REVIEW ARTICLE

Orphan LuxR regulators of quorum sensing

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Received 27 August 2008; revised 9 January 2009; accepted 12 January 2009.
Final version published online 13 February 2009.

DOI:10.1111/j.1574-6976.2009.00163.x

Editor: Miguel Camara

Keywords
orphan LuxR; quorum sensing; LuxR regulators; transcriptional regulators; bacterial communication.

Abstract

Bacteria can modulate their behavior by releasing and responding to the accumulation of signal molecules. This population co-ordination, referred to as quorum sensing, is prevalent in Gram-negative and Gram-positive bacteria. The essential constituents of quorum-sensing systems include a signal producer, or synthase, and a cognate transcriptional regulator that responds to the accumulated signal molecules. With the availability of bacterial genome sequences and an increased elucidation of quorum-sensing circuits, genes that code for additional transcriptional regulators, usually in excess of the synthase, have been identified. These additional regulators are referred to as 'orphan' regulators, because they are not directly associated with a synthase. Here, we review orphan regulators characterized in various Gram-negative bacteria and their role in expanding the bacterial regulatory network.

Introduction

Bacteria are often social organisms that form communities in their natural environments. Within these communities bacteria are subjected to constant changes in external conditions, such as the availability of nutrients or the presence of toxic compounds. Therefore, it is critical that bacteria co-ordinate their behavior in order to adapt and survive. The regulation of genes mediated by signaling molecules and regulatory proteins in a population density-dependent manner is referred to as quorum sensing. This phenomenon enables bacteria not only to sense members of their own species but others as well (Miller & Bassler, 2001; Waters & Bassler, 2005). Since it was first described in marine bacteria by Nealson *et al.* (1970), quorum sensing has been well characterized in both Gram-negative and Gram-positive microorganisms where it has been reported to play a role in human pathogenesis (*Pseudomonas aeruginosa*), symbiosis (*Sinorhizobium meliloti*), plasmid conjugation (*Agrobacterium tumefaciens*), and competence (*Bacillus subtilis*) (Nealson *et al.*, 1970; Farrand *et al.*, 2002; Marketon *et al.*, 2003; Pappas & Winans, 2003; Smith & Iglewski, 2003; Comella & Grossman, 2005).

In Gram-negative quorum-sensing systems, the bacterial autoinducer synthase produces a signal molecule, which accumulates as the population density increases. On achieving a threshold critical concentration, this signal interacts with specific response regulators and the resulting complex binds to a DNA sequence located upstream of the target gene promoter, resulting in transcriptional regulation (Fuqua *et al.*, 2001; Whitehead *et al.*, 2001). *N*-acyl homoserine lactones (AHL) are the predominant type of signals in quorum-sensing systems, though a wide array of molecules have also been identified as population density-dependent signals in Gram-negative bacteria (Flavier *et al.*, 1997; Holden *et al.*, 1999; Pesci *et al.*, 1999; Surette *et al.*, 1999; Wang *et al.*, 2004; He *et al.*, 2006; Park *et al.*, 2006; Rader *et al.*, 2007; Schaefer *et al.*, 2008). Though autoinducers differ in their chemical and structural properties, they have a common role: to bind to response regulators and mediate transcription.

The *Vibrio fischeri* quorum-sensing network serves as the paradigm for most Gram-negative bacterial quorum-sensing networks (Fuqua *et al.*, 1996). In this system, the synthase and response regulator were named 'LuxI' and 'LuxR', respectively (Fuqua *et al.*, 1996). Similar proteins

subsequently identified in other bacteria are referred to as LuxI- and LuxR-type proteins (Fuqua *et al.*, 1994, 1996). The detailed molecular mechanisms of various components of quorum sensing have been described previously in several exhaustive reviews (Fuqua *et al.*, 1996, 2001; Miller & Bassler, 2001; Whitehead *et al.*, 2001; González & Marketon, 2003; Newton & Fray, 2004; Waters & Bassler, 2005). In this review we focus on the response regulator component, which in conjunction with the autoinducer controls gene expression.

Response regulators

Genetic and biochemical evidence identified the LuxR protein of *V. fischeri* and its homologs as the autoinducer receptors (Engebrecht & Silverman, 1984). The LuxR-type proteins are comprised of two functional domains, a signal-binding and a DNA-binding domain. The amino-terminal region of the LuxR-type protein binds its activating signal, and the carboxy-terminal region contains a helix-turn-helix domain that interacts with DNA (Choi & Greenberg, 1992; Fuqua *et al.*, 2001). The nature of the DNA–protein interaction determines if the response regulator functions as an activator or a repressor of transcription (Luo & Farrand, 1999; Cases & de Lorenzo, 2005; Nasser & Reverchon, 2007). Transcriptional activation occurs when the AHL/LuxR-type protein complex binds upstream of the transcriptional start site and recruits RNA polymerase through direct contact (Vannini *et al.*, 2002; Zhang *et al.*, 2002). The autoinducer–regulator complex binds specifically to DNA sequences of dyad symmetry called *lux* boxes at quorum sensing-regulated promoters centered at about −40 from the transcriptional start site (Whitehead *et al.*, 2001). Though the presence of a *lux* box is necessary for DNA-binding in the case of LuxR and TraR (*tra* box) of *A. tumefaciens*, other LuxR-type proteins, like LasR of *P. aeruginosa*, do not seem to have a requirement for dyad symmetry within the promoters of their target genes (Egland & Greenberg, 1999; Zhu & Winans, 1999; Schuster *et al.*, 2004).

In the absence of AHLs, some LuxR-type proteins, such as EsaR of *Pantoea stewartii* and ExpR of *Erwinia* sp., act as repressors by binding promoters of target genes, where they prevent transcription by blocking access to the RNA polymerase (Minogue *et al.*, 2002; von Bodman *et al.*, 2003b). Binding to an AHL causes conformational changes that release the repressor from DNA and relieve repression (Minogue *et al.*, 2002; von Bodman *et al.*, 2003b). Therefore, either via activation or repression, the LuxR-type response regulators serve to modulate the behavior of the entire bacterial population.

Protein sequence comparisons have revealed that LuxR-type proteins share only 18–25% end-to-end identity, although their functional domains share much higher sequence conservation (Whitehead *et al.*, 2001; Nasser & Reverchon, 2007). Genetic and structural analyses of the TraR of *A. tumefaciens* have identified the amino acid residues that are directly involved in autoinducer and DNA binding (Vannini *et al.*, 2002; Zhang *et al.*, 2002; Chai & Winans, 2004). Sequence alignments of TraR with other LuxR-type proteins indicated that nine residues are identical in at least 95% of LuxR-type proteins (Table 1) (Whitehead *et al.*, 2001; Zhang *et al.*, 2002). Six of those residues in the N-terminal domain (W57, Y61, D70, P71, W85, and G113) were involved in binding to the cognate autoinducer while three in the C-terminal domain (E178, L182, and G188) were associated with DNA binding (Vannini *et al.*, 2002; Zhang *et al.*, 2002; Nasser & Reverchon, 2007). Overall, structure–function analysis in TraR and other response regulators have indicated that the DNA-binding domain is largely conserved, while the autoinducer-binding domain tends to vary in several LuxR-type proteins, perhaps to accommodate the variety of activating signals (Vannini *et al.*, 2002; Zhang *et al.*, 2002; Yao *et al.*, 2006; Bottomley *et al.*, 2007).

Orphan LuxR homologs

Characterization of quorum sensing in different bacteria has led to the identification of several LuxR and LuxI homologs. Typically, the genes for these proteins lie in close proximity to each other on their genome and are referred to as the cognate *luxR/I* pair (Table 2). As more bacterial genomes are sequenced, the presence of additional LuxR homologs has become evident. Many of these do not have an associated synthase on their genome and are therefore referred to as orphan LuxR homologs (Table 2) (Fuqua, 2006). Their predicted protein sequences have the amino-terminal-binding domain and carboxy-terminal domain, typical of the LuxR family of proteins. Unlike synthase-associated LuxR proteins, orphan LuxR homologs do not directly control the synthesis of autoinducers, but can interact with them to expand the existing regulatory network of the bacterium. AHLs appear to be the most prevalent activating signal for orphan LuxR homologs, though other mechanisms of regulatory action such as heterodimer formation or activation by plant signals also exist (Oger *et al.*, 1998; Ledgham *et al.*, 2003; Ferluga *et al.*, 2007; Zhang *et al.*, 2007).

This review describes the current state of knowledge about functionally characterized orphan LuxR-type regulators. Here we define orphan LuxR regulators as those whose encoding gene is not associated with a synthase on the genome, which contain specific domain organizations (i.e. helix-turn-helix at the carboxy-terminus and signal-binding domain at the amino terminus), and whose predicted protein sequences do not contain any other functional domains. Because this pattern of domain

Table 1. Comparison of residues conserved among > 95% of LuxR-type proteins

	Conserved amino acid residues of $TraR_{A.\ tumefaciens}$								
	Autoinducer-binding domain						DNA-binding domain		
LuxR-type proteins	W_{57}	Y_{61}	D_{70}	P_{71}	W_{85}	G_{113}	E_{178}	L_{182}	G_{188}
Cognate LuxR homologs									
$LuxR_{V.\ fischeri}$	W	Y	D	P	W	G	E	L	G
$LasR_{P.\ aeruginosa}$	W	Y	D	P	W	G	E	L	G
$SinR_{S.\ meliloti}$	**A**	Y	D	P	W	G	E	L	G
$CinR_{R.\ etli}$	W	Y	D	P	W	*N*	E	L	G
$RhlR_{P.\ aeruginosa}$	W	Y	D	P	W	G	E	L	G
$Expr1_{Erwinia\ sp.}$	W	Y	D	P	W	G	E	L	G
$SmaR_{Serratia\ sp.}$	W	Y	D	P	W	G	E	L	G
Orphan LuxR homologs									
$TrlR_{A.\ tumefaciens}$	W	Y	D	P	W	G	E	S*	A*
$TrlR_{A.\ tumefaciens}$*	W	Y	D	P	W	G	E	L*	G*
$QscR_{P.\ aeruginosa}$	W	Y	D	P	W	G	E	L	G
$VirR/ExpR2_{Erwinia\ sp.}$	W	Y	D	P	W	G	E	L	G
$CarR_{Serratia\ sp.}$	**C**	Y	D	P	W	G	E	L	G
$CarR_{Erwinia\ sp.}$	W	Y	D	P	W	G	E	L	G
$BisR_{R.\ leguminosarum}$	W	Y	D	P	W	*N*	E	L	G
$SdiA_{E.\ coli}$	W	Y	D	P	W	G	E	L	G
$SdiA_{S.\ enterica}$	W	Y	D	P	W	G	E	L	G
$OryR_{X.\ oryzae}$	**M**	*W*	D	P	W	G	E	L	G
$XccR_{X.\ campestris}$	**M**	*W*	D	P	W	G	E	L	G
$NesR_{S.\ meliloti}$	**M**	*W*	D	P	W	G	E	L	G
$ExpR_{S.\ meliloti}$	W	Y	D	P	W	G	E	L	G
$SMc00878_{S.\ meliloti}$	W	Y	*S*	P	*F*	G	E	L	G
$AvhR_{A.\ vitis}$	W	Y	*S*	P	*Y*	G	E	L	G
$AviR_{A.\ vitis}$	W	Y	D	P	W	G	E	L	G
$VjbR_{B.\ melitensis}$	W	Y	D	P	**S**	**F**	E	L	G
$BlxR_{B.\ melitensis}$	W	Y	D	P	W	G	E	L	G
$BmaR4_{B.\ mallei}$	W	Y	D	P	W	G	E	L	G
$BpmR4_{B.\ pseudomallei}$	W	Y	D	P	W	G	E	L	G
$BtaR4_{B.\ thailandensis}$	W	Y	D	P	W	G	E	L	G
$BmaR5_{B.\ mallei}$	W	Y	D	P	**L**	G	E	L	G
$BpmR5_{B.\ pseudomallei}$	W	Y	D	P	**L**	G	E	L	G
$BtaR5_{B.\ thailandensis}$	W	Y	D	P	**L**	G	E	L	G
Putative-orphan LuxR homologs									
$VisN_{S.\ meliloti}$	**V**	*W*	*S*	**G**	**R**	G	E	L	G
$VisR_{S.\ meliloti}$	**C**	**A**	D	**E**	W	G	E	L	G
$SMc00877_{S.\ meliloti}$	**L**	Y	*S*	*T*	**V**	*A*	E	L	G
$SMc00658_{S.\ meliloti}$	**L**	Y	*S*	**E**	**G**	**H**	E	L	G
$VqsR_{P.\ aeruginosa}$	**L**	Y	**P**	**F**	**H**	**E**	E	L	G

*Frameshift mutation of TrlR when corrected restores appropriate conserved residues. Bold residues are substitutions of conserved residues, while italicized residues are substitutions with similar residues, with respect to $TraR_{A.\ tumefaciens}$. The proteins were aligned using the VECTOR NTI ADVANCE 10 (Invitrogen) software.

organization is common among transcriptional regulators, we have taken into consideration the extent of conservation to the nine amino acids considered critical during structure–function analysis of TraR of *A. tumefaciens* (Table 1) (Vannini *et al.*, 2002; Zhang *et al.*, 2002; Nasser & Reverchon, 2007). In this review, we discuss orphan LuxR-type proteins with a minimum number of substitutions ($\leq$ 2) in the nine conserved residues observed to be homologous among > 95% of previously characterized LuxR proteins (Table 1). We have excluded from further discussion putative-orphan LuxR-type proteins that exhibit a lower level of conservation (i.e. VisN, VisR, or VqsR) when compared with classical LuxR homologs (Table 1) (Sourjik *et al.*, 2000; Juhas *et al.*, 2005).

Below we discuss many of the well-characterized orphan LuxR regulators identified so far in various Gram-negative

Table 2. Orphan LuxR homologs in Gram-negative bacteria

Organism	Orphan LuxR homolog	Function	Related proteins or orthologs*	Cognate LuxR/I pair	Function	References
A. tumefaciens	TrlR	Inhibit conjugation of Ti plasmid	TraR of *Agrobacterium* sp.	TraR/TraI	Conjugation of Ti plasmid	Fuqua & Winans (1994), Oger *et al*. (1998)
P. aeruginosa	QscR	Inhibit premature activation of the *las*/*rhl* regulon, virulence factor production	QscR of *Pseudomonas* sp., *Burkholderia* sp. (Q2T5X2, Q62LJ8)	LasR/I	Virulence, biofilm	Chugani *et al*. (2001)
Erwinia sp.	VirR/ExpR2	Production of plant cell wall-degrading enzymes	ExpR of *Erwinia* sp.	ExpR/I	Enzyme production	Barnard & Salmond (2007)
	CarR	Antibiotic production	CarR of *Serratia* sp.			
Serratia sp. ATCC39006	CarR	Antibiotic production	CarR of *Erwinia* sp.	SmaR/I	Antibiotic and pigment production	Coulthurst *et al*. (2005)
R. leguminosarum bv. *viciae*	BisR	Symbiotic plasmid conjugation	CinR of *Rhizobium* sp., *Mesorhizobium* sp. (Q11IH2), *Rhodopseudomonas palustris* (Q11IH2)	CinR/I	Symbiotic plasmid acquisition, growth inhibition	Downie & González (2008), Lithgow *et al*. (2000), Rodelas *et al*. (1999), Wilkinson *et al*. (2002)
				TraR/I	Symbiotic plasmid conjugation	
				RhiR/I	Nodulation efficiency	
S. meliloti	ExpR	Exopolysaccharide production, repression of motility	*R. etli* (Q2K341), *A. tumefaciens* (Q7CWE0), *R. leguminosarum* (Q1MAB6)	SinR/I	Timing of plant nodulation	Downie & González (2008), Galibert *et al*. (2001), Marketon *et al*. (2002), Pellock *et al*. (2002), Patankar *et al*. (2009)
	NesR	Stress adaptation, competition for plant nodulation	*R. etli* (Q2K212), *P. syringae* (Q4ZNM6), *R. leguminosarum* (Q1M918)			
	SMc00878	Putative role in the denitrification pathway	*R. etli* (Q2KBW8, Q2KB21), *A. tumefaciens* (Q7D0V8), *R. leguminosarum* (Q1MKT8)			
A. vitis	AviR	Necrosis in grape, hypersensitive response in tobacco	*R. etli* (Q2K341), *A. tumefaciens* (Q7CWE0), *M. loti* (Q98BC5)	AvsR/I	Necrosis in grape, hypersensitive response in tobacco	Hao & Burr (2006), Hao *et al*. (2005); Zheng *et al*. (2003)
	AvhR		*R. leguminosarum* (Q1MKT8), *R. etli* (Q2KBW8), *A. tumefaciens* (Q7D0V8)			
S. enterica	SdiA	Resistance to host defenses	*Shigella* sp. (Q322M3, Q3Z2U0)	none	–	Ahmer (2004)
E. coli	SdiA	Transcription of cell division genes, resistance to antibiotics	*Shigella* sp. (Q322M3, Q3Z2U0, Q83R45)	none	–	Ahmer (2004)
X. campestris	XccR	Plant pathogenesis	*A. hydrophila* (P0A3J5), *R. etli* Q2K212, *P. syringae*	none	–	Zhang *et al*. (2007)

Table 2. Continued.

Organism	Orphan LuxR homolog	Function	Related proteins or orthologs*	Cognate LuxR/I pair	Function	References
			(Q4ZNM6), *R. leguminosarum* (Q1M918),			
X. oryzae	OryR	Plant pathogenesis	*A. hydrophila* (P0A3J5), *R. etli* (Q2K212), *P. syringae* (Q4ZNM6), *R. leguminosarum* (Q1M918)	none	–	Ferluga *et al*. (2007)
B. melitensis	VjbR	Virulence	*Brucella* sp., *S. meliloti* (Q92S02)	none	–	Delrue *et al*. (2005), Rambow-Larsen *et al*. (2008)
	BlxR	Virulence	*Brucella* sp., *Burkholderia* sp. (Q398E5)			
B. mallei	BmaR4, BmaR5	Virulence	*Burkholderia* sp., *P. putida* (Q8GEL8), *R. solanacearum* (Q8XRQ3)	BmaR/I1, BmaR/I3	Virulence	Ulrich *et al*. (2004b)
B. pseudomallei	BpmR4, BpmR5	Virulence	*Burkholderia* sp., *P. putida* (Q8GEL8), *R. solanacearum* (Q8XRQ3)	BmlR/I, BpmR/I2, BpmR/I3	Virulence	Ulrich *et al*. (2004a)
B. thailandensis	BtaR4, BtaR5	Virulence, metabolism	*Burkholderia* sp., *P. aeruginosa* (Q9RMS5), *R. solanacearum* (P58590, Q8XRQ3), *B. cepacia* (Q9AHP7)	BtaR/I1, BtaR/I2, BtaR/I3	Virulence	Ulrich *et al*. (2004c)

*UniProt accession numbers of relevant orthologs (in addition to the ones mentioned in the text) deduced from BLASTP analyses are indicated.

bacteria and their contributions to the regulatory circuits of those bacteria (Table 2).

TrlR of *A. tumefaciens*

Agrobacterium tumefaciens is a plant pathogen that causes crown gall tumors mediated by the virulence genes from its tumor inducing (Ti) plasmid. Transfer of the Ti plasmid by conjugation to other agrobacteria is critical for increasing the overall number of pathogenic bacteria. Conjugation of the Ti plasmid is controlled by quorum sensing in *A. tumefaciens* and the genes required for both plasmid transfer and for quorum sensing reside on the Ti plasmid. Conjugation is initiated by the plant tumor-produced compounds called octopines, which activate the transcriptional regulator, OccR, on the Ti plasmid. OccR, in turn, activates transcription of the quorum-sensing response regulator *traR* (Fuqua & Winans, 1996b). Moreover, the cognate autoinducer synthase, TraI, produces the signal 3O-C_8-HSL. This signal accumulates as the bacterial population increases and in conjunction with TraR, activates the transcription of genes required for conjugation (the *tra* and *trb* operons) (Fig. 1) (Fuqua & Winans, 1996a; Fuqua *et al*., 1996; Farrand *et al*., 2002). In addition to octopines, the plant tumor also produces mannopines, which serve mostly as a source of nutrition. Octopine-type Ti plasmids code for genes involved in the catabolism of mannopines in the *mot* (mannityl opine catabolism) operon. A gene of the *mot* operon called *trlR* (traR-like regulator) highly resembles the *traR* gene (Oger *et al*., 1998). The first 181 amino acids of TrlR and TraR show 88% identity, whereas the remaining region of 31 residues of TrlR lacks homology (Oger *et al*., 1998). DNA sequence analysis of *trlR* identified a frameshift mutation after the 542nd residue, and restoration of the mutation increases homology with *traR* to 90% (Oger *et al*., 1998). Because of the location of the mutation in the carboxy-terminus of the protein, TrlR cannot bind to DNA, but its amino-terminus can still bind AHLs. TrlR forms heterodimers with TraR and prevents its activity as a regulator, and this in turn inhibits conjugation (Chai *et al*., 2001). Mannopines activate expression of the *mot* operon, and though they were not initially considered to play a role

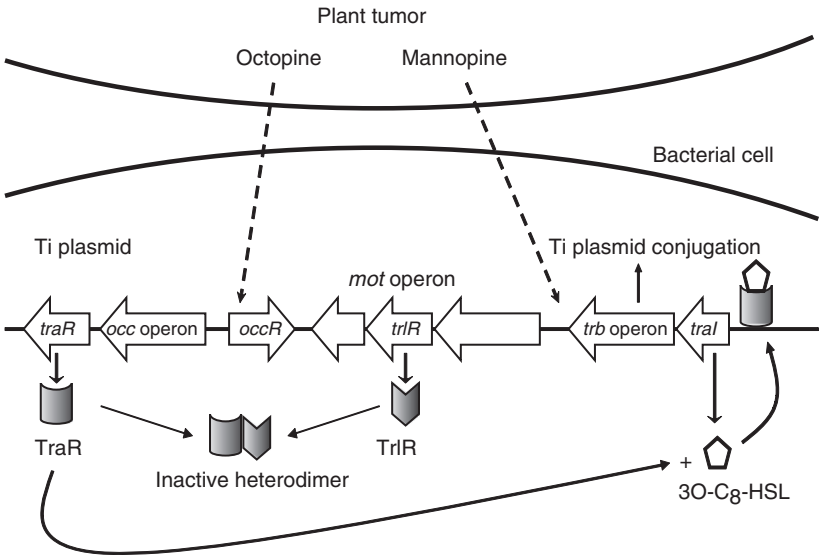

Fig. 1. TrlR of *Agrobacterium tumefaciens*. Conjugation of the Ti plasmid in *A. tumefaciens* is controlled by quorum sensing, where TraR and TraI serve as the cognate response regulator and synthase, respectively (Fuqua & Winans, 1994; Farrand *et al.*, 2002). Quorum sensing is activated by a plant tumor-produced compound called octopine, which initiates transcription of *traR* via OccR (Fuqua & Winans, 1996b). Mannopine, another compound secreted by the plant tumor, initiates transcription of the mannopine catabolism operon (*mot* operon), including the orphan LuxR, *trlR* of this operon (Oger *et al.*, 1998). In the absence of better sources of carbon other than mannopines, formation of heterodimers between TrlR and TraR prevents initiation of the energy-intensive process of conjugation (Chai *et al.*, 2001).

in conjugation, it is now evident that mannopines inhibit conjugation indirectly by controlling the expression of the TrlR inactivator (Chai *et al.*, 2001). When richer sources of carbon and energy are available, (i.e. succinate), mannopine catabolism is repressed, leading to reduced accumulation of TrlR and ensuring that the energy-expensive process of conjugation occurs only during nutritionally conducive conditions (Chai *et al.*, 2001).

QscR of *P. aeruginosa*

In the opportunistic pathogen *P. aeruginosa*, two LuxI-type proteins, LasI and RhlI, synthesize 3O-C_{12}-HSL and C_4-HSL, respectively. These AHLs bind to their cognate response regulators, LasR and RhlR, and regulate a number of virulence factors including the production of elastases, rhamnolipids, and biofilm formation (Chugani *et al.*, 2001; Ledgham *et al.*, 2003). In addition, *P. aeruginosa* has a third LuxR-type orphan quorum-sensing regulator, QscR (quorum sensing control repressor) for which no cognate LuxI-type gene has been identified (Fig. 2). Mutants of *qscR* are hypervirulent, express quorum-sensing-controlled genes early, and form blue colonies due to overproduction of the phenazine pigment (Chugani *et al.*, 2001). QscR has been shown to delay the expression of several quorum-sensing-controlled virulence factors such as phenazine and hydrogen peroxide by forming inactive heterodimers with LasR and RhlR (Ledgham *et al.*, 2003; Lequette *et al.*, 2006). Furthermore, QscR responds to the 3O-C_{12}-HSL produced by LasI and controls expression of genes independent of the LasR/I or RhlR/I systems (Lequette *et al.*, 2006). In the *P. aeruginosa* genome, *qscR* is flanked by the phenazine operon (*phz*) and the gene *PA1897*, which codes for a hypothetical protein. QscR activates transcription of *PA1897* specifically in response to 3O-C_{12}-HSL and represses the *phz* operon due to formation of inactive heterodimers with LasR.

The quorum-sensing systems of *P. aeruginosa* are hierarchical, where the *las* system controls the expression of the *rhl* system at the level of transcription. The *las* system also indirectly controls QscR, because the 3O-C_{12}-HSL produced by the *las* system is required by QscR to control gene expression (Lequette *et al.*, 2006). In addition to 3O-C_{12}-HSL, QscR can respond to other long chain AHLs such as 3O-C_{10}-HSL, C_{10}-HSL, and C_{12}-HSL, suggesting that it may respond to signals produced by other bacteria (Lee *et al.*, 2006). Thus QscR serves to expand the regulatory network of *P. aeruginosa* by utilizing the existing components of the resident quorum-sensing systems.

In *P. aeruginosa*, a fourth LuxR-like protein, VqsR, was characterized as the regulator of several quorum-sensing genes. Although this protein contains the DNA-binding domain of typical LuxR proteins, it lacks the conventional autoinducer-binding domain and does not fall within our definition of an orphan LuxR-type regulator (Table 1) (Juhas *et al.*, 2005; Schuster & Greenberg, 2006).

VirR/ExpR2 of *Erwinia* species

Bacteria of the *Erwinia* sp. are Gram-negative plant pathogens that cause soft rot disease in their hosts (Barnard & Salmond, 2007). Quorum-sensing-based regulation of virulence has been identified in several species of *Erwinia*, but only strains that code for orphan LuxR homologs are discussed below. A detailed review of quorum sensing in *Erwinia* is described elsewhere (von Bodman *et al.*, 2003a; Barnard & Salmond, 2007). The strains that code for cognate autoinducer-response regulator pairs include *Erwinia carotovora* ssp. *carotovora* (*Ecc*) strain SCC3193

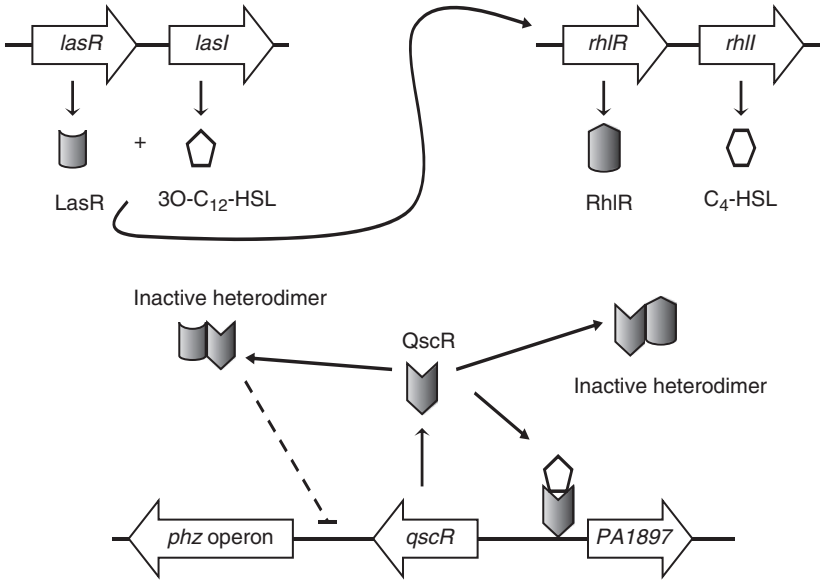

Fig. 2. QscR of *Pseudomonas aeruginosa*. In the hierarchy of the quorum-sensing systems of *P. aeruginosa*, the *las* system controls the *rhl* and *qscR* systems. At low population densities, in the absence of accumulated *lasI*-produced autoinducer (3O-C_{12}-HSL), QscR, an orphan LuxR, forms heterodimers with LasR and RhlR to prevent the premature activation of their regulons (Chugani *et al.*, 2001; Ledgham *et al.*, 2003). For example, the phenazine biosynthetic operon (*phz*) is a part of the *las* regulon but is inhibited by QscR heterodimers at low population densities. As the population density increases, inhibition is relieved as QscR is released from the heterodimers, making LasR available for activation. Concurrently, QscR binds to 3O-C_{12}-HSL from the *las* system and regulates its independent regulon (*PA1897*) (Lequette *et al.*, 2006).

(*expI*/*expR1*), *Ecc* strain ATCC39048 (*carI*/*expR*), *Ecc* strain SCR1193 (*expI*/*expR*), *Ecc* strain 71 (*ahlI*/*expR*), and *E. carotovora* ssp. *atroseptica* (*Eca*) strain SCR11043 (*expI*/*expR*) (von Bodman *et al.*, 2003a; Chatterjee *et al.*, 2005; Burr *et al.*, 2006). All five strains carry an orphan LuxR homolog termed VirR in *Ecc*, *Ecc* strain ATCC39048 and in *Eca* strain SCR11043 or ExpR2 in *Ecc* strain SCC3193 and in *Ecc* strain 71 (Chatterjee *et al.*, 2005; Burr *et al.*, 2006; Sjöblom *et al.*, 2006). Strains of *Erwinia* also differ in the type of AHLs they produce. *Ecc* strain SCC3193 predominately synthesizes 3O-C_8-HSL and minor amounts of 3O-C_6-HSL (Chatterjee *et al.*, 2005). On the other hand, *Ecc* ATCC39048, *Ecc* strain SCR1193, *Ecc* strain 71, and *Eca* SCR11043 make only 3O-C_6-HSL (Chatterjee *et al.*, 2005). Mutants of the AHL synthase exhibit reduced expression of virulence factors such as the plant wall-degrading exoenzymes, and increasing evidence indicates that regulation of the virulence factors is mediated by the orphan LuxR homologs in conjunction with the AHLs (Barnard & Salmond, 2007). Unlike most quorum-sensing systems, where binding of an autoinducer to a response regulator leads to activation of transcription, in the *Erwinia* sp., binding of the 3O-C_8-HSL or 3O-C_6-HSL autoinducers to the VirR/ExpR2 regulators relieves repression of transcription. At low population densities (i.e. in the absence of AHLs), VirR/ExpR2 activates the transcription of the global repressor *rsmA* (Chatterjee *et al.*, 2005; Sjöblom *et al.*, 2006). RsmA in turn represses exoenzyme production. As the population density increases and AHLs accumulate, the binding of VirR/ExpR2 to the *rsmA* promoter is reduced (Chatterjee *et al.*, 2005). Production of virulence factors in *Erwinia* is also responsive to plant cell wall products; therefore, in order to mount an effective attack, synthesis of exoenzymes is co-ordinated with the presence of a suitable host and a sufficient number of bacteria (Barnard & Salmond, 2007).

CarR of *Erwinia* and *Serratia* species

Carbapenems are members of the β-lactam family of antibiotics (Coulthurst *et al.*, 2005). They inhibit the cross-linking of peptidoglycan and are active against both Gram-positive and Gram-negative bacteria. Strains of the Gram-negative plant pathogen *Ecc* ATCC39048 and the opportunistic pathogen *Serratia* sp. strain ATCC39006, produce the carbapenem antibiotic 'Car' (1-carbapen-2-em-3-carboxylic acid) (Coulthurst *et al.*, 2005). In both strains, antibiotic production is controlled by an orphan LuxR homolog, CarR, which lies upstream of the *carA-H* operon and activates the operon in a population density-dependent manner (Fig. 3). CarA-E produces the antibiotic, whereas CarF and CarG are required for generating intrinsic resistance to the antibiotic, while the function of CarH is unknown (Coulthurst *et al.*, 2005). In *Ecc* ATCC39048, CarR activates transcription of the *car* operon by binding to 3O-C_6-HSL and activating carbapenem production. The product of *carI* results in the synthesis of the AHL, but its cognate regulator ExpR (see preceding section) is not involved in the production of the antibiotic (Coulthurst *et al.*, 2005; Barnard & Salmond, 2007).

In *Serratia* sp. ATCC39006, although the *carR* and *carA-H* operon are highly homologous to that of *Ecc*, its mode of regulation differs. Here, both genes of the *smaI/R* locus are involved in antibiotic production. SmaR inhibits the transcription of *carR* in the absence of AHLs (Fig. 3). As the population density increases, accumulation of C_4-HSL produced by the *smaI* gene relieves this repression by

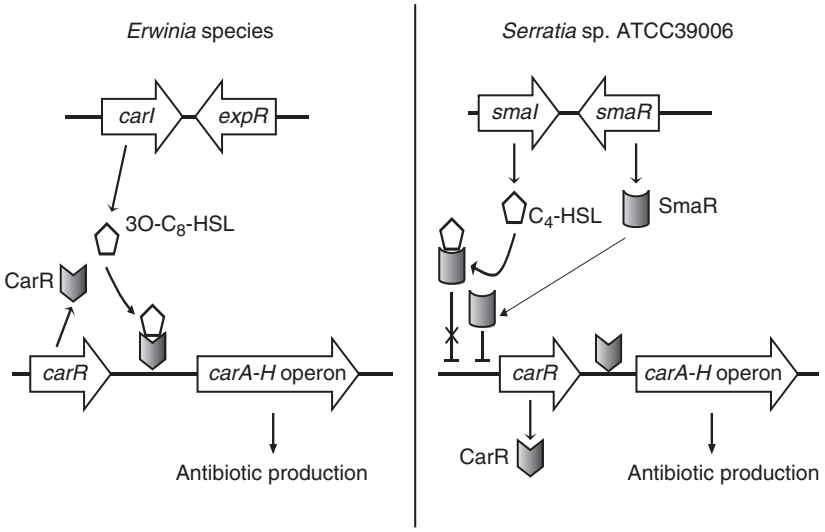

Fig. 3. CarR of *Erwinia* and *Serratia* species. Carbapenem antibiotic production is under the control of the orphan LuxR CarR that regulates the *carA-H* antibiotic biosynthetic operon. In the *Erwinia* sp., CarR interacts with 3O-C_6-HSL produced by the cognate pair of *carI/expR* and binds upstream of the *car* operon to activate expression. However, in the *Serratia* sp., CarR binds to DNA independent of autoinducers. At low population densities SmaR from the cognate SmaI/R pair binds upstream of *carR* to repress its transcription. As population density increases, C_4-HSL produced by SmaI disassociates SmaR, releasing *carR* expression (Coulthurst *et al.*, 2005; Barnard & Salmond, 2007).

sequestering SmaR. Transcription of liberated *carR* thus allows for antibiotic production (Thomson *et al.*, 2000). Uniquely, CarR in *Serratia* activates transcription in an autoinducer-independent manner. The role of the AHL in this case is to remove the repression exerted by SmaR on the expression of CarR (Cox *et al.*, 1998; Slater *et al.*, 2003).

Antibiotic production in both organisms is responsive to environmental nutritional cues. Carbon sources like glycerol inhibit expression of *carI* in *Ecc*, and phosphate-limiting conditions activate transcription of *smaI* in *Serratia* (Slater *et al.*, 2003; Coulthurst *et al.*, 2005). The role of the Car antibiotic production in bacteria is not well understood, but it probably serves to defend against neighboring organisms. It is speculated that because quorum sensing in *Ecc* also controls production of plant cell wall-degrading enzymes, which leads to the creation of a nutrition-rich environment, synchronizing antibiotic production at the same time could help stave off other competing bacteria (Coulthurst *et al.*, 2005).

BisR of *Rhizobium leguminosarum*

Rhizobium leguminosarum bv. *viciae* forms a symbiotic association with pea, lentils, and field bean plants, and most of the genes required for this association reside on the plasmid pRL1JI (Downie & González, 2008). In *R. leguminosarum*, conjugation of this symbiotic plasmid is dependent on its multiple quorum-sensing systems. The chromosomally located *cinR/I* locus is at the top of an intricate regulatory cascade, where CinI synthesizes 3OH-$C_{14:1}$-HSL, and CinR, in response to this AHL, positively regulates *cinI* (Lithgow *et al.*, 2000; Downie & González, 2008). The pRL1JI plasmid carries two quorum-sensing systems. RhiI is involved in the production of C_6, C_7, and C_8-HSL, which in conjunction with RhiR regulates genes involved in the rhizosphere interaction of *R. leguminosarum* with pea plants (Rodelas *et al.*, 1999). The second system on the plasmid, the Tra system, resembles the conjugation-controlling systems found in several plant-associated rhizobia and agrobacteria (Fig. 4) (Danino *et al.*, 2003; Downie & González, 2008). The Tra locus consists of *traI*, which codes for the autoinducer 3O-C_8-HSL, the *trb* operon, which is required for plasmid transfer, and two LuxR homologs, BisR and TraR (Wilkinson *et al.*, 2002). The cognate regulator of TraI is TraR, which, along with 3O-C_8-HSL, activates the transcription of *traI* and the *trb* operon, thereby increasing plasmid transfer frequencies (Wilkinson *et al.*, 2002; Danino *et al.*, 2003). BisR is an orphan LuxR-type protein, which is highly homologous to CinR (59%) (Wilkinson *et al.*, 2002). BisR regulates the expression of *traR* upon sensing 3OH-$C_{14:1}$-HSL, and mutants of *bisR* or *traR* have reduced plasmid transfer frequencies (Fig. 4) (Wilkinson *et al.*, 2002). Paradoxically, BisR also represses production of 3OH-$C_{14:1}$-HSL by binding to the promoter of *cinI* (McAnulla *et al.*, 2007). BisR serves a unique role in *R. leguminosarum* in that it controls recipient-induced plasmid transfer. In recipient strains that lack the pRL1JI plasmid, the chromosomal *cinR/I* locus produces and accumulates 3OH-$C_{14:1}$-HSL. Donor strains containing the plasmid do not produce 3OH-$C_{14:1}$-HSL because plasmid-encoded BisR represses *cinI*. In response to the 3OH-$C_{14:1}$-HSL produced by the recipient strains, BisR activates transcription of *traR*, which eventually induces conjugation by activating the *traI-trb* operon in response to population density (Danino *et al.*, 2003). Therefore, in the absence of 3OH-$C_{14:1}$-HSL, BisR acts as a repressor of *cinI*, and in its presence acts as an activator of *traR*.

SdiA of *Salmonella enterica* and *Escherichia coli*

Salmonella enterica sv. Typhimurium and *E. coli* code for only one LuxR-type response regulator, SdiA, and have no

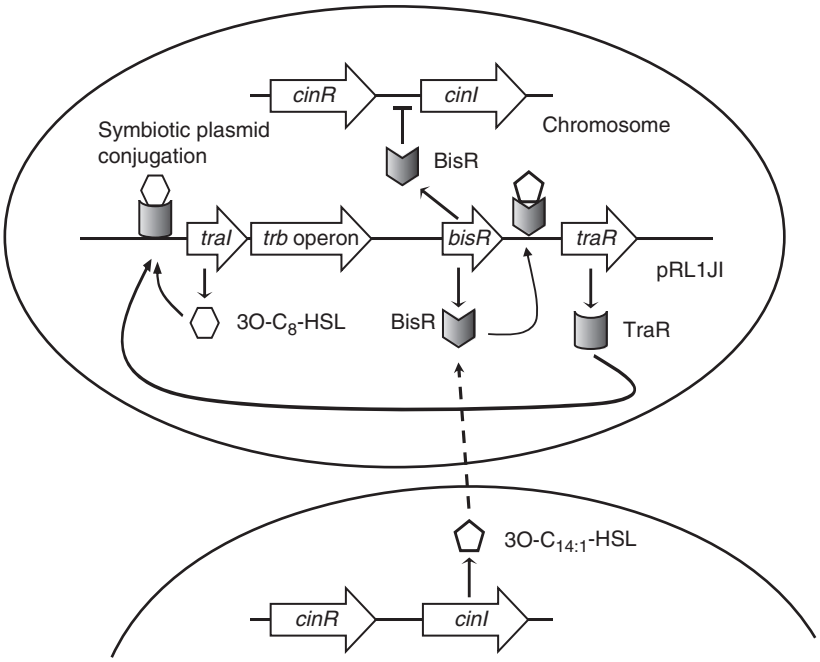

Fig. 4. BisR of *Rhizobium leguminosarum*. In *R. leguminosarum* transfer of the symbiotic plasmid pRL1JI is controlled by quorum sensing. In cells that have the plasmid, BisR, an orphan LuxR homolog, represses expression of the chromosomal *cinI*. In cells that lack the plasmid, the functional *cinR/I* produce and regulate 3O-$C_{14:1}$-HSL. BisR is responsive to 3O-$C_{14:1}$ produced by other cells. Binding of the autoinducer–BisR complex to the *traR* promoter activates production of TraR, which, in conjunction with 3O-C_8-HSL, activates the *trb* operon required for conjugation (Danino *et al*., 2003; McAnulla *et al*., 2007; Downie & González, 2008).

known autoinducer synthase (Ahmer, 2004). The role of SdiA in *S. enterica* is better characterized, where it has been shown to regulate the *rck* (resistance to complement killing) operon and *srgE* (*sdiA*-regulated gene), a gene of unknown function (Ahmer *et al*., 1998). The *rck* operon resides on a virulence plasmid and has been shown to play a role in bacterial binding to extracellular matrix proteins and epithelial cells, and also in the avoidance of the host complement responses (Ahmer *et al*., 1998; Ahmer, 2004). Testing for the presence of autoinducers using supernatants of cultures to activate biosensors or SdiA-regulated genes have so far yielded no candidates (Ahmer, 2004). However, in response to either synthetic AHLs or AHLs from other bacteria, SdiA can regulate both *rck* and *srgE*, suggesting that SdiA probably responds to external stimuli (Michael *et al*., 2001; Ahmer, 2004). SdiA recognizes 3O-C_8-HSL and 3O-C_6-HSL in the physiologically relevant concentrations of 1–5 nM (Michael *et al*., 2001).

The role of SdiA in *E. coli* is not well understood, especially because *E. coli* does not possess the *rck* operon or the *srgE* gene. In *E. coli*, *sdiA* was identified in screens for genes which, when expressed on plasmids, could bypass inhibition of cell division (Wang *et al*., 1991). SdiA stands for suppression of division of inhibition, and in response to AHLs activates transcription of the *ftsQAZ* operon involved in cell division (Wang *et al*., 1991; Sitnikov *et al*., 1996). SdiA in *E. coli* has also been shown to confer resistance to antibiotics like mitomycin C and quinolones, and repress expression of motility and chemotaxis genes (Wei *et al*., 2001; Rahmati *et al*., 2002). *Salmonella enterica* and *E. coli* are enteropathogens; therefore, their perception of autoinducers from surrounding bacteria could serve to signal their arrival to the appropriate host environment (Ahmer, 2004; Hughes & Sperandio, 2008). AHLs have been detected in the rumen content of cattle, though they have not yet been characterized from human gut bacteria (Erickson *et al*., 2002). Another role of SdiA in *E. coli* has been established in biofilm formation. In response to the extracellular interspecies signal indole, SdiA was shown to decrease biofilm formation and this effect was more pronounced at 30 °C than at 37 °C (Lee *et al*., 2007, 2008).

XccR of *Xanthomonas campestris* and OryR of *Xanthomonas oryzae*

Virulence-factor production in the plant pathogens *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae* is controlled by quorum sensing. These species do not produce any AHLs as autoinducers and instead use the signal factors DSF and DF for gene regulation (Wang *et al*., 2004). Though their genomes do not encode any AHL synthases, different *Xanthomonas* species code for LuxR-type proteins. Recently two such orphan LuxR homologs, XccR and OryR, were characterized in *X. campestris* pv. *campestris* and *X. oryzae* pv. o*ryzae*, respectively (Ferluga *et al*., 2007; Zhang *et al*., 2007). Both XccR and OryR are homologous to each other and are required for virulence in their respective host plants. Fusions of *xccR* did not respond to synthetic AHLs, and overexpressed OryR protein did not solubilize in the presence of synthetic AHLs, indicating that the genes are not

activated by AHLs and that their functional proteins do not bind AHLs. Interestingly, host–plant exudates activate expression of *xccR* and *oryR*, indicating that these genes could serve in modulating interkingdom communication. The *xccR* locus in *X. campestris* is flanked by a *pip* (proline iminopeptidase) gene. Pip is widely distributed in bacteria and catalyzes the removal of N-terminal proline residues from peptides, but its biological function is largely unclear (Sarid *et al.*, 1959; Medrano *et al.*, 1998). In *X. campestris* it is now evident that XccR in conjunction with plant exudates controls expression of *pip*, and that along with *xccR* the *pip* locus is also required for virulence (Zhang *et al.*, 2007). The pattern of a *luxR*-type gene flanking a *pip* gene is observed in several plant-associated species of rhizobia and pseudomonads (Zhang *et al.*, 2007).

Orphan LuxR homologs of *S. meliloti*

Sinorhizobium meliloti exists as a free-living soil bacterium or in a symbiotic association with alfalfa, its leguminous plant host. In *S. meliloti*, the *sinR/I* locus is involved in the production and regulation of a range of AHLs (C_{12}-HSL, C_{14}-HSL, 3O-C_{14}-HSL, $C_{16:1}$-HSL, 3O-$C_{16:1}$-HSL, and C_{18}-HSL) (Fig. 5) (Marketon *et al.*, 2002; González & Marketon, 2003; Teplitski *et al.*, 2003). Mutations in the Sin system result in impaired nodulation of host plants (Marketon *et al.*, 2002). In addition to the *sinR/I* genes, *S. meliloti* strain 8530 has an orphan LuxR-type response regulator called ExpR (Pellock *et al.*, 2002). The ExpR regulator was shown to control genes in the production of the symbiotically important exopolysaccharide EPS II (Pellock *et al.*, 2002; Marketon *et al.*, 2003). Furthermore, microarray studies have shown that together, SinR/I and ExpR control a myriad of genes involved in motility, chemotaxis, and low-molecular weight succinoglycan production, another symbiotically relevant exopolysaccharide produced by *S. meliloti* (Hoang *et al.*, 2004, 2008; Glenn *et al.*, 2007). Interestingly, the control of motility by ExpR is mediated through two LuxR-type proteins called VisN and VisR (Sourjik *et al.*, 2000; Bahlawane *et al.*, 2008; Hoang *et al.*, 2008). Both proteins are believed to form heterodimers for their functional activity. Although both proteins are considered to belong to the LuxR family of proteins, their autoinducer-binding domains are highly variable compared with typical LuxR proteins indicating that they may operate using a novel mechanism of action (Table 1) (Sourjik *et al.*, 2000). At low population densities, VisN/VisR are global gene activators of flagellar, motor, and chemotaxis genes (Sourjik *et al.*, 2000; Hoang *et al.*, 2008). Recently, Bahlawane *et al.* (2008) demonstrated a cascade of regulation, where at high population density, ExpR in conjunction with AHL binds to the promoter of *visN*, thereby inhibiting expression of motility and chemotaxis.

In addition to the above described response regulators, the sequenced *S. meliloti* 1021 genome indicated the presence of other LuxR homologs (*SMc04032*, *SMc00878*, *SMc00877*, and *SMc00658*), which shared the highest level of homology to the classical *V. fischeri* LuxR protein and to the *S. meliloti* SinR and ExpR proteins (Galibert *et al.*, 2001). The predicted protein sequences of these regulators contain the signature response regulatory domain at the amino-terminus and DNA-binding helix-turn-helix domain at the carboxy terminus and they are not associated with a synthase on the genome (Galibert *et al.*, 2001). SMc00877

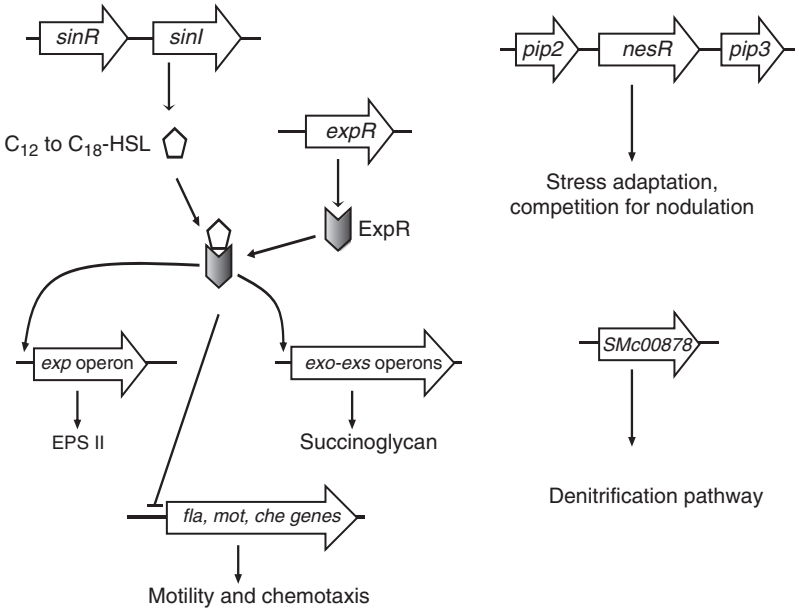

Fig. 5. Orphan LuxR homologs of *Sinorhizobium meliloti*. The SinR/I system of *S. meliloti* generates long-chain AHLs (C_{12} to C_{18}-HSL) that bind the orphan quorum-sensing regulator, ExpR, to activate expression of the symbiotically important exopolysaccharides (succinoglycan and EPS II) and repress genes involved in motility and chemotaxis via VisN/VisR (Sourjik *et al.*, 2000; Marketon *et al.*, 2002, 2003; Pellock *et al.*, 2002; Hoang *et al.*, 2004, 2008; Glenn *et al.*, 2007; Bahlawane *et al.*, 2008). In addition, *S. meliloti* has genes that code for other orphan LuxR proteins. The regulatory role of *nesR* (*SMc04032*) was determined to affect stress adaptation and competition for nodulation, whereas *SMc00878* controls the transcription of genes from the denitrification pathway of *S. meliloti*. The *nesR* (*SMc04032*) gene is flanked by two proline iminopeptidase genes (*pip2* and *pip3*) (Galibert *et al.*, 2001). Association of PIP with orphan-LuxR proteins is observed in several plant-related bacteria, including *Xanthomonas* species, where it has been shown to control plant virulence (Zhang *et al.*, 2007).

and SMc00658 contain several modifications within their autoinducer-binding domains (with respect to conserved residues) and were therefore grouped with 'putative-orphan' LuxR homologs (Table 1). Through expression and phenotypic analysis, it was deduced that the *SMc04032* (*nesR*) locus is involved in stress adaptation and competition for nodulation (Fig. 5) (A. Patankar & J. González, in press). The functional processes affected by *SMc00878* mostly fall within the denitrification pathway of *S. meliloti* (Fig. 5) (A. Patankar & J. González, unpublished data). The known AHLs of *S. meliloti* (Sin AHLs) do not serve as the effector molecules for these additional orphan LuxR homologs. The cell-signaling mechanism involved in activating these orphan LuxR homologs in *S. meliloti* remains to be identified. Interestingly, the SMc04032 (NesR) predicted protein sequence is highly homologous to the plant signal-activated XccR and OryR of *Xanthomonas*, while SMc00878 and SMc00877 are highly homologous to AviR of *Agrobacterium vitis* (Zheng *et al*., 2003; Ferluga *et al*., 2007; Zhang *et al*., 2007). Moreover, analyses of bacterial genomes from the *Rhizobiaceae* family reveals that the abundance of orphan LuxR-type proteins is conserved in most of its members (Case *et al*., 2008; Crossman *et al*., 2008). *Rhizobium etli* and *R. leguminosarum* are predicted to code for 11 and nine orphan LuxR homologs, respectively, and most of these are orthologs of the *S. meliloti* orphan LuxR proteins (Table 2) (Crossman *et al*., 2008).

AviR and AvhR of *A. vitis*

Agrobacterium vitis causes crown gall disease and necrosis in grape plants and induces a hypersensitive-like response (HR) in nonhost plants like tobacco (Burr & Otten, 1999; Zheng *et al*., 2003). *Agrobacterium vitis* contains a cognate *avsR/I* locus, which is involved in the production of long-chain AHLs and induction of tobacco HR and grape necrosis (Hao & Burr, 2006). The *avsR/I* locus is most homologous to the *sinR/I* locus of *S. meliloti*. Both loci are involved in the production and regulation of AHLs. In addition, two orphan LuxR regulators, AviR and AvhR, were identified in *A. vitis* by Tn*5* mutagenesis. Mutants of AviR are completely defective in necrosis and HR and produce fewer long-chain AHLs (Zheng *et al*., 2003). Interestingly, AviR is highly homologous to the well-characterized orphan LuxR homolog ExpR of *S. meliloti* and a putative LuxR homolog (AGR-c-4942) of *A. tumefaciens* C58 (Zheng *et al*., 2003). AviR is involved in the production of AHLs, though unlike its homolog in *S. meliloti* (ExpR), it is not involved in the production of exopolysaccharides in *A. vitis*. Mutants of AvhR cause partial necrosis of grape plants, induce HR, and are not involved in the production of AHLs (Hao *et al*., 2005). AvhR is also highly homologous to the orphan LuxR homologs of *S. meliloti* (SMc00878 and SMc00877) and *A. tumefaciens* C58 (AGR-c-1279) (Hao *et al*., 2005).

VjbR and BlxR of *Brucella melitensis*

Brucella melitensis is a Gram-negative facultative intracellular pathogen and the causative agent of the zoonotic disease brucellosis (Corbel, 1997). Brucellosis affects both humans and economically important livestock and is easily spread through aerosols (Corbel, 1997). Pathogenesis is established by the ability of the bacteria to survive and replicate within phagocytic and nonphagocytic host cells (Corbel, 1997). The type IV secretion system (coded by the *virB* operon) and the ability to produce flagella are critical for virulence, and recently these phenotypes were shown to be regulated by quorum sensing (Delrue *et al*., 2004). A quorum-sensing signal, C_{12}-HSL, has been identified in spent culture supernatants of *B. melitensis*, though the identity of the gene involved in its production is not yet known (Taminiau *et al*., 2002). The C_{12}-HSL autoinducer has been shown to repress the expression of the *virB* operon (Taminiau *et al*., 2002). In contrast, the orphan LuxR homolog, VjbR, was shown to activate expression of the *virB* operon and the flagellar genes (Delrue *et al*., 2005). Delrue *et al*. (2005) demonstrated that the effect of C_{12}-HSL on virulence determinants was mediated by inhibition of VjbR activity. In addition, VjbR also regulates exopolysaccharide production and outer-membrane proteins, both of which could be required for host–bacterial interactions (Uzureau *et al*., 2007). The genomic organization of *vjbR* flanking flagella genes mirrors the organization of the *luxR*-like *visN/visR* from *S. meliloti*, and its regulation of flagellar genes seems to parallel that of *S. meliloti* as well (Sourjik *et al*., 2000; Delrue *et al*., 2005). In *B. melitensis*, VjbR activates expression of flagellar genes via the two-component response regulator, FtcR (Leonard *et al*., 2007). Orthologs of these proteins conduct similar functions in *S. meliloti*, where VisN/VisR regulate flagella biosynthesis through the response regulator, Rem (Rotter *et al*., 2006). Mutants of VjbR cause an attenuation of virulence in animal models, and this property has been used in the development of an effective vaccine treatment (Arenas-Gamboa *et al*., 2008).

The role of a second orphan LuxR homolog, BlxR, in modulating virulence was recently characterized in *B. melitensis* (Rambow-Larsen *et al*., 2008). Analogous to VjbR, BlxR was required for regulation of the type IV secretion system and flagellar gene expression (Rambow-Larsen *et al*., 2008). Moreover, microarray analysis revealed other regulatory targets of BlxR, which included the LysR family of transcriptional regulators and cell envelope proteins (Rambow-Larsen *et al*., 2008). In addition to the overlap of regulatory control, BlxR and VjbR cross regulate transcription of each, suggesting a convergence of

quorum-sensing regulatory pathways in *B. melitensis* (Rambow-Larsen *et al.*, 2008). Homologs of both VjbR and BlxR exist in other *Brucella* species as well as in other proteobacterial species (Table 2) (Delrue *et al.*, 2005; Rambow-Larsen *et al.*, 2008).

Orphan LuxR homologs of *Burkholderia* species

Bacteria of the genus *Burkholderia* are Gram-negative opportunistic human pathogens (Eberl, 2006). Quorum sensing has been implicated in pathogenesis due to its role in the production of exoenzymes (Eberl, 2006). Strains of *Burkholderia mallei* contain two, whereas *Burkholderia pseudomallei* and *Burkholderia thailandensis* contain three pairs of cognate LuxR/I homologs that are involved in production of AHLs ranging from C_8 to C_{14}-HSL (Table 2) (Ulrich *et al.*, 2004a, b, c). Additionally, each of these strains contains two orphan response regulators, *bmaR4* and *bmaR5* in *B. mallei*, *bpmR4* and *bpmR5* in *B. pseudomallei*, and *btaR4* and *btaR5* in *B. thailandensis* (Ulrich *et al.*, 2004a, b, c). The role of these orphan regulators has not been extensively characterized, but it was observed that they seem to affect different processes in different strains. In *B. mallei* and *B. pseudomallei*, mutants of *bmaR5* and *bpmR5* exhibit reduced virulence in animal models (Ulrich *et al.*, 2004a, b). In *B. thailandensis*, mutations in *btaR5* affect virulence factor (lipase) production and metabolism of various carbon sources (e.g. arabinose, glucose 6-phosphate) (Ulrich *et al.*, 2004c).

Evolutionary perspectives

In natural habitats, bacteria exist as members of communities that interact with each other. Communication via quorum sensing within these communities modulates the behavior of an entire population, thus imparting a multicellularity character to unicellular organisms. The co-ordination and regulation of gene expression is viewed as an evolutionary adaptation to survive in a changing environment (Cases *et al.*, 2003). Bacteria exposed to variable niches, such as soil or aquatic environments, typically have larger genomes and devote more of their genes to regulation (*P. aeruginosa*, genome size = 6.3 Mb; *S. meliloti*, genome size = 6.6 Mb) (Galibert *et al.*, 2001; Winsor *et al.*, 2005). On the other hand, intracellular pathogens that face more stable environments have much smaller genomes with only a small proportion of genes devoted to regulation (*Rickettsia* sp., genome size = 1 Mb) (Cases & de Lorenzo, 2005). The pressures exerted by a constantly changing environment select for genes that equip the bacteria with a repertoire of appropriate responses.

Two main mechanisms exist for the expansion of transcriptional networks. The first consists of internal reorganization of genes primarily by duplication and then divergence (Cases & de Lorenzo, 2005). The second method includes horizontal gene transfer from the large selection that is available in their diverse niches (McAdams *et al.*, 2004). Additionally, transcriptional regulatory circuits are believed to evolve independently from the gene or operons that they will ultimately control (Cases & de Lorenzo, 2005). A combination of these and other events probably occurred in the evolution of orphan LuxR homologs (Fig. 6). Though *trlR* is located in the *mot* operon, it is not homologous to any of the other genes in the operon; instead, it is highly homologous to *traR* (Oger *et al.*, 1998). If the point mutation of TrlR is rescued, its homology to TraR is 90%, indicating that *trlR* probably arose from duplication and then divergence from *traR* (Oger *et al.*, 1998). The high homology between TrlR and TraR forms the basis of heterodimer formation, which prevents regulatory activity of TraR (Fig. 6) (Chai *et al.*, 2001). Phylogenetic studies also support the idea that TrlR was the result of gene duplication (Gray & Garey, 2001). In *R. leguminosarum* bv. *viciae*, BisR is 59% homologous to CinR and both proteins are not more than 30% homologous to other LuxR-type proteins, implying that BisR was probably a result of a duplication of CinR, after which its sequence diverged, an idea that is supported by phylogenetic analysis (Fig. 6) (Gray & Garey, 2001; Wilkinson *et al.*, 2002; Case *et al.*, 2008). Additionally, CinR and BisR both bind to 3O-$C_{14:1}$-HSL to regulate expression of genes, another factor suggestive of common ancestry (Wilkinson *et al.*, 2002). Given the high homology between the orphan LuxR homologs of *S. meliloti*, *A. vitis*, and putative LuxR homologs of *A. tumefaciens* C58, it seems likely that they came from common ancestors and then diversified in each bacteria to control different phenotypes such as exopolysaccharide production by ExpR in *S. meliloti* or necrosis and hypersensitivity induction by AviR in *A. vitis* (Fig. 6) (Table 2) (Pellock *et al.*, 2002; Marketon *et al.*, 2003; Hao *et al.*, 2005). Sequence comparisons indicate that the plethora of orphan LuxR homologs in these strains is also present in other members of *Rhizobiaceae*, indicating that they too were perhaps initially acquired from common ancestors (Case *et al.*, 2008; Crossman *et al.*, 2008).

In *E. carotovora* ssp. *carotovora* (*Ecc*), CarR is involved in antibiotic production and is linked to the antibiotic biosynthetic operon. Though both ExpR and CarR of *Ecc* bind to 3O-C_6-HSL made by CarI, they have different roles within the bacteria. The cognate regulator ExpR is maintained for its normal role in regulation whereas the ability to produce antibiotic served as a strong selective pressure to maintain the *car* genes (Lerat & Moran, 2004). Thus, CarR of *Ecc* was effectively integrated into the regulatory circuit of *Erwinia* by utilizing a pre-existing signal to modulate newer beneficial regulons. Phylogenetic analysis suggests that the CarR of *Ecc* protein is more homologous to CarR of *Serratia* sp. than to other LuxR-type proteins from *Erwinia* sp.

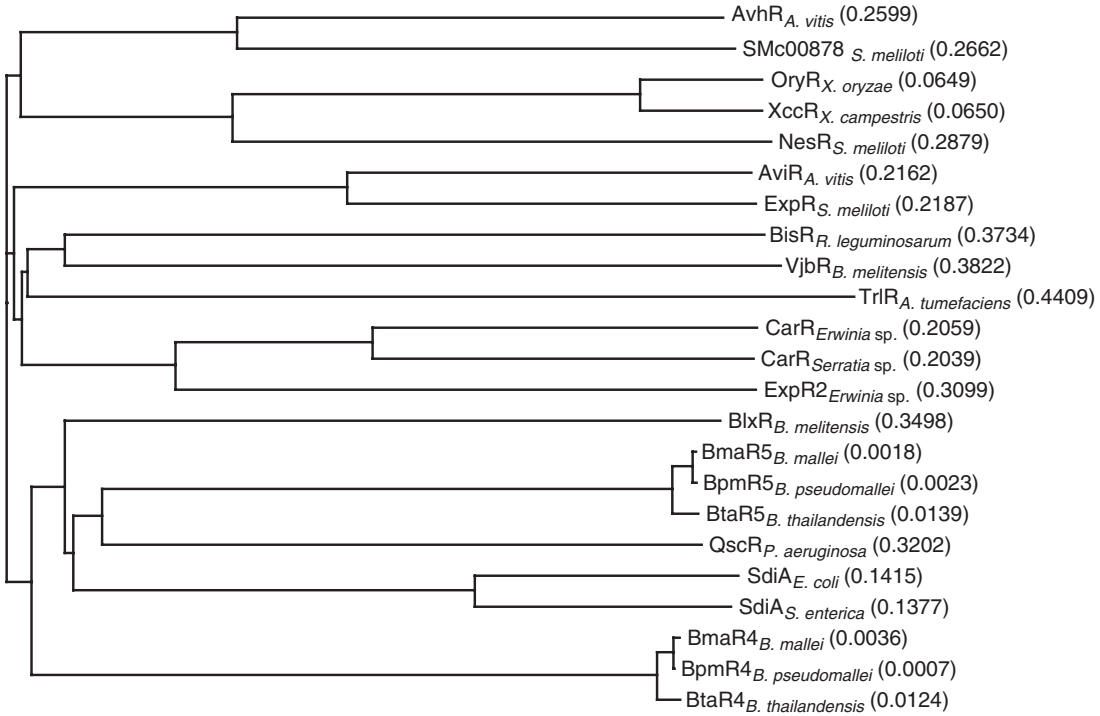

Fig. 6. Phylogenetic tree of orphan LuxR homologs. Protein sequences were aligned using the neighbor-joining method with the VECTOR NTI ALIGNX program (Invitrogen). The calculated distances, related to the degree of divergence between the sequences, are indicated in parenthesis.

indicating that horizontal gene transfer may have occurred between *Erwinia* and *Serratia* sp. (Fig. 6) (Gray & Garey, 2001; Lerat & Moran, 2004). Interestingly, phylogenetic studies show that SdiA of *S. enterica* and *E. coli* grouped with the RhlR sequences of *P. aeruginosa* instead of enterobacterial homologs, suggesting a horizontal gene transfer from *Pseudomonas* into these bacteria (Gray & Garey, 2001). Moreover, even though they belong to different classes of *Proteobacteria*, the orphan LuxR homologs of *Burkholderia* species also group with *Pseudomonas*, indicating evolution through horizontal gene transfer (Fig. 6) (Case *et al.*, 2008).

Though LuxI- and LuxR-type proteins are related by function, they belong to distinct protein families that probably coevolved (Gray & Garey, 2001). In phylogenetic genome comparisons of 68 *Proteobacteria*, 45 bacteria contained orphan LuxR homologs but no additional LuxI homologs (Case *et al.*, 2008). Several factors can justify the increased prevalence of orphan LuxR regulators in quorum-sensing systems of bacteria. These regulators can utilize the existing quorum-sensing signal in the bacteria and alleviate the cost associated with making additional signal molecules. Gain of response regulators also leads to expansion of the existing regulatory networks. For instance, ExpR of *S. meliloti* and QscR of *P. aeruginosa* utilize the existing AHL signal molecules to extend their regulatory control beyond that of the cognate LuxR/I pair (Hoang *et al.*, 2004; Lequette *et al.*, 2006). Moreover, orphan LuxR regulators could be recruited for eavesdropping or perceiving exogenous signals for intercellular communication. Several instances of intercellular communication have been reported for orphan LuxR regulators. In *Serratia* sp. ATCC39006, carbapenem synthesis by CarR is modulated by the interspecies communication system of LuxS/AI-2 (Coulthurst *et al.*, 2005). Other examples include QscR of *P. aeruginosa* and SdiA of *E. coli*, which sense signals not produced by their respective hosts (Ahmer, 2004; Lee *et al.*, 2006). QscR responds to autoinducer signals, 3O-C_{10}-HSL and C_{10}-HSL, neither of which are generated by *P. aeruginosa* (Lee *et al.*, 2006). SdiA responds to AHLs and the metabolic signal indole, both of which could be present in its extracellular milieu (Ahmer, 2004; Lee *et al.*, 2007). Furthermore, having additional LuxR homologs may increase the potential to respond to non-native interkingdom signals. For instance, XccR and OryR of *Xanthomonas* sp. mediate regulatory activity by responding to host–plant exudates (Ferluga *et al.*, 2007; Zhang *et al.*, 2007). Therefore, they represent a novel class of LuxR-type proteins that have perhaps evolved to sense plant-derived signal molecules (Fig. 6) (Ferluga *et al.*, 2007; Zhang *et al.*, 2007). Thus it is

likely that orphan LuxR homologs have adapted to play an important role in perception of exogenous signals from their environmental niches.

Concluding remarks

Bacteria exist as part of dynamic microbial communities within various environmental niches. In this context, bacteria use quorum sensing as an effective means to translate environmental cues into global gene regulation. Using the three basic components of the signal producer, the signal itself, and the signal response regulator, bacteria are able to control an extensive set of biological processes. The wide scope of regulation is aided by the optimal utilization of the quorum-sensing components, such as use of additional LuxR-type proteins called orphan LuxR regulators. Evolution and environmental pressures have selected for gain of these orphan response regulators, and their presence helps in fine tuning the existing quorum-sensing regulatory network while opening up possibilities of controlling newer independent regulons. Moreover, they could potentially respond to external environmental stimuli and be a part of intercellular/interkingdom communication. The fact that the orphan LuxR regulators are maintained after acquisition and efficiently integrated into the bacterial regulatory systems points toward the advantageous contributions and the competitive advantage afforded by these regulators to the bacteria.

Acknowledgements

We thank Brandon McKethan for assistance with the figures in this manuscript. We also thank the members of the laboratory for their thoughtful insights and input during the preparation of this review. The work in our laboratory is supported by the National Science Foundation grant MCB-9733532 and by the National Institutes for Health grant 1R01GM069925 to J.E.G.

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