Microbiology (2009), **155**, 1377–1385 DOI 10.1099/mic.0.026849-0

Mini-Review

LuxR-family 'solos': bachelor sensors/regulators of signalling molecules

Sujatha Subramoni and Vittorio Venturi

International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Correspondence
Vittorio Venturi
venturi@icgeb.org

N-Acylhomoserine lactone (AHL) quorum-sensing (QS) signalling is the best-understood chemical language in proteobacteria. In the last 15 years a large amount of research in several bacterial species has revealed in detail the genetic, molecular and biochemical mechanisms underlying AHL signalling. These studies have revealed the role played by protein pairs of the AHL synthase belonging to the LuxI family and cognate LuxR-family AHL sensor–regulator. Proteobacteria however commonly possess a QS LuxR-family protein for which there is no obvious cognate LuxI synthase; these proteins are found in bacteria which possess a complete AHL QS system(s) as well as in bacteria that do not. Scientists are beginning to address the roles played by these proteins and it is emerging that they could allow bacteria to respond to endogenous and exogenous signals produced by their neighbours. AHL QS research thus far has mainly focused on a cell-density response involving laboratory monoculture studies. Recent findings on the role played by the unpaired LuxR-family proteins highlight the need to address bacterial behaviour and response to signals in mixed communities. Here we review recent progress with respect to these LuxR proteins, which we propose to call LuxR 'solos' since they act on their own without the need for a cognate signal generator. Initial investigations have revealed that LuxR solos have diverse roles in bacterial interspecies and interkingdom communication.

Introduction

Bacteria in the wild mainly live in close association with many different bacteria and eukaryotic hosts, meaning that they constantly need to monitor and communicate with their neighbours. It is now established that bacteria produce signalling molecules which are believed to allow them to monitor their population density in a process called quorum sensing (QS) (Fuqua *et al.*, 1994). The predominant QS signalling molecules produced and detected by proteobacteria are *N*-acylhomoserine lactones (AHLs). QS research has thus far largely concentrated on monocultures and has shown that AHLs are constantly produced and accumulate as the bacterial population increases. When the bacterial population reaches a certain size (a quorum), the bacteria respond to AHLs and synchronize community behaviour through regulation of their gene expression. These investigations have linked AHL QS with phenotypes that benefit the AHL-producing community, including production of virulence factors, motility, nodulation, plasmid transfer, antibiotic production, bioluminescence and biofilm formation (Bassler, 2002; Waters & Bassler, 2005; Whitehead *et al.*, 2001).

A typical AHL QS system is most commonly mediated by two proteins, belonging to the LuxI and LuxR protein families (Fig. 1a). LuxI-type proteins synthesize AHLs which interact directly with the cognate LuxR-type protein, and this complex usually allows them to bind specific promoter sequences called *lux*-boxes affecting expression of QS target genes (Fuqua & Greenberg, 2002). AHLs can also be synthesized by the AinS/LuxM (belonging to *Vibrio fischeri* and *Vibrio harveyi* respectively) family, and these do not display any similarity to the LuxI protein family (Bassler *et al.*, 1993; Gilson *et al.*, 1995). The AHLs they produce differ in length of the acyl-chain moiety and substitution at position C-3, which can either be part of a methylene group or carry an oxo- or hydroxyl group. QS LuxR proteins bind and respond optimally to the AHL produced by the cognate LuxI-family protein, guaranteeing a good degree of selectivity. In most cases *luxI/luxR* pairs are actually genetically linked; however there are examples where the *luxI/luxR* functional pairs are distantly located in the bacterial chromosome or plasmid. LuxR proteins which belong to a LuxI/LuxR functional pair can however sometimes bind structurally closely related AHLs, albeit with less efficiency.

AHL QS research now needs to more insistently address the role in bacterial interspecies communication and understand whether AHLs take part in interkingdom signalling. A recent observation related to these roles is that numerous sequenced proteobacterial genomes have QS-related LuxR AHL sensors/regulators which lack a cognate LuxI AHL synthase (Case *et al.*, 2008). These

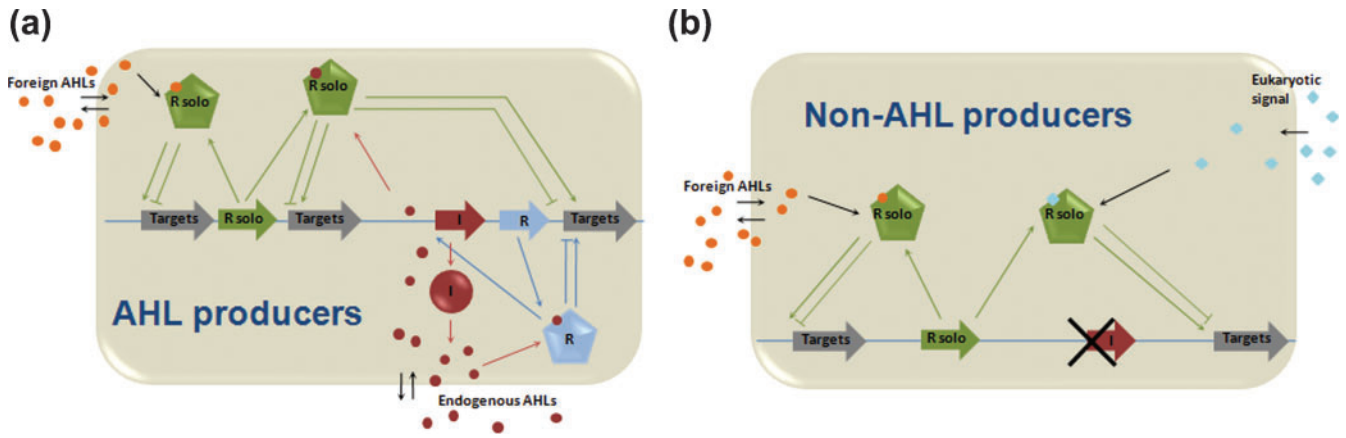

Fig. 1. Possible roles of LuxR solos in AHL-producing and non-AHL-producing proteobacteria. In AHL-producing bacteria AHLs can bind either endogenous or exogenous AHLs; in non-AHL-producing bacteria LuxR solos can bind exogenous AHLs or other signals also originating in eukaryotes. LuxR solos can then control different sets of target gene expression.

unpaired QS LuxR-family proteins have been called orphans (Fuqua, 2006) and possess the typical modular structure having an AHL-binding domain at their N-terminus and a helix–turn–helix DNA-binding domain at their C-terminus. Some, however, differ in their length and others lack conserved amino acids within the AHL-binding domain (Fuqua & Greenberg, 2002; Fuqua, 2006). In this mini-review we assess current knowledge and discuss the possible roles of these proteins, which we propose to designate as LuxR-family 'solos'. We believe 'solo' is a more appropriate term than 'orphan', since in bacterial genetics 'orphan' is often used to describe a putative gene of unknown function which does not have any homologues. The name 'solo' is therefore more suitable to describe this growing novel family of LuxR proteins (Table 1).

Working models for LuxR solos

Genetically, thus far AHL QS has been found exclusively in proteobacteria, and analysing 265 proteobacterial genomes revealed that 68 of them possess at least one LuxI and one LuxR homologue (Case *et al.*, 2008). Interestingly, however, as many as 45 of these 68 genomes possess more LuxR than LuxI homologues, indicating that they probably possess LuxR solos (as depicted in Fig. 1a). A further 45 genomes do not possess an AHL synthase gene, but they contain LuxR solos. Nevertheless, it cannot be excluded that some bacteria which do not possess *luxI* homologues are able produce AHLs. LuxR solos are therefore widespread in bacteria which possess a complete AHL QS system(s) as well as in bacteria that apparently do not synthesize AHLs. LuxR solos present in AHL-producing bacteria could be sensing the endogenously produced AHL(s) and thus extending the AHL QS regulon to other gene targets. Alternatively, they could have a more relaxed or different specificity towards AHLs or possibly other ligands produced by different bacterial neighbours. The presence of LuxR solos in non-AHL-producing bacteria, on the other hand, could allow them to respond to AHLs produced by other bacteria, possibly allowing them to team up or maybe switch to a competitive behaviour towards their neighbours. It cannot be excluded that some LuxR solos which lack conserved amino acid residues in the AHL domain are functioning in a ligand-independent way. Then again, these imperfect LuxR solos could be binding to signals which are not AHLs but are produced by nearby bacteria or eukaryotic cells. If they respond to compounds produced by eukaryotes, they are not necessarily involved in QS but rather in informing bacteria of their whereabouts. Recent investigations have revealed that the processes described by these different models are actually taking place (summarized in Figs 1 and 2), highlighting the diverse roles in bacterial interspecies and interkingdom communication played by these proteins.

LuxR solos in AHL-producing bacteria

ExpR of *Sinorhizobium meliloti*, BisR of *Rhizobium leguminosarum* bv. *viciae* and QscR of *Pseudomonas aeruginosa* are LuxR solos in AHL-producing bacteria which have been well characterized. All of these bacterial species actually possess multiple complete AHL QS systems and produce several types of AHL molecules. The functional features of these three LuxR solos are integrated with the resident AHL QS regulatory networks (as depicted in Fig. 1a). A better understanding of the role of these LuxR solos could provide insights into the rationale for production of several AHLs by these bacteria and other closely related species that occupy a similar ecological niche.

ExpR of *Sinorhizobium meliloti*

ExpR was initially identified as being essential for the synthesis of symbiotically active galactoglucan (EPSII) in *S.*

Table 1. Functionally characterized LuxR solos

LuxR solo	Organism	AHL QS systems	Binding molecule(s)	Functions regulated	Reference
AvhR	*Agrobacterium vitis*	AvsI/R	Not yet determined	Ability to cause necrosis on grapes and HR* on tobacco plants	Hao *et al.* (2005)
AviR	*Agrobacterium vitis*	AvsI/R	Not yet determined	Ability to cause necrosis on grapes and HR on tobacco plants	Zheng *et al.* (2003)
BisR	*Rhizobium leguminosarum* bv. *viciae*	CinI/Rl, RhiI/Rl, RaiI/Rl, TraI/R	3OH-C14 : 1-HSL	Symbiotic plasmid pRL1J1 transfer and growth inhibition in the presence of 3OH-C14 : 1-HSL	Danino *et al.* (2003); Wilkinson *et al.* (2002)
BlxR	*Brucella melitensis*	None	Not yet determined	Regulation of virulence factors like type IV secretion system and flagella	Rambow-Larsen *et al.* (2008)
CarR	*Serratia marcescens*	*Sma*I/R	Ligand independent	Carbapenem antibiotic production	Cox *et al.* (1998)
ExpR	*Sinorhizobium meliloti*	SinI/SinR Mel	C14-HSL, 3-oxo-C14-HSL, C16 : 1-HSL, 3-oxo-C16-HSL, C18-HSL	Production of symbiotically active EPSII, succinoglycan production, motility, chemotaxis, nitrogen fixation, metal transport, etc.	Bartels *et al.* (2007); Hoang *et al.* (2004); McIntosh *et al.* (2008); Rambow-Larsen *et al.* (2008)
NesR	*Sinorhizobium meliloti*	SinI/SinR Mel	C14-HSL, 3-oxo-C14-HSL, C16 : 1-HSL, 3-oxo-C16-HSL, C18-HSL	Nutritional and environmental stress response, plant nodulation	Patankar & Gonzalez (2009)
OryR	*Xanthomonas oryzae* pv. *oryzae*	None	Rice signal molecule	Proline iminopeptidase (*pip*) gene expression; virulence on rice	Ferluga *et al.* (2007); Ferluga & Venturi (2009)
QscR	*Pseudomonas aeruginosa*	LasI/R RhlI/R	3-Oxo-C12-HSL, 3-oxo-C10-HSL	Timing of expression of AHL production and virulence factors	Chugani *et al.* (2001); Lequette *et al.* (2006)
SdiA	*Escherichia coli*, *Salmonella enterica* serovar Typhimurium	None	3-Oxo-C8-HSL, 3-oxo-C6-HSL, 3-oxo-C4-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, C6-HSL, C8-HSL	Functions involved in adhesion and resistance to complement killing	Ahmer (2004); Michael *et al.* (2001); Yao *et al.* (2006)
VjbR	*Brucella melitensis*	None	C12-HSL	Regulation of virulence factors like type IV secretion system and flagella	Delrue *et al.* (2005)
XccR	*Xanthomonas campestris* pv. *campestris*	None	Plant signal molecule	Proline iminopeptidase (*pip*) gene expression; virulence on cabbage	Zhang *et al.* (2007)

*HR, hypersensitive response.

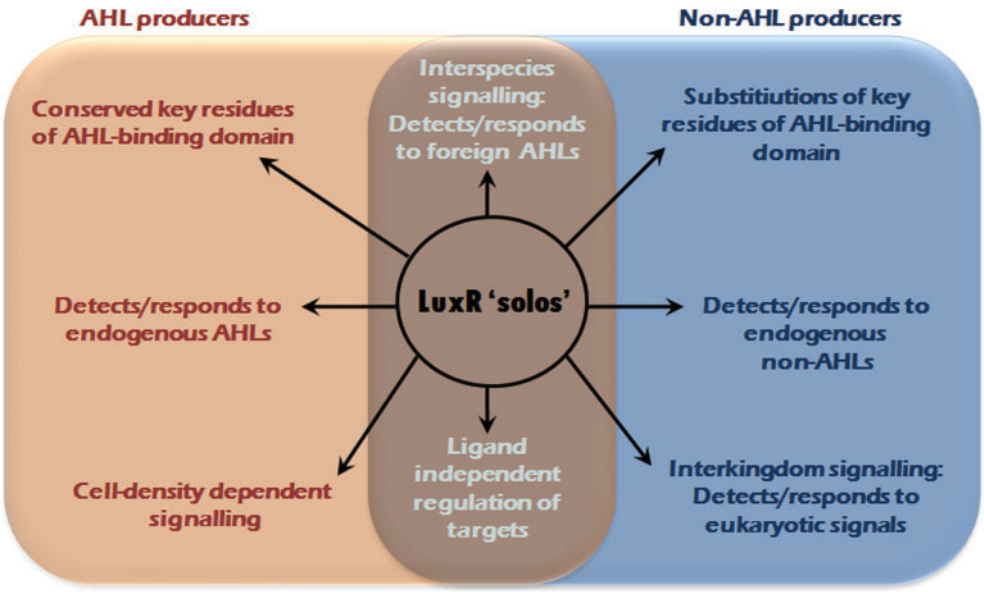

Fig. 2. Functional and structural features of LuxR solos in proteobacteria: summary of the features and characteristics of LuxR solos determined so far in AHL-producing and non-AHL-producing bacteria. There are distinct roles in the two scenarios, however; some properties are also shared in both situations.

meliloti Rm1021. The wild-type strain produces negligible EPSII due to an inactive copy of *expR* with an insertion element (ISRm2011-1); precise spontaneous excision of this insertion element results in functional *expR*, inducing production of EPSII (Pellock *et al.*, 2002). The SinI/R system of *S. meliloti* Rm1021 produces several long-chain AHLs ranging from C_{12}- to C_{18}-HSL and the presence of another system (designated Mel) has been hypothesized (i.e. this system has not been identified) which could produce short-chain AHLs (Gonzalez & Marketon, 2003). ExpR is not associated with a LuxI homologue; hence it is a solo and regulates expression of its targets in response to SinI-AHLs (Marketon *et al.*, 2003; McIntosh *et al.*, 2008).

Comparison of *S. meliloti* strains differing in the functional status of *expR*, *sinI* or *sinR* in various combinations showed that the regulation of the majority of target genes by the SinI/R system requires the presence of an active ExpR (Hoang *et al.*, 2004, 2008). Significantly, ExpR regulates a separate set of genes, including those involved in nitrogen fixation and metal transport; expression of several of these genes is independent of the SinI-AHLs (Hoang *et al.*, 2004).

ExpR is different from other typical QS LuxR regulators in being soluble and stable in the absence of AHLs (LuxR-family proteins when overexpressed are usually highly insoluble in the absence of the AHL ligand) as well as not binding to a typical *lux*-box; in fact it has been reported to bind to a 24 bp sequence located upstream of the putative *lux*-box of the *sinI* promoter (Bartels *et al.*, 2007; McIntosh *et al.*, 2008). ExpR also specifically binds to the promoter regions of galactoglucan biosynthesis genes *wgaA* and *wgeA* (previously known as *exp* genes) in a 3-oxo-C14-HSL-dependent manner, while binding to *exoI* and *exsH* (genes involved in succinoglycan biosynthesis) promoters is independent of the presence of AHL (McIntosh *et al.*, 2008). Also, succinoglycan production is induced in the presence of SinI-AHLs and repressed without these AHLs in an *expR*$^+$ strain. It is however produced independently of AHLs in the absence of a functional ExpR (Glenn *et al.*, 2007). ExpR is very versatile and either activates or represses target genes depending on the presence or absence of AHL molecules. Interestingly *S. meliloti* Rm1021 possesses four additional putative LuxR solos; recently one of these, designated NesR, has been shown to be involved in nutritional and stress responses as well as in nodulation (Patankar & Gonzalez, 2009). NesR is not integrated with the SinI/R system and its homology to the xanthomonad LuxR solos OryR and XccR suggests that the ligand for NesR might be an uknown plant compound (see below). Understanding ExpR-mediated regulation and the identification of signal molecules sensed by the other LuxR solos present in *S. meliloti* would help in deciphering this rather complex QS signalling network.

BisR of *Rhizobium leguminosarum* bv. *viciae*

BisR (bacteriostasis induction sensor) is a LuxR solo of *R. leguminosarum* bv. *viciae* strain A34 (a symbiont of pea and vetch) which contains the symbiotic plasmid pRL1J1. Strain A34 has four complete QS systems and produces a number of different AHLs (Gonzalez & Marketon, 2003). The *bisR* gene is located on symbiotic plasmid pRL1J1 and is responsible for controlling growth inhibition in the

presence of 3OH-C14 : 1-HSL (initially called small bacteriocin; Wilkinson *et al.*, 2002) and transfer frequency of pRL1JI (Wilkinson *et al.*, 2002). BisR represses the expression of *cinI*, which is responsible for the synthesis of 3OH-C14 : 1-HSL as well as inducing the expression of *triR* (encoding the LuxR regulator TriR, now designated TraR) in response to 3OH-C14 : 1-HSL (Danino *et al.*, 2003; Wilkinson *et al.*, 2002). Danino *et al.* (2003) revealed an elegant system in which BisR represses the expression of *cinI* and thereby maintains minimal amounts of 3OH-C14 : 1-HSL. Upon sensing of a critical concentration of 3OH-C14 : 1-HSL produced by an optimum number of recipient cells, the plasmid transfer functions are induced by BisR. In contrast to its role in plasmid transfer, not much is known about the mechanism of BisR-mediated growth inhibition of *R. leguminosarum* bv. *viciae* in the presence of 3OH-C14 : 1-HSL. Future work will probably focus on identification of additional target genes of BisR and its interaction with the resident complete AHL QS systems.

QscR of *Pseudomonas aeruginosa*

QscR is a well-studied LuxR solo of *P. aeruginosa*, which has two complete AHL QS systems, the LasI/R and RhlI/R systems. LasI directs the synthesis of 3-oxo-C12-HSL whereas RhlI synthesizes C4-HSL. The two systems are intimately connected, the LasI/R system regulating *rhlI/R* forming a cascade, and together are involved in the regulation of many virulence factors (Smith & Iglewski, 2003).

The QscR solo responds to endogenously LasI-produced 3-oxo-C12-HSL and one of its roles is to time and prevent premature expression of endogenous AHL signals and virulence factors (Chugani *et al.*, 2001; Lequette *et al.*, 2006). In response to endogenous 3-oxo-C12-HSL, QscR also regulates an independent set of target genes distinct from those regulated by the LasI/R and RhlI/R systems (Lequette *et al.*, 2006). This role of QscR extends the AHL QS regulon to other targets, probably allowing a more timely and efficient response to cell density by *P. aeruginosa*. However, QscR can also respond well to 3-oxo-C10-HSL, demonstrating a more relaxed specificity for AHL binding than LasR and RhlR (Lee *et al.*, 2006). *P. aeruginosa* produces 3-oxo-C10-HSL via LasI; however, 3-oxo-C12-HSL is the dominant signal produced. The more relaxed specificity towards AHLs is an indication that QscR could also respond to exogenous AHLs produced by neighbouring bacteria.

Other LuxR solos in AHL-producing bacteria

Other LuxR solos such as AviR and AvhR of *Agrobacterium vitis* have been reported (Hao *et al.*, 2005; Zheng *et al.*, 2003). *A. vitis* strain F2/5 most likely possesses two complete QS systems: the AvsI/R system responsible for the production of long-chain AHLs and another system that directs the production of short-chain AHLs (Hao & Burr, 2006). Both AviR and AvhR solos are required for induction of necrosis in grapes and hypersensitive response on non-host tobacco plants. Mutants in *aviR* produced lower levels of many AHLs while mutations in *avhR* resulted in higher intensity of the signals, suggesting contrasting effects of these two regulators on genes responsible for AHL production (Hao *et al.*, 2005; Zheng *et al.*, 2003). In AvhR, three of the six conserved amino acid residues of the AHL-binding domain have substitutions; these include the important residues Trp57, Asp70 and Trp85 designated with reference to TraR, where they are involved in AHL binding (Hao *et al.*, 2005). Therefore the possibility that AvhR binds to a non-AHL molecule or functions without a signalling molecule cannot be ruled out.

CarR of *Serratia marcescens* is an example of a LuxR solo that regulates target gene expression in a ligand-independent manner (Cox *et al.*, 1998). *S. marcescens* CarR functionally complements an *Erwinia carotovora* subsp. *carotovora carI carR* double mutant without the need for 3-oxo-C6-HSL, whereas its homologue CarR of *E. carotovora* regulates production of carbapenem antibiotic in a 3-oxo-C6-HSL-dependent manner. *S. marcescens* is an AHL producer; the LuxI/LuxR pair designated SmaI/R constitutes the QS system, which produces and responds to C4-HSL and C6-HSL, regulating several cellular functions. Current understanding of these proteins indicates that SmaR acts as a repressor of *carR* and carbapenem biosynthetic genes at low cell densities; repression is possibly released by binding of *S. marcescens* AHLs to SmaR (Slater *et al.*, 2003).

Finally, many members of the genera *Pseudomonas*, *Burkholderia*, *Rhizobium* and *Agrobacterium* which have complete AHL QS systems possess LuxR solos (Case *et al.*, 2008). The function of most of these is currently unknown; however, among those LuxR solos that have been studied up to now, many are interconnected with the resident AHL QS systems. No clear indications have yet emerged as to whether they are involved in interspecies signalling; interestingly, several members of these genera share similar habitats.

LuxR solos in non-AHL-producing bacteria

Since many non-AHL-producing proteobacteria (almost 20 %) possess LuxR solos, this could imply that in communities with AHL producers and non-producers some of the members can detect and respond to molecular signals produced by others. Surprisingly, to our knowledge only two LuxR solos in non-AHL-producing bacteria have thus far been investigated in some detail. One, called SdiA, is present in members of the genera *Salmonella*, *Escherichia* and *Klebsiella* and is able to bind and detect AHLs produced by others (as depicted in Fig. 1b). The other is from plant-pathogenic *Xanthomonas* spp. and is also very well conserved in other plant-associated bacteria. In two *Xanthomonas* species it is involved in virulence upon

binding an unknown plant-produced low-molecular-mass compound which is not an AHL. This extends the role of LuxR solos to interkingdom signalling by detecting non-AHL compounds produced by eukaryotes (as depicted in Fig. 1b).

The SdiA LuxR solo

SdiA detects an unusually wide variety of AHLs when compared to other LuxR homologues; it binds most specifically to 3-oxo-C8-HSL and then with decreasing efficiency to 3-oxo-C6-HSL, 3-oxo-C4-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, C6-HSL and C8-HSL (Janssens *et al.*, 2007; Michael *et al.*, 2001; Yao *et al.*, 2006). It responds to physiologically relevant concentrations of some of these AHLs whereas it needs higher concentrations for others. For this reason, the precise role of *in vivo* detection for the different AHLs could be significantly different.

The function of SdiA is best understood in *Salmonella enterica* serovar Typhimurium, where it has been determined that it regulates two *Salmonella*-specific loci in response to AHLs (Ahmer, 2004; Michael *et al.*, 2001; Smith & Ahmer, 2003). They are the *rck* operon (resistance to complement killing), which is located on the *S.* Typhimurium virulence plasmid, and the *srgE* (*sdiA* regulated gene) locus located in the chromosome (Ahmer, 2004; Smith & Ahmer, 2003). The *rck* operon consists of six genes (*pefI*, *srgD*, *srgA*, *srgB*, *rck* and *sgrC*), most of which are believed to be involved in adhesion and resistance to complement killing. The chromosomal *srgE* locus on the other hand consists of a single gene which encodes a predicted protein of unknown function containing a coiled-coil domain. *Salmonella sdiA* mutants are not attenuated in virulence in several animal models and thus SdiA is believed to regulate only accessory virulence factors (Ahmer, 2004). Scientists now need to determine whether SdiA is sensing AHLs *in vivo* since the intestinal environment contains a large number of different microbial species. Along these lines, Smith *et al.* (2008) recently observed that SdiA-mediated AHL detection did not occur in the intestinal tract of several animals (e.g. mouse, chicken, pig, rabbit and cow); however, SdiA of *Salmonella* did detect AHLs during transit through the intestinal tract of turtles which harboured AHL-producing *Aeromonas hydrophila*.

Unlike the SdiA of *Salmonella*, no experiments have been performed on the *sdiA* gene of *Klebsiella* spp., whilst the data currently available for *Escherichia coli* are rather difficult to interpret. The *sdiA* gene in *E. coli* has been isolated on three occasions for conferring different phenotypes when overexpressed in a plasmid (Ahmer, 2004). It increases transcription of the cell division operon *ftsQAZ* and increases resistance to certain antibiotics and quinolones, probably through activation of efflux pumps (Ahmer, 2004; Rahmati *et al.*, 2002; Sitnikov *et al.*, 1996). *E. coli* SdiA has been shown to be able to activate *Salmonella* and *E. coli* gene promoters in an AHL-dependent manner (Ahmer, 2004; Sitnikov *et al.*, 1996). Functionally, however, no *E. coli* gene has thus far been shown to be regulated by the single chromosomal copy of *sdiA*. Overexpression of SdiA in an enterohaemorrhagic *E. coli* strain also resulted in repression of certain virulence factors (Kanamaru *et al.*, 2000). Apparently this repression is dependent on an uncharacterized *E. coli*-produced factor present in spent culture supernatants. This opens the door to the prospect that SdiA also binds to an as yet unidentified factor produced by *E. coli*.

SdiA was the first LuxR solo in non-AHL-producing bacteria shown to be able to bind AHLs. However, open questions remain, including whether SdiA also binds/responds to an *E. coli*-produced factor and what are its functional roles. These questions might be answered by studying the interaction of *E. coli* and *Salmonella* with other bacterial species, since waste products, metabolites or molecular signals produced by neighbours can influence the functioning of SdiA. This approach makes sense since in nature both *Salmonella* spp. and *E. coli* live in close association with many other bacterial species. Finally it must be noted that recently, indole has been identified as a signalling compound produced by *E. coli* (reviewed by Walters & Sperandio, 2006). Indole signalling has been shown to be important for regulating biofilm formation and research is also now focusing on determining whether indole and SdiA interact and if they are in some way connected (Lee *et al.*, 2007). SdiA could possibly be involved with the response to at least two different kinds of signalling molecules – AHLs (exogenous) and indole (endogenous) – and modulate cellular functions appropriately. Further studies are needed to confirm these possible roles of SdiA in intra- and interspecies signalling in *E. coli*.

The ability of the SdiA solo to bind and respond to AHLs interferes with the practical use of *E. coli* as an AHL detector/biosensor strain. *E. coli* has been engineered by several research groups via the introduction of plasmids harbouring heterologous *luxR* genes, target gene promoters and reporter systems in order to detect and respond to the presence of exogenously provided AHLs (Steindler & Venturi, 2007). It has been observed that SdiA interferes with these experiments and hence the best results are obtained when an *E. coli sdiA* knockout mutant is used for these experiments (Lindsay & Ahmer, 2005).

The LuxR solo of xanthomonads and other plant-associated bacteria

The plant-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas campestris* pv. *campestris* (*Xcc*) possess a LuxR solo, designated OryR and XccR, which is required for full virulence to rice and cabbage, respectively. Both OryR and XccR do not bind or respond to AHLs and in their AHL-binding domain they lack two of the several conserved residues involved in AHL binding (Ferluga *et al.*, 2007; Zhang *et al.*, 2007). XccR regulates *in planta* the neighbouring proline iminopeptidase (*pip*) virulence gene.

It associates with a plant factor and functions as a transcriptional activator by binding to a *lux*-box present in the promoter of the *pip* gene (Zhang *et al.*, 2007). Similarly, OryR binds a compound present in the rice plant. This was concluded following the observation that OryR was not solubilized by many of the structurally different AHLs but OryR solubilization was achieved in the presence of rice extract (Ferluga *et al.*, 2007). The concentration of the molecule in rice which interacts with OryR increases when rice is infected with *Xoo*, meaning that rice most probably increases its production upon pathogen attack (Ferluga & Venturi, 2009). Just like XccR, OryR also positively regulates expression of the adjacent *pip* gene upon exposure to the rice signal molecule. It is probable that OryR and XccR solos participate in interkingdom signalling, whereby they facilitate successful infection by detecting and binding to a host signal molecule(s).

Orthologues of OryR and XccR are present in the genomes of several other plant-associated bacteria which are both beneficial and pathogenic, for example *Pseudomonas fluorescens* and *Pseudomonas syringae* (Zhang *et al.*, 2007). Since some strains of these species do produce AHLs, it cannot be excluded that these solos can also respond to AHLs. It is postulated however that probably a subclass of LuxR solos are employed in interkingdom signalling between bacteria and plant; this is not so surprising, as bacteria have co-evolved with plants for many years. Plants have been reported to produce compounds that are able to act as agonists or antagonists to bacterial AHL QS systems and these have been called AHL mimics (Bauer & Mathesius, 2004). AHL mimics from several plants, including rice, are able to stimulate gene expression via LuxR-family AHL sensors/regulators. To date the structures of these plant compounds are unknown and it cannot be excluded that similar molecules are involved in interkingdom signalling with the XccR/OryR subgroup of LuxR solos.

Other LuxR solos in non-AHL-producing proteobacteria

In *Brucella melitensis,* two LuxR solos called VjbR and BlxR have been shown to be involved in regulating virulence loci as well as cross-regulating their gene expression (Delrue *et al.*, 2005; Rambow-Larsen *et al.*, 2008; Uzureau *et al.*, 2007). Surprisingly, *B. melitensis* has been shown to produce C12-HSL even though it does not have a *luxI* homologue in its genome (Taminiau *et al.*, 2002). C12-HSL is believed to interact with VjbR and control virulence-associated loci (Delrue *et al.*, 2005). As mentioned previously, it is therefore possible that some bacteria which do not possess *luxI* homologues are able produce AHLs.

Predicted LuxR solos in non-AHL-producing bacteria are present in several other bacterial genera, including *Pseudomonas* and *Shigella* (Case *et al.*, 2008). It is probable that scientists are currently studying these regulators and we will therefore have insights into their mode of action in the near future. Most often LuxR solos within a genus are closely related, indicating a probable conserved function.

Concluding remarks

Bacteria most probably are constantly vigilant of the living organisms present in the surroundings as this will affect decisions on their programme of gene expression. Consequently, an important question that we need to address in the future is how often bacteria are involved in interspecies and interkingdom signalling. Initial studies with LuxR solos indicate that some of these may be important players in this process (Table 1 summarizes the LuxR solos studied to date).

The few LuxR solos studied so far highlight that they could be highly diverse in the type of ligands they respond to, as well as their mechanism of regulation of target genes (Fig. 2). Generally, this probably depends on their occurrence in an AHL-producing or non-AHL-producing bacterium and the immediate surrounding environment. It is clear that LuxR solos extend bacterial participation to processes other than just cell-density-dependent signalling. As QS research now enters the next phase of studying mixed bacterial communities, it is important to recognize the functional resourcefulness of LuxR solos. Any future intervention strategies based on disruption/manipulation of bacterial communication networks will require a better understanding of these proteins.

Acknowledgements

S. S. is beneficiary of an ICGEB fellowship. The activities in the laboratory of V. V. are supported by ICGEB, Fondazione Italiana per la Ricerca sulla Fibrosis Cistica and by the Fondazione Cassamarca. We thank Clay Fuqua for the suggestion to name the proteins LuxR solos.

References

Ahmer, B. M. (2004). Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica. Mol Microbiol* **52**, 933–945.

Bartels, F. W., McIntosh, M., Fuhrmann, A., Metzendorf, C., Plattner, P., Sewald, N., Anselmetti, D., Ros, R. & Becker, A. (2007). Effector-stimulated single molecule protein-DNA interactions of a quorum-sensing system in *Sinorhizobium meliloti. Biophys J* **92**, 4391–4400.

Bassler, B. L. (2002). Small talk. Cell-to-cell communication in bacteria. *Cell* **109**, 421–424.

Bassler, B. L., Wright, M., Showalter, R. E. & Silverman, M. R. (1993). Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol Microbiol* **9**, 773–786.

Bauer, W. D. & Mathesius, U. (2004). Plant responses to bacterial quorum sensing signals. *Curr Opin Plant Biol* **7**, 429–433.

Case, R. J., Labbate, M. & Kjelleberg, S. (2008). AHL-driven quorum-sensing circuits: their frequency and function among the Proteobacteria. *ISME J* **2**, 345–349.

Chugani, S. A., Whiteley, M., Lee, K. M., D'Argenio, D., Manoil, C. & Greenberg, E. P. (2001). QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa. Proc Natl Acad Sci U S A* **98**, 2752–2757.

Cox, A. R., Thomson, N. R., Bycroft, B., Stewart, G. S., Williams, P. & Salmond, G. P. (1998). A pheromone-independent CarR protein controls carbapenem antibiotic synthesis in the opportunistic human pathogen *Serratia marcescens*. *Microbiology* **144**, 201–209.

Danino, V. E., Wilkinson, A., Edwards, A. & Downie, J. A. (2003). Recipient-induced transfer of the symbiotic plasmid pRL1JI in *Rhizobium leguminosarum* bv. *viciae* is regulated by a quorum-sensing relay. *Mol Microbiol* **50**, 511–525.

Delrue, R. M., Deschamps, C., Leonard, S., Nijskens, C., Danese, I., Schaus, J. M., Bonnot, S., Ferooz, J., Tibor, A. & other authors (2005). A quorum-sensing regulator controls expression of both the type IV secretion system and the flagellar apparatus of *Brucella melitensis*. *Cell Microbiol* **7**, 1151–1161.

Ferluga, S. & Venturi, V. (2009). OryR is a LuxR-family protein involved in inter-kingdom signaling between pathogenic *Xanthomonas oryzae* pv. *oryzae* and rice. *J Bacteriol* **191**, 890–897.

Ferluga, S., Bigirimana, J., Hofte, M. & Venturi, V. (2007). A LuxR homologue of *Xanthomonas oryzae* pv. *oryzae* is required for optimal rice virulence. *Mol Plant Pathol* **8**, 529–538.

Fuqua, C. (2006). The QscR quorum-sensing regulon of *Pseudomonas aeruginosa*: an orphan claims its identity. *J Bacteriol* **188**, 3169–3171.

Fuqua, C. & Greenberg, E. P. (2002). Listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* **3**, 685–695.

Fuqua, W. C., Winans, S. C. & Greenberg, E. P. (1994). Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* **176**, 269–275.

Gilson, L., Kuo, A. & Dunlap, P. V. (1995). AinS and a new family of autoinducer synthesis proteins. *J Bacteriol* **177**, 6946–6951.

Glenn, S. A., Gurich, N., Feeney, M. A. & Gonzalez, J. E. (2007). The ExpR/Sin quorum-sensing system controls succinoglycan production in *Sinorhizobium meliloti*. *J Bacteriol* **189**, 7077–7088.

Gonzalez, J. E. & Marketon, M. M. (2003). Quorum sensing in nitrogen-fixing rhizobia. *Microbiol Mol Biol Rev* **67**, 574–592.

Hao, G. & Burr, T. J. (2006). Regulation of long-chain *N*-acyl-homoserine lactones in *Agrobacterium vitis*. *J Bacteriol* **188**, 2173–2183.

Hao, G., Zhang, H., Zheng, D. & Burr, T. J. (2005). *luxR* homolog *avhR* in *Agrobacterium vitis* affects the development of a grape-specific necrosis and a tobacco hypersensitive response. *J Bacteriol* **187**, 185–192.

Hoang, H. H., Becker, A. & Gonzalez, J. E. (2004). The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in *Sinorhizobium meliloti* gene expression. *J Bacteriol* **186**, 5460–5472.

Hoang, H. H., Gurich, N. & Gonzalez, J. E. (2008). Regulation of motility by the ExpR/Sin quorum-sensing system in *Sinorhizobium meliloti*. *J Bacteriol* **190**, 861–871.

Janssens, J. C., Metzger, K., Daniels, R., Ptacek, D., Verhoeven, T., Habel, L. W., Vanderleyden, J., De Vos, D. E. & De Keersmaecker, S. C. (2007). Synthesis of *N*-acyl homoserine lactone analogues reveals strong activators of SdiA, the *Salmonella enterica* serovar Typhimurium LuxR homologue. *Appl Environ Microbiol* **73**, 535–544.

Kanamaru, K., Kanamaru, K., Tatsuno, I., Tobe, T. & Sasakawa, C. (2000). SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157 : H7. *Mol Microbiol* **38**, 805–816.

Lee, J. H., Lequette, Y. & Greenberg, E. P. (2006). Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. *Mol Microbiol* **59**, 602–609.

Lee, J., Jayaraman, A. & Wood, T. K. (2007). Indole is an inter-species biofilm signal mediated by SdiA. *BMC Microbiol* **7**, 42.

Lequette, Y., Lee, J. H., Ledgham, F., Lazdunski, A. & Greenberg, E. P. (2006). A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. *J Bacteriol* **188**, 3365–3370.

Lindsay, A. & Ahmer, B. M. (2005). Effect of *sdiA* on biosensors of *N*-acylhomoserine lactones. *J Bacteriol* **187**, 5054–5058.

Marketon, M. M., Glenn, S. A., Eberhard, A. & Gonzalez, J. E. (2003). Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti*. *J Bacteriol* **185**, 325–331.

McIntosh, M., Krol, E. & Becker, A. (2008). Competitive and cooperative effects in quorum-sensing-regulated galactoglucan biosynthesis in *Sinorhizobium meliloti*. *J Bacteriol* **190**, 5308–5317.

Michael, B., Smith, J. N., Swift, S., Heffron, F. & Ahmer, B. M. (2001). SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol* **183**, 5733–5742.

Patankar, A. V. & Gonzalez, J. E. (2009). An orphan LuxR homolog of *Sinorhizobium meliloti* affects stress adaptation and competition for nodulation. *Appl Environ Microbiol* **75**, 946–955.

Pellock, B. J., Teplitski, M., Boinay, R. P., Bauer, W. D. & Walker, G. C. (2002). A LuxR homolog controls production of symbiotically active extracellular polysaccharide II by *Sinorhizobium meliloti*. *J Bacteriol* **184**, 5067–5076.

Rahmati, S., Yang, S., Davidson, A. L. & Zechiedrich, E. L. (2002). Control of the AcrAB multidrug efflux pump by quorum sensing regulator SdiA. *Mol Microbiol* **43**, 677–685.

Rambow-Larsen, A. A., Rajashekara, G., Petersen, E. & Splitter, G. (2008). Putative quorum-sensing regulator BlxR of *Brucella melitensis* regulates virulence factors including the type IV secretion system and flagella. *J Bacteriol* **190**, 3274–3282.

Sitnikov, D. M., Schineller, J. B. & Baldwin, T. O. (1996). Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. *Proc Natl Acad Sci U S A* **93**, 336–341.

Slater, H., Crow, M., Everson, L. & Salmond, G. P. (2003). Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol Microbiol* **47**, 303–320.

Smith, J. N. & Ahmer, B. M. (2003). Detection of other microbial species by *Salmonella*: expression of the SdiA regulon. *J Bacteriol* **185**, 1357–1366.

Smith, R. S. & Iglewski, B. H. (2003). *P. aeruginosa* quorum-sensing systems and virulence. *Curr Opin Microbiol* **6**, 56–60.

Smith, J. N., Dyszel, J. L., Soares, J. A., Ellermeier, C. D., Altier, C., Lawhon, S. D., Adams, L. G., Konjufca, V., Curtiss, R., 3rd & other authors (2008). SdiA, an *N*-acylhomoserine lactone receptor, becomes active during the transit of *Salmonella enterica* through the gastrointestinal tract of turtles. *PLoS One* **3**, e2826.

Steindler, L. & Venturi, V. (2007). Detection of quorum-sensing *N*-acyl homoserine lactone signal molecules by bacterial biosensors. *FEMS Microbiol Lett* **266**, 1–9.

Taminiau, B., Daykin, M., Swift, S., Boschiroli, M. L., Tibor, A., Lestrate, P., De Bolle, X., O'Callaghan, D., Williams, P. & Letesson, J. J. (2002). Identification of a quorum-sensing signal molecule in the facultative intracellular pathogen *Brucella melitensis*. *Infect Immun* **70**, 3004–3011.

Uzureau, S., Godefroid, M., Deschamps, C., Lemaire, J., De Bolle, X. & Letesson, J. J. (2007). Mutations of the quorum sensing-dependent regulator VjbR lead to drastic surface modifications in *Brucella melitensis*. *J Bacteriol* **189**, 6035–6047.

Walters, M. & Sperandio, V. (2006). Quorum sensing in *Escherichia coli* and *Salmonella*. *Int J Med Microbiol* **296**, 125–131.

Waters, C. M. & Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* **21**, 319–346.

Whitehead, N. A., Barnard, A. M., Slater, H., Simpson, N. J. & Salmond, G. P. (2001). Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol Rev* **25**, 365–404.

Wilkinson, A., Danino, V., Wisniewski-Dye, F., Lithgow, J. K. & Downie, J. A. (2002). *N*-acyl-homoserine lactone inhibition of rhizobial growth is mediated by two quorum-sensing genes that regulate plasmid transfer. *J Bacteriol* **184**, 4510–4519.

Yao, Y., Martinez-Yamout, M. A., Dickerson, T. J., Brogan, A. P., Wright, P. E. & Dyson, H. J. (2006). Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. *J Mol Biol* **355**, 262–273.

Zhang, L., Jia, Y., Wang, L. & Fang, R. (2007). A proline iminopeptidase gene upregulated *in planta* by a LuxR homologue is essential for pathogenicity of *Xanthomonas campestris* pv. *campestris*. *Mol Microbiol* **65**, 121–136.

Zheng, D., Zhang, H., Carle, S., Hao, G., Holden, M. R. & Burr, T. J. (2003). A *luxR* homolog, *aviR*, in *Agrobacterium vitis* is associated with induction of necrosis on grape and a hypersensitive response on tobacco. *Mol Plant Microbe Interact* **16**, 650–658.