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A structural perspective on the mechanisms of *quorum sensing* activation in bacteria

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ABSTRACT

Bacteria are able to synchronize the population behavior in order to regulate gene expression through a cell-to-cell communication mechanism called *quorum sensing*. This phenomenon involves the production, detection and the response to extracellular signaling molecules named autoinducers, which directly or indirectly regulate gene expression in a cell density-dependent manner. *Quorum sensing* may control a wide range of biological processes in bacteria, such as bioluminescence, virulence factor production, biofilm formation and antibiotic resistance. The autoinducers are recognized by specific receptors that can either be membrane-bound histidine kinase receptors, which work by activating cognate cytoplasmic response regulators, or cytoplasmic receptors acting as transcription factors. In this review, we focused on the cytosolic *quorum sensing* regulators whose three-dimensional structures helped elucidate their mechanisms of action. Structural studies of *quorum sensing* receptors may enable the rational design of inhibitor molecules. Ultimately, this approach may represent an effective alternative to treat infections where classical antimicrobial therapy fails to overcome the microorganism virulence.

Key words: autoinducer, bacteria, *quorum sensing*, receptor, structure.

INTRODUCTION

Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to regulate the expression of specific sets of genes in response to alterations in population density. The perception of population density is mediated by signaling molecules called autoinducers (AIs) synthesized by individual cells. Bacterial growth leads to a proportional increase in the AI extracellular concentration. Once a critical threshold is reached (namely quorum), the bacterial population detects the AI and responds to it through the coordinated expression of specific genes (Fuqua et al. 1994).

QS controls activities that are unproductive when conducted by an individual cell, but become effective when performed collectively (Novick et al. 1995, Seed et al. 1995). A wide range of biological processes are regulated by QS, including bioluminescence, gene transfer, sporulation, antibiotic production and resistance, biofilm formation, pathogen/host interaction and virulence factor secretion (Novick and Geisinger 2008, Ng and Bassler 2009).

A diverse chemical vocabulary has been developed by bacteria, so that Gram-negative

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and Gram-positive bacteria signal QS through structurally distinct molecules. Gram-negative bacteria utilize small organic molecules as AIs, mainly N-acylhomoserine lactones (AHLs) (Bassler 1999, Fuqua et al. 2001). Due to their amphiphilic nature, AHLs diffuse freely across the bacterial plasma membrane (Fuqua and Greenberg 1998). On the contrary, Gram-positive bacteria communicate using oligopeptides named autoinducing peptides (AIPs). These are typically 5 to 17 amino acids in length which may present unusual modifications in their sequences (Mayville et al. 1999). In contrast to Gram-negative AHLs, AIPs are actively secreted into the extracellular environment in a process facilitated by cell surface transporters (Ansaldi et al. 2002).

The detection of AIs and the resulting changes in gene expression are specific to each QS system (Miller and Bassler 2001). At sufficiently high concentrations, Gram-negative AHLs bind to cytoplasmic receptors that also act as transcription factors. The receptor:AI complex then modulates transcription of QS-responsive genes (Zhu and Winans 1999, 2001). On the other hand, Gram-positive AIPs are detected by two-component signal transduction systems composed of a sensor kinase and a response regulator (Havarstein et al. 1995). When the quorum threshold is reached, AIPs bind to extracellular domains of specific membrane histidine kinase receptors. This interaction activates the kinase activity of the receptor, which phosphorylates itself and a cognate cytoplasmic response regulator allowing the expression of QS genes (Solomon et al. 1996). Certain Gram-negative bacterial QS systems also use histidine kinase receptors that function similarly to those described for Gram-positive bacteria. Moreover, Gram-positive AIPs can also be transported back into the cell cytoplasm and interact with specific transcription factors leading to the expression of particular QS-controlled genes (Rutherford and Bassler 2012).

QS was first described in the marine Gram-negative bacterium *Vibrio fischeri*, a symbiont of the Hawaiian bobtail squid, where it controls bioluminescence. (Ruby 1996). This regulatory system is composed of a receptor (LuxR), a target DNA (*lux* operon) and an AI (N-(3-oxo-hexanoyl)-homoserine lactone). The AI, which is synthesized by the enzyme LuxI, diffuses freely between the intra- and extracellular environments. When the bacterial population density increases, the AI binds to the cytoplasmic protein LuxR. This LuxR:AI complex then binds to the operator sequence in the *lux* operon and activates the expression of bioluminescence genes, including the luciferase enzyme (Ruby 1996, Hastings and Greenberg 1999).

Several clinically-relevant bacteria regulate virulence factor production by QS systems, and multidrug resistance is characteristic of these infections (Hentzer et al. 2003, Clatworthy et al. 2007, Geske et al. 2007, Kievit and Iglewski 2000). Thus, the development of antimicrobials that target QS pathways represents an important therapeutic strategy, since QS disturbance attenuates the bacteria pathogenicity without being lethal (Wu et al. 2004, Rasmussen and Givskov 2006a). In addition, a significant advantage of this approach is the absence of selective pressure that results in drug resistance, often seen in traditional antibiotic therapies (Rasmussen and Givskov 2006b, Bjarnsholt and Givskov 2007).

GRAM-NEGATIVE LUXR-TYPE RECEPTORS

Gram-negative LuxR-type receptors comprise about 250 amino acid residues arranged into two functional modules: the N-terminal ligand-binding domain (LBD) and the C-terminal DNA-binding domain (DBD) (Choi and Greenberg 1991, Fuqua and Greenberg 1998). In the absence of the AI, most LuxR-type proteins do not fold correctly, which leads to proteolytic degradation or accumulation in inclusion bodies (Pinto and Winans 2009, Swem et al. 2009, Zhang et al. 2002), while LuxR:AI

complexes are stable and can exert their cellular functions (Hussain et al. 2008, Piper et al. 1993).

On the structural aspect, very little is known about the large family of LuxR-type QS receptors to date. Currently, only TraR (*Agrobacterium tumefaciens*) (Vannini et al. 2002), LasR (*Pseudomonas aeruginosa*) (Bottomley et al. 2007), QscR (*P. aeruginosa*) (Lintz et al. 2011), CviR (*Chromobacteriumviolaceum*) (Chen et al. 2011) and SdiA (*Escherichia coli*) (Kim et al. 2014) have had their three-dimensional structures determined (Table I). However, just TraR, QscR, CviR and SdiA have been crystallized as full-length proteins. For LasR, only the LBD structure has been reported. The receptors tertiary structures are quite similar but their quaternary organization is highly dynamic, making it difficult to establish a general mechanism for their function. This highlights the need for further structural studies on other AHL-binding QS systems.

TraR

The plant pathogen *Agrobacterium tumefaciens* is able to transfer the tumor-inducing plasmid responsible for plant crown gall disease by controlling the LuxI/LuxR-type QS system TraI/TraR. The TraR:N-(3-oxo-octanoyl)-L-homoserine lactone complex binds to promoter elements on the

TABLE I
Three-dimensional structures of *quorum sensing* receptors currently available in the literature.

	QS receptor	Microorganism	Oligomerization state	PDBid	Reference
Gram-negative	TraR	*Agrobacterium tumefaciens*	Dimer (holo)	1H0M	Vannini et al. 2002
				1L3L	Zhang et al. 2002
	LasR	*Pseudomonas aeruginosa*	Dimer (holo)	2UV0	Bottomley et al. 2007
			Dimer (holo)	3IX3	Zou and Nair 2009
				3IX4	
				3IX8	
				3JPU	
	CviR	*Chromobacterium violaceum*	Dimer (holo)	3QP1	Chen et al. 2011
				3QP2 3QP4 3QP5 3QP6	
				3QP8	
	QscR	*Pseudomonas aeruginosa*	Dimer (holo)	3SZT	Lintz et al. 2011
	SdiA	*Escherichia coli*	Dimer (holo)	4LFU	Kim et al. 2014
				4LGW	
			Monomer (holo)	2AVX	Yao et al. 2006
Gram-positive	PrgX	*Enterococcus faecalis*	Tetramer (apo)	2AXU	Shi et al. 2005
			Dimer (holo)	2AXZ	
				2AW6	
	PlcR	*Bacillus thuringiensis*	Dimer (holo)	2QFC	Declerck et al. 2007
			Dimer (apo)	4FSC	Grenha et al. 2013
			Dimer (holo)	3U3W	
	NprR	*Bacillus cereus*	Tetramer (holo)	4GPK	Zouhir et al. 2013

Ti plasmid called *tra boxes*, activating conjugal genes transcription (Zhu et al. 2000).

The structure of TraR bound to its cognate AI and its specific DNA sequence was solved by x-ray crystallography (Vannini et al. 2002). The ternary complex structure reveals an asymmetric homodimer where one monomer is more elongated than the other (Fig. 1a). Such arrangement is possible due to differences in conformation of a linker region that leads to distinct interactions between the N- and C-terminal domains of each monomer. Surprisingly, the AI is not directly involved in receptor dimerization. Instead, the AI is completely buried in an enclosed cavity present in each N-terminal ligand-binding domain (Fig. 1a). In addition, a helix-turn-helix (HTH) motif mediates the interaction of the C-terminal domain with the DNA duplex (Fig. 1a) (Vannini et al. 2002).

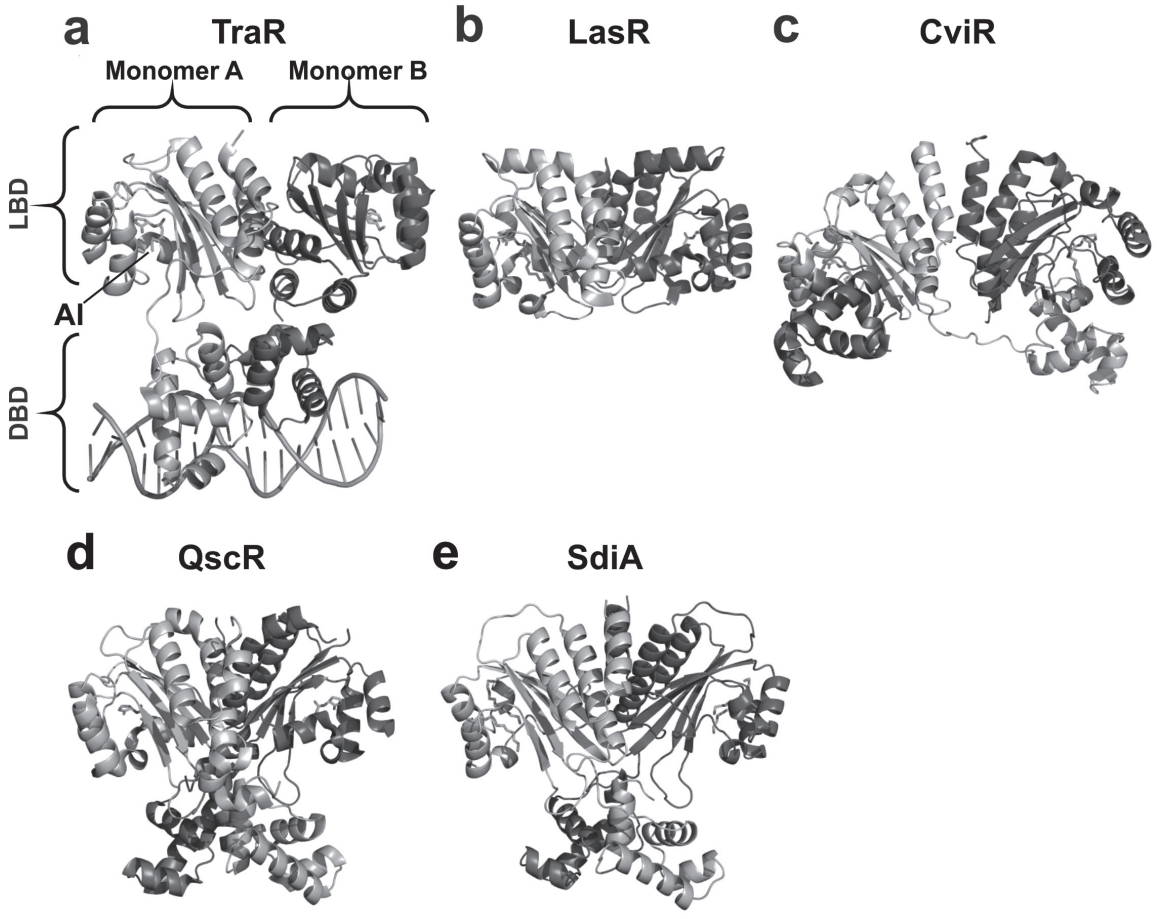

Figure 1 - Three-dimensional structures of Gram-negative LuxR-type QS receptors. (**a**) Ribbon diagram of the TraR:3-oxo-C8-HSL:DNA ternary complex structure (PDB: 1H0M); (**b**) Ribbon diagram of the LasR-LBD:3-oxo-C12-HSL complex structure (PDB: 2UV0); (**c**) Ribbon diagram of the CviR:chlorolactone complex structure (PDB: 3QP5); (**d**) Ribbon diagram of the QscR:3-oxo-C12-HSL complex structure (PDB: 3SZT). (**e**) Ribbon diagram of the SdiA dimer structure. The AI-binding site is occupied by two low-molecular weight PEG molecules derived from the crystallization condition. All structures are oriented in respect to the LBD structure of TraR monomer A. N-terminal ligand-binding domains are colored in blue, while C-terminal DNA-binding domains are colored in salmon. Monomers are represented either by light or dark colors. AI structures are displayed in stick representation and colored in green.

The N-terminal ligand-binding domain (1-162) is composed of a five-stranded antiparallel β-sheet surrounded by three α-helices on each side (Fig. 1a). The AI interaction site is located between the central β-sheet and helices α3 (54-64), α4 (70-79) and α5 (97-107). A cluster of aromatic and hydrophobic amino acids forms a cavity that accommodates the AI without any solvent contact. Hydrogen bond interactions with residues Trp57 and Asp70 stabilize the AI molecule in the cavity. The homoserine lactone (HSL) portion of the AI interacts with residues Trp57, Asp70, Val72, Val73, Trp85, Phe101, Tyr102, Ala105 and Ile110, while the acyl moiety makes hydrophobic contacts with residues Tyr53, Leu40, Tyr61 and Phe62. The dimer interface is located on the opposite side of the AI interaction site. Hydrophobic interactions between the two α6 helices from each monomer stabilize the TraR dimer (Vannini et al. 2002).

The C-terminal DNA-binding domain (176-243) consists of four α-helices: α7 (176-188), α8 (119-198), α9 (203-217), and α10 (223-230). The typical HTH motif is stabilized by a large hydrophobic cluster and a conserved salt bridge between residues Glu178 and Arg215. The DNA-binding domain is a dimer in the crystal and interacts with two binding sites on *tra box* (Vannini et al. 2002). Helices α8 and α9 directly interact with DNA, working as "scaffold" and "recognition" sites, respectively. Five residues from helix α9 make direct contacts with the major groove of the DNA. The specificity of this interaction is mediated by the side chains of two neighboring arginine residues, Arg206 and Arg210 (Vannini et al. 2002).

The most striking feature of the [TraR:3-oxo-C8-HSL]$_2$:*tra box* structure is that the AI is completely embedded in a hydrophobic cavity in the N-terminal domain. This suggests a key role played by the AI in the correct folding of the receptor. The current hypothesis for TraR ligand-binding activation includes the participation of the AI in the stabilization of nascent TraR. Once properly folded, TraR becomes dimeric and protease-resistant. Receptor dimerization through the exposure of hydrophobic amino acids in helix a6 results in specific interaction of the DNA-binding domain dimers with *tra box* sequences.

LasR

The LuxI/LuxR-homologous QS system LasI/LasR is responsible for QS-coordinated activities in *P. aeruginosa* (Kiratisin et al. 2002, Schuster et al. 2004). Thus, structure resolution of the LasR regulator was an important step toward a molecular understanding of QS signal transduction in this microorganism. The crystal structure of the LasR ligand-binding domain (1-175) in complex to its cognate AI N-(3-oxo-dodecanoyl)-L-homoserine lactone reveals a symmetrical dimer with one AI deeply inserted in each monomer (Fig. 1b). The overall structure of the LasR monomer is very similar to that of TraR, consisting of a three α-helices/five-stranded antiparallel β-sheet/three α-helices sandwich (Fig. 1b) (Bottomley et al. 2007).

As observed for other LuxR-type receptors, a cavity formed between the β-sheet and helices α3, α4 and α5 hides the AI from the solvent (Fig. 1b). The HSL moiety of the AI is stabilized by six hydrogen bond interactions with residues Tyr56, Trp60, Arg61, Asp73, Thr75 and Ser129. These residues are strictly conserved among the LuxR-type family, reflecting a common mode of interaction with the AI headgroup. On the other hand, the acyl chain extends into a pocket formed by a cluster of hydrophobic residues. Some of them only occur in LasR, such as Leu40, Tyr47, Cys79 and Thr80 (Bottomley et al. 2007). These structural features provide the molecular basis for the high ligand specificity of LasR. Moreover, structural differences in the mode of interaction of TraR and LasR with their cognate AIs provide additional information about the specificity of QS receptors.

Despite the similarities among the monomer structures of TraR and LasR ligand-binding domains, their quaternary organization differ

considerably. The most notable difference between the structures of TraR and LasR is the dimerization interface. In both structures, helix α9 is responsible for the majority of hydrophobic contacts and hydrogen bond interactions that contribute to dimer stabilization. However, the two α9 helices are oriented parallel to each other in the structure of TraR (Vannini et al. 2002), while they are almost perpendicular in LasR (Bottomley et al. 2007). This causes a 90º shift in orientation of one monomer with regards to the other when comparing both structures. This difference in orientation of the N-terminal domains may lead to a difference in conformation of the two C-terminal domains and result in a distinct mode of DNA recognition by LasR. Nevertheless, the model of AI-induced activation that arises from the structure of the LasR:3-oxo-C12-HSL complex is reminiscent to that of other LuxR-type receptors and requires the binding of the AI to nascent LasR and the stabilization of its fold. Subsequently, LasR dimerization can occur, enabling specific binding to DNA promoters and transcriptional activation of QS-controlled genes.

CviR

The human pathogen *Chromobacterium violaceum* uses QS to control biofilm formation, cyanide production and purple pigment violacein synthesis (McClean et al. 1997). The cognate AI of the LuxR-type receptor CviR is the *N*-(hexanoyl)-L-homoserine lactone molecule. The crystal structure of full-length CviR bound to its antagonist (chlorolactone—CL) was determined (Chen et al. 2011). The structures of the independent domains of CviR resemble those of TraR, however, their overall organization is markedly different. In contrast to TraR, the CviR:CL complex shows a cross-subunit architecture where the DNA-binding domain of each monomer is positioned underneath the ligand-binding domain of the opposite monomer, making extensive LBD-DBD interactions (Fig. 1c) (Chen et al. 2011). This cross-subunit conformation leads to a separation of the two DNA-binding helices in each C-terminal domain by about 60 Å (Fig. 1c). This distance is twice the ~30 Å separation required for efficient operator binding, which may explain, at a molecular level, the decreased DNA-binding affinity displayed by the CviR:CL complex (Chen et al. 2011). Because the full-length CviR structure was determined in complex with its antagonist, it is possible that the cross-subunit structure may be a consequence of this interaction. Therefore, the accepted hypothesis for CviR inhibition by CL involves the binding of CL to the AI cavity and the induction of a closed conformation unable to bind DNA.

QscR

QscR is a LuxR homolog also found in *P. aeruginosa* that binds various AHLs including 3-oxo-C12-HSL, which is recognized by LasR (Oinuma and Greenberg 2011, Lee et al. 2006). The crystal structure of full-length QscR bound to its AI showed a symmetrical dimer with a domain configuration different than those observed in the previously reported structures of LuxR-type receptors (Lintz et al. 2011).

QscR contains general structural features that are characteristic of the LuxR family. It is formed by an N-terminal ligand-binding domain and a C-terminal DNA-binding domain. The LBD adopts the typical α-β-α sandwich architecture composed of a five-stranded β-sheet surrounded by five α-helices, while the DBD is formed by a canonical HTH motif. The AI binding site is located in a hydrophobic cavity formed between the β-strand and helices α3, α4 and α5. Nonetheless, QscR forms a symmetrical homodimer with a unique cross-subunit configuration (Fig. 1d) (Lintz et al. 2011).

The HSL moiety of the AI makes a number of hydrogen bond interactions with QscR conserved

residues Tyr58, Trp62, and Asp75, as well as the nonconserved residues Ser38 and Ser56. In addition, numerous hydrophobic contacts between the AI acyl chain and QscR residues Arg42, Tyr52, Val78, Leu82, and Ile125 are observed. These residues are in close proximity to the cross-subunit LBD-DBD dimerization interface and thus may be involved in QscR ligand-binding specificity (Lintz et al. 2011). Interestingly, the conformation of the AI molecule and the shape of the binding pocket in QscR are similar to those of LasR but are distinct from TraR and CviR, suggesting that QscR and LasR recognizes their cognate AHLs in similar ways.

The cross-subunit configuration of the QscR:3-oxo-C12-HSL dimer is a remarkable feature among the members of the LuxR-type family. In this conformation, the LBD of one monomer makes extensive interactions with the LBD and the DBD of the other monomer (Fig. 1d) (Lintz et al. 2011). Polar and hydrogen bond contacts between residues Glu84, Ser147, and Lys121 of one monomer and the same residues in the opposite monomer stabilize the LBD-LBD dimer interface. In addition, hydrogen bond interactions between residues Arg42 and Arg79 of monomer A and Asn237 of monomer B stabilize the LBD-DBD interface. The LBD-LBD dimerization interface of QscR resembles that of LasR and is distinct from those of TraR and CviR. On the other hand, the QscR DBDs form a dimer that is most similar to the one seen in TraR (Lintz et al. 2011). This suggests that, in contrast to CviR, the cross-subunit structure of QscR is prompt for DNA binding.

SdiA

The QS receptor SdiA is found in commensal and pathogenic bacteria of the intestine, such as *Escherichia coli*. The structure of full length SdiA was determined by x-ray crystallography (Kim et al. 2014) and the crystal structures of SdiA have shown a similar fold to the mean NMR structure of the SdiA LBD reported previously (Yao et al. 2006).

Like other LuxR-type receptors, SdiA is formed by two functionally different domains: an N-terminal domain that binds the AI and a C-terminal domain that recognizes specific DNA sequences. These domains are connected by a linker and form a symmetrical dimer (Fig. 1e). The LBD exhibits an α/β/α topology composed of α-helices surrounding each side of a central antiparallel five-stranded β-sheet, which has a concave surface, where the AI-binding site is located (Fig. 1e). The DBD has four α-helices and is characterized by a typical HTH DNA-binding motif formed by helices α8 and α9 (Fig. 1e) (Kim et al. 2014). The overall folding of the SdiA LBD and DBD are well conserved when compared to other LuxR-type receptors, as opposed to the orientation of the two domains, which is significantly different. This observation is in agreement with the theory proposed by a previous study of an evolutionary combination of the two ancestral domains (Aravind and Ponting 1997). To date, it is still unclear why each domain of LuxR-type receptors presents different relative orientations. It is assumed that each receptor defines the relative orientation of each domain since the residues located in the interdomain interface are not conserved among LuxR-type proteins. The conformation and function of the DBD remain unchanged upon AHL binding, which suggests that AHL may regulate the transcriptional activity of SdiA by increasing its stability.

The *E. coli* genome does not code for a LuxI-type QS synthetase (Ahmer 2004), and it is known that SdiA is able to respond to indole as well as various AHL molecules released from other bacterial species. Therefore, SdiA may aid in space and competition with other microorganisms by being capable of recognizing a variety of AIs (Michael et al. 2001). This broad ligand-binding specificity of SdiA, compared to other LuxR-type receptors, may be due to a wide AI-binding site that is open to the solvent and can easily accommodate

a wide range of ligands. Nevertheless, several studies have reported that *E. coli* SdiA has some selectivity to AI, especially for ligands with short chain length. This may occur because the occluded residues Phe59 and Gln72 of the AI-binding cavity limit the chain length of the acyl moiety to eight carbon atoms (Kim et al. 2014).

Dimeric SdiA has two Cys residues in the DBD conferring inter-cysteine distance within the range of disulphide bond formation (5.7 Å), which suggests that an intersubunit disulphide bond may be relevant to modulation of DNA-binding activity (Kim et al. 2014). Furthermore, the binding affinity of SdiA for the *uvrY* promoter and the transcriptional activity of SdiA may be reduced under oxidative conditions. This cross-talk between QS and ROS signaling has also been reported in the QS arg system, which is characterized by an intramolecular disulfide redox switch (Sun et al. 2012).

The structural analysis of the intact *E. coli* SdiA provides an understanding not only of its broad ligand selectivity, but also for the general role of AHL-dependent QS receptors, where it is likely that regulation of SdiA may occur through multiple independent signals involving not only AIs but also the oxidative state of the cell.

GRAM-POSITIVE RNPP QS RECEPTORS

Gram-positive bacteria utilize oligopeptides as QS signaling molecules named autoinducing peptides (AIPs) (Miller and Bassler 2001). These are grouped according to the subcellular localization of their cognate receptors, at the cell surface or inside the cell (Williams 2007). The first group consists of peptides that interact with the external domain of membrane histidine kinases, resulting in further signaling to a final cytoplasmic effector, called indirect QS systems. The second group reflects a direct QS system, comprising peptides that are reimported from the vicinity and regulate the QS response by interacting directly with cytoplasmic receptors (Lyon and Novick 2004, Lazazzera 2001, Slamti and Lereclus 2002). The AIP-binding receptors of the direct QS systems have been grouped in the new RNPP protein family (Rap, NprR, PlcR and PrgX) (Declerck et al. 2007). Rap proteins are phosphatases and transcriptional anti-activators (Parashar et al. 2011), while NprR, PlcR, and PrgX are DNA-binding transcription factors (Wintjens and Rooman 1996, Aravind et al. 2005).

The RNPP protein family is characterized by a C-terminal domain presenting several tetratricopeptide repeats (TPRs), a structural motif of 34 residues that mediates protein–protein and protein–peptide interactions (Blatch and Lassle 1999, D'Andrea and Regan 2003). With the exception of Rap proteins, all RNPP members contain an N-terminal HTH-type DNA-binding domain and exhibit transcriptional activity (Wintjens and Rooman 1996, Aravind et al. 2005). To date, the only Gram-positive AIP-binding QS receptors that were described at the molecular level are PrgX (*Enterococcus faecalis*) (Shi et al. 2005), PlcR (*Bacillus cereus*) (Declerck et al. 2007) and NprR (*Bacillus cereus*) (Zouhir et al. 2013) (Table I). Although the AIP-binding mode is similar among the RNPP family members, the peptide-induced conformational changes are specific for each receptor (Declerck et al. 2007).

PrgX

The QS receptor PrgX regulates the pCF10 plasmid conjugative transfer in *Enterococcus faecalis* (Bae et al. 2000). PrgX recognizes two DNA binding sites on pCF10, leading to transcriptional repression of the *prgQ* operon. AIP binding causes a decrease in the PrgX oligomerization state that alleviates transcriptional repression, resulting in expression of conjugation genes. The three-dimensional structures of full-length PrgX in its free form and bound to its cognate AIP cCF10 were determined by x-ray crystallography (Shi et al. 2005). Structure determination of PrgX:cCF10 complexes were

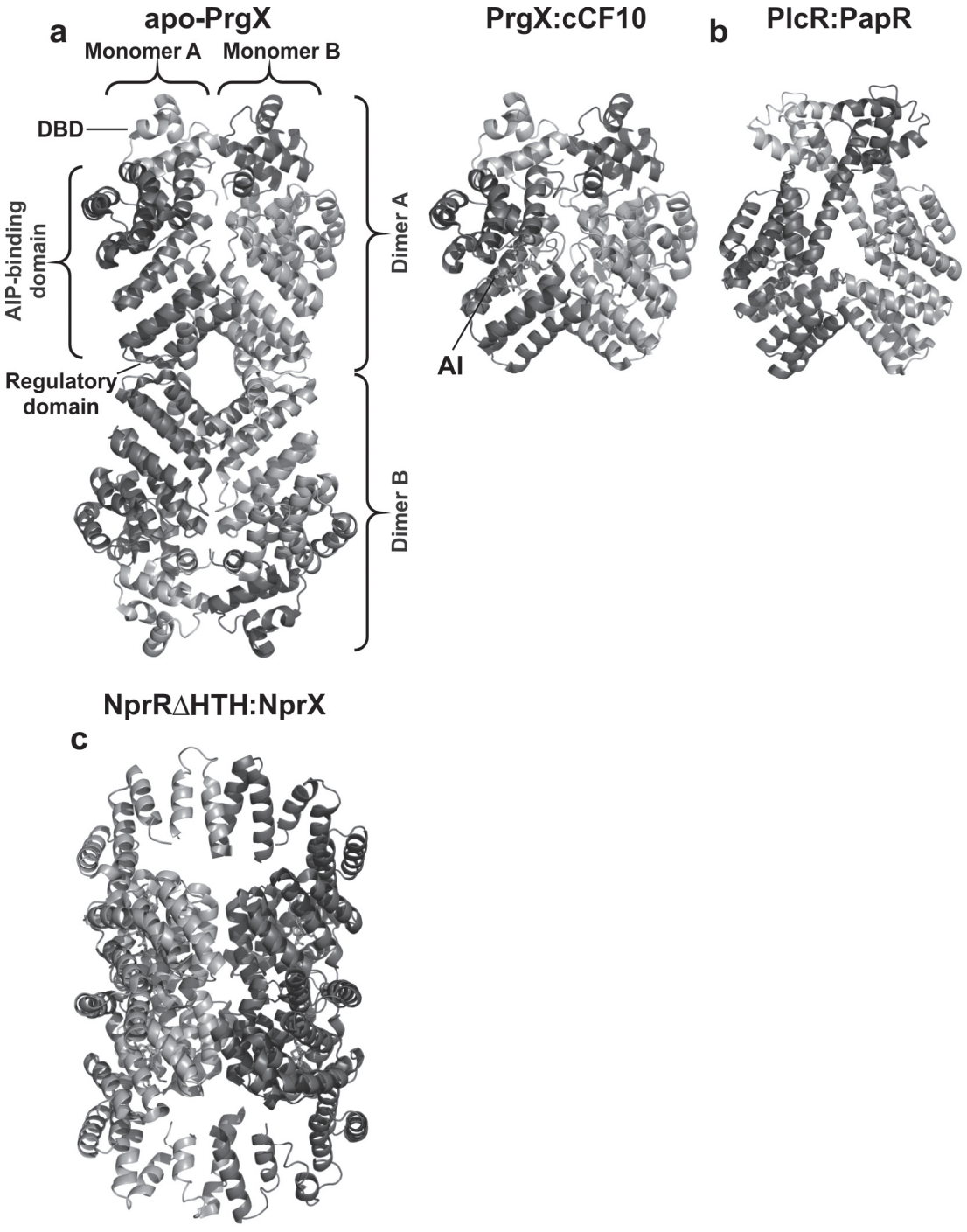

Figure 2 - Three-dimensional structures of Gram-positive RNPP QS receptors. (**a**) Ribbon diagram of apo-PrgX (PDB: 2AXU) (*left*) and the PrgX:cCF10 complex (PDB: 2AXZ) (*right*) structures; (**b**) Ribbon diagram of the PlcR:PapR complex structure (PDB: 2QFC); (**c**) Ribbon diagram of the NprRDHTH:NprX complex structure (PDB: 2GPK). N-terminal DNA-binding domains are colored in blue, central dimerization and AIP-binding domains are colored in salmon, and regulatory C-terminal domains are colored in orange. Monomers are represented either by light or dark colors. AI structures are displayed in stick representation and colored in green.

essential to elucidate the mechanism by which AIP binding leads to receptor activation.

The crystal structures of apo-PrgX reveal a symmetrical tetramer composed by two homodimers that interact in a tail-to-tail manner (Bae et al. 2002) (Fig. 2a). Each dimer cooperatively binds to one operator site on the *prgQ* operon. In the repressed state, both DNA-binding sites are occupied by the PrgX tetramer. PrgX adopts a helical structure that can be divided into three functional modules: a N-terminal DNA-binding domain, a central dimerization and AIP-binding domain, as well as a regulatory C-terminal domain (Fig. 2a) (Shi et al. 2005).

The N-terminal domain (1–68) is composed by five α-helices, including α1 (3–15), α2 (18–24), α3 (28–37), α4 (43–55) and α5 (57–65) (Fig. 2a). DNA binding is mediated by helices α2 and α3 that form a typical HTH motif. The PrgX dimer adopts a crossed-subunit architecture, where the N-terminal domain of one monomer simultaneously interacts with the N-terminal and central domains of the other monomer (Fig. 2a). Structural comparison between PrgX and other prokaryotic transcriptional regulators enabled the construction of a model for the PrgX:DNA complex. In this model, the DNA duplex loops around the PrgX tetramer, interacting with the swapped N-terminal domains of each dimer (Shi et al. 2005).

The central domain (69-283) consists of 11 antiparallel α-helices arranged in a two-layered super helix that mediates both receptor dimerization and AIP binding (Orengo et al. 1997). The dimerization interface is composed by helices α14 and α15 from each monomer. Residues Leu234, Ile263, Ile266, and Ile267 form a hydrophobic core surrounded by hydrogen bond interactions that stabilize the PrgX dimer. In addition, the PrgX tetramer is stabilized by van der Walls and hydrogen bond interactions between the C-terminal domain loop (287–294) of one dimer and a loop comprising residues 248–255 of the opposite dimer (Shi et al. 2005). The cCF10 peptide binds into a pocket in PrgX composed by helices α6, α8, α10, α12, α14 and α16. Residues Ile82, Phe86, Ile200, Ile283 and Leu282, located on each side of the AIP-binding site, establish hydrophobic interactions with cCF10 side chains (Shi et al. 2005).

The C-terminal domain (287–305) consists of a loop region and helix α17 that regulates the *prgQ* operon transcription (Grenha et al. 2013). The crystal structures of PrgX:cCF10 complexes reveal that cCF10 binding induces a conformational change in the C-terminal domain loop. In the bound conformation, this loop is rotated 120° away from the tetramerization interface, leading to disruption of the PrgX tetramer (Fig. 2a) (Shi et al. 2005). cCF10-induced tetramer dissociation decreases the affinity of PrgX for the second operator site, resulting in disruption of the DNA-loop structure, RNA polymerase binding, and increased expression of *prgQ*-encoded genes.

PlcR

The QS receptor PlcR regulates pathogenicity in *Bacillus cereus* (Lereclus et al. 1996). In the presence of its cognate AIP PapR, PlcR activates the expression of virulence factors (Slamti and Lereclus 2002). The structure of PlcR bound to PapR was determined by x-ray crystallography. The PlcR:PapR complex structure displays a symmetrical dimer that is most similar to the dimeric unit of the PrgX:cCF10 tetramer structure (Fig. 2b). Each PlcR monomer is composed of an N-terminal DNA-binding domain and a regulatory C-terminal AIP-binding and dimerization domain (Fig. 2b) (Declerck et al. 2007).

The N-terminal domain consists of a 5-helix HTH motif that mediates DNA interaction (Fig. 2b). Helix α3 inserts itself into the DNA major groove, working as "recognition" helix. As in PrgX, the PlcR dimer adopts a crossed conformation formed by two monomers with their N-terminal domains swapped (Fig. 2b). Conversely, PlcR N-terminal domains are directly attached to the first C-terminal domain helix, resulting in higher mobility than in PrgX (Declerck et al. 2007).

The C-terminal regulatory domain is strikingly similar to the central domain of PrgX. It consists of eleven α-helices forming five tetratricopeptide repeats (TPR) and a capping C-terminal helix arranged in a superhelix (Fig. 2b). The dimer interface is composed by the two TPR domains. The AIP-binding site is located in the middle of the TPR superhelix, between helices α5 and α7. Residues Asn159, Asn201 and Lys204 make hydrogen bond interactions with the backbone of PapR. Moreover, hydrophobic interactions between two PapR Phe residues and PlcR side chains from the binding pocket stabilize the AIP-bound conformation (Declerck et al. 2007).

In contrast to PrgX, PlcR lacks the C-terminal domain loop that mediates receptor tetramerization. Consequently, apo-PlcR is dimeric in solution, with an overall dimension comparable to the crystallographic PlcR:PapR dimer, as revealed by SAXS measurements (Declerck et al. 2007). In contrast, the PlcR:PapR complex is capable of forming higher order oligomers in solution (Declerck et al. 2007). This leads to the current hypothesis regarding the activation mechanism of PlcR by PapR. In this hypothesis, PapR binding increases the curvature of the TPR domain of PlcR. This domain closure separates the two HTH motifs and triggers PlcR multimerization. In this PlcR:PapR multimer, all the HTH domains are mobile and thus suited for DNA binding.

NprR

Adaptive gene expression response of *Bacillus cereus* is controlled by the QS receptor NprR (Perchat et al. 2011). The crystal structure of a truncated form of NprR, named NprRΔHTH (HTH motif deleted), in complex to its cognate AIP NprX has been determined (Zouhir et al. 2013).

The structure of the NprRΔHTH:NprX complex reveals a tetramer, consisting of a dimer of dimers. Each dimer comprises two monomers arranged in a head-to-tail fashion (Fig. 2c) (Zouhir et al. 2013). The NprRΔHTH:NprX monomer is composed of 18 α-helices arranged in a two-layered, right-handed superhelix formed by 9 TPR motifs (Fig. 2c). The dimer interface is mainly stabilized by van der Waals interactions between residues located in loops α8-α9 from one monomer and α10-α11 from the other monomer. On the other hand, the tetramer interface is stabilized by hydrophobic contacts between residues located on helices a9 and α18 from opposite dimers (Zouhir et al. 2013).

As in other RNPP family members, the AIP binds into a cleft located in the concave side of the TPR domain (Fig. 2c). NprX backbone atoms make van der Waals and hydrogen bond interactions with NprR amino acids from the binding pocket. In addition, a hydrogen bond between NprX Asp residue and the side chain of the conserved NprR Asn126 stabilizes the complex, playing a key role in the mechanism of NprR activation upon NprX binding (Zouhir et al. 2013).

In contrast to the NprRΔHTH:NprX tetramer structure, apo-NprR, both full-length and NprRΔHTH, is a dimer in solution, as evidenced by size-exclusion chromatography and dynamic light scattering (Zouhir et al. 2013). NprX binding shifts the dimer-tetramer equilibrium toward the higher order state. Therefore, NprX binding induces the association of two apo-NprR dimers, resulting in the formation of an active NprR:NprX tetramer. Comparison of the NprRΔHTH:NprX structure with the structures of the RNPP QS receptors PrgX and PlcR bound to their cognate AIPs enabled the building of a model for the full-length NprR:NprX complex. In this model, the two HTH-type DNA-binding domains point toward opposite sides of the tetramer, suggesting that NprR and PrgX interact with their target DNA sequences in a similar manner (Zouhir et al. 2013).

CONCLUSIONS

Despite the fact that each QS system may present specific mechanistic details, the emerging scenario is that the Gram-negative LuxR-type QS

receptors are activated by their cognate AIs through a folding-switch mechanism. In the absence of AHL, these receptors are intrinsically unstable, and thus may be quickly degraded or stored in inclusion bodies. Moreover, the interaction with AHLs promotes the receptor's correct folding, increasing its stability and leading to the appropriate conformation and oligomerization state required for specific interaction with its respective target DNA. On the other hand, the activation of Gram-positive RNPP QS receptors occurs through an allosteric regulatory mechanism, which is triggered by the interaction with its respective AIP. The receptor oligomerization is regulated by AIP binding, where it may either lead to dissociation, as seen with PrgX, or multimerization, which is the case of PlcR, and this regulation reflects a specific interaction of these receptors with their DNA sequences.

It is important to highlight that the structural studies of QS receptor:AI interactions are important to elucidate the molecular network underlying the mechanisms of quorum sensing activation in bacteria, as the structural characterization may bring new insights into how these receptors work. This knowledge is crucial for the development of strategies involving the rational design of QS inhibitors. As an example, the available 3D structural data can be used to guide a virtual screening of potential QS receptors antagonists. In addition, a complete molecular description of the interaction between the receptor and its AI may facilitate the development of electrophile compounds that covalently bind to the AI-binding pocket, specifically inhibiting the QS receptor. Furthermore, these potential inhibitors, also known as quorum quenching molecules, may become a promising new generation of antimicrobials.

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RESUMO

Bactérias são capazes de sincronizar seu comportamento populacional a fim de regular a expressão gênica por meio de um mecanismo de comunicação célula-a-célula denominado *quorum sensing*. Este fenômeno envolve a produção, detecção e resposta a moléculas sinalizadoras extracelulares chamadas de auto-indutores, que direta ou indiretamente regulam a expressão de genes de maneira dependente da densidade celular. *Quorum sensing* controla uma grande variedade de processos biológicos em bactérias, tais como bioluminescência, produção de fatores de virulência, formação de biofilme e resistência a antibiótico. Os auto-indutores são reconhecidos por receptores específicos, que podem ser tanto receptores histidina quinases associados a membranas, que funcionam através da ativação de reguladores de resposta citoplasmáticos cognatos, quanto receptores citoplasmáticos que atuam como fatores de transcrição. Nesta revisão, nós focamos nos reguladores de *quorum sensing* citosólicos, cujas estruturas tridimensionais auxiliaram na elucidação dos seus mecanismos de ação. Estudos estruturais de receptores de *quorum sensing* podem viabilizar o desenho racional de moléculas inibitórias. Finalmente, esta abordagem pode representar uma alternativa eficaz no tratamento de infecções onde a terapia antimicrobiana clássica é incapaz de superar a virulência do micro-organismo.

Palavras-chave: auto-indutor, bactéria, *quorum sensing*, receptor, estrutura.

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