC) and a DNA-binding HTH domain. The expression of *cupE* is directly upregulated by the PprAB system, with the PprB response regulator binding to the *cupE* promoter. The absence of *cupE* expression in shaken cultures can be overcome by PprB overproduction

Table 1. Common rules and specific regulation features controlling the expression of *cup* genes in *P. aeruginosa*.

			H-NS		
	TCS	c-di-GMP	Transcriptional level	Translational level	Others
cupA	No	SiaD, MorA, WspR, PA1120, PvrR, PA2133 D'argenio *et al.* (2002) Castang and Dove (2010) Klebsenberger *et al.* (2009) Li *et al.* (2009) Vallet *et al.* (2004) Vallet-Gely *et al.* (2005; 2007) Meissner *et al.* (2007) McManus and Dove (2011)	Yes Vallet *et al.* (2004)	n.d.	Cgr proteins Sia transduction module Wsp chemosensory system Phase variation D'argenio *et al.* (2002) Klebsenberger *et al.* (2009) Vallet *et al.* (2004) McManus and Dove (2011)
cupB	RocS1 and RocS2 Kulasekara *et al.* (2005) Ruer *et al.* (2007) Sivaneson *et al.* (2011)	n.d.	Yes Vallet *et al.* (2004)	No S. Ruer and S. de Bentzmann, personal data	Not found in high-throughput screen Kulasekara *et al.* (2005)
cupC	RocS1 and RocS2 Kulasekara *et al.* (2005) Ruer *et al.* (2007) Sivaneson *et al.* (2011)	RocR Kulasekara *et al.* (2005)	Yes Vallet *et al.* (2004)	No S. Ruer and S. de Bentzmann, personal data	Not found in high-throughput screen Kulasekara *et al.* (2005)
cupD	RscBC and PvrSR Mikkelsen *et al.* (2009) Nicastro *et al.* (2009)	PvrR Mikkelsen *et al.* (2009)	n.d.	n.d.	n.d.
cupE	PprAB Giraud *et al.* (2011)	No C. Giraud and S. de Bentzmann, personal data	Yes Vallet *et al.* (2004)	n.d.	Not found in high-throughput screen Giraud *et al.* (2011)

n.d., not determined.

(Giraud *et al*., 2011). Induction of *cupE* expression occurs before attachment in biofilm-like conditions and signals such as low oxygen tension or an absence of shearing forces are probably responsible for activating *cupE* expression through the PprAB system.

The hypothesis that PprB-dependent activation of *cupE* expression, like in other CU loci in *P. aeruginosa*, is antagonized by c-di-GMP has been tested. The overproduction of EAL domain-containing proteins, such as PA2133 and RocR, in a recipient strain naturally overproducing PprB only slightly decreases *cupE* expression (C. Giraud and S. de Bentzmann, pers. data) and further investigations are required to ensure that c-di-GMP is not involved in *cupE* expression.

Conclusions

From the different studies presented in this review, it appears that genes encoding the various **CU** systems in *P. aeruginosa* share general trends (Table 1) that include (i) a fine tuned and poor expression in laboratory conditions, (ii) a regulation dependent on signals from the environment, mostly unknown except oxygen paucity, a signal consistent with the role of these structures in *P. aeruginosa* biofilm formation (demonstrated for *cupA* and *cupE* genes) and lowering temperature (demonstrated for *cupA* and *cupD* genes), (iii) a TCS-dependent regulation (demonstrated for *cupB*, *cupC*, *cupD* and *cupE* genes), (iv) a c-di-GMP-dependent regulation with high level of the intracellular second messenger related to induced expression of CU systems (effective for *cupA*, *cupC* and *cupD* genes, not demonstrated for *cupB*, but uneffective for *cupE* genes), and (v) an HNS-dependent repression of *cup* gene expression (demonstrated for *cupA*, *cupB*, *cupC* and *cupE* gene transcription, but invalidated at the translational level for *cupB*, *cupC*). Additionally and uniquely, *cupA* gene expression is controlled by a variety of systems including the Wsp chemosensory system, the Sia transduction module responsive to SDS exposure, the Cgr-related proteins, and by a phase variation mechanism.

These various CU loci responsible for the assembly of fimbriae are regulated by different and specific regulatory pathways, for which the environmental activating signals are therefore mostly unknown. Further studies are now required to identify the signal or combination of signals responsible for triggering these regulatory pathways. All the CU loci of the γ4 fimbrial clade (*cupA*, *cupD*, *cupB* and *cupC*) display complex regulation through combinations of signals sensed by various TCS or chemosensory pathways and c-di-GMP-dependent regulation. The single archaic CU locus of the σ fimbrial clade, *cupE*, displays a simple pattern of regulation dependent on a single classical TCS that is not dependent on c-di-GMP. Furthermore, a comparison of the regulation of *cupA* and *cupD* (*cupD* is thought to have been generated from *cupA* by duplication) also illustrates how the genetic redundancy of systems, as observed for the CU pathway in *P. aeruginosa*, may be an advantage for dealing with different environments.

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