Quorum Sensing and LuxR Solos in *Photorhabdus*

Sophie Brameyer and Ralf Heermann

Abstract Bacterial communication via small diffusible molecules to mediate group-coordinated behaviour is commonly referred to as 'quorum sensing'. The prototypical quorum sensing system of Gram-negative bacteria consists of a LuxI-type autoinducer synthase that produces acyl-homoserine lactones (AHLs) as signals and a LuxR-type receptor that detects the AHLs to control expression of specific genes. However, many bacteria possess LuxR homologs but lack a cognate LuxI-type AHL-synthase. Those LuxR-type receptors are designated as 'LuxR orphans' or 'solos'. Entomopathogenic bacteria of the genus *Photorhabdus* all harbour a large number of LuxR solos, more than any other bacteria examined so far. Two novel quorum sensing systems were found to regulate cell clumping in *Photorhabdus* and therefore affect pathogenicity. In *Photorhabdus luminescens* and *Photorhabdus temperata* the LuxR solo PluR senses α-pyrones named 'photopyrones' instead of AHLs, which are produced by the pyrone synthase PpyS. In contrast, *Photorhabdus asymbiotica*, a closely related insect and human pathogen, has the PluR homolog PauR, which senses dialkylresorcinols produced by the DarABC pathway to regulate pathogenicity. All three *Photorhabdus* species harbour at least one LuxR solo with an intact AHL-binding motif, which might also allow sensing of exogenous AHLs. However, the majority of the LuxR solos in all *Photorhabdus* species have a PAS4 signal-binding domain. These receptors are assumed to detect eukaryotic compounds and are proposed to be involved in host sensing. Overall, because of the large number of LuxR solos they encode, bacteria of the genus *Photorhabdus* are ideal candidates to study and to identify novel bacterial communication networks.

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1 Quorum Sensing in Gram-Negative Bacteria

Bacteria occupy complex environments in nature and often live in close association with other organisms. In order to survive in complex mixed communities, bacteria constantly need to monitor and communicate with their surroundings and to adapt their behaviour accordingly. To process external information and to generate an intracellular response, bacteria have developed several systems, which act either at single cell level, like chemotaxis or two-component systems, or at the population level, like quorum-sensing systems. Regulation of bacterial behaviour in a population density-dependent manner via the use of small signalling molecules is referred to as 'quorum sensing' (QS) (Nealson and Hastings 1979). QS pathways are widespread among microorganisms and several processes are regulated via QS systems in bacteria, such as bioluminescence, antibiotic production, virulence, biofilm formation, motility, and sporulation (Waters and Bassler 2005). The major signalling molecules used for QS can be classified into two different groups among Gram-negative and Gram-positive bacteria. Typically, Gram-negative bacteria use small diffusible molecules, the acyl-homoserine lactones or 'AHLs' derived from fatty acids, whereas Gram-positive bacteria use peptide derivatives for QS-dependent communication. However, one exception is the Gram-positive bacterium *Exiguobacterium* sp. isolated from marine water, which was found to use AHLs for quorum sensing (Biswa and Doble 2013). As *Photorhabdus* species are Gram-negative, here we focus on quorum sensing and LuxR-type receptors in Gram-negative bacteria.

The prototypical quorum sensing system of Gram-negative bacteria consists of a LuxI-like autoinducer synthase that produces AHLs as signals and a LuxR-type receptor that detects the AHLs to control expression of specific genes (Waters and Bassler 2005). AHLs are constantly synthesised by LuxI at a low basal level and sensed by the cognate LuxR-like receptor when they exceed a certain threshold concentration. Upon AHL-binding, LuxR binds to the promoter/operator regions of the target genes/operons adapting expression of several genes in response to the cell count (Fig. 1). Thus, bacteria respond to AHLs and adapt bacterial group-behaviour

by regulation of gene expression when bacterial cell density reaches a certain level or 'quorum'. LuxI/LuxR-based quorum sensing systems have been intensively studied. The first system was discovered in *Vibrio fischeri* demonstrating that AHLs are used to regulate light production dependent on cell density (Nasser and Reverchon 2006). Furthermore, LuxR-based cell–cell communication is medically relevant as many pathogenic bacteria use these quorum-sensing systems for effective infection (Rutherford and Bassler 2012). Traditionally, it was thought that each LuxR-type receptor requires a cognate LuxI-like homolog producing the AHL-signalling molecule. However, many proteobacterial genomes encode LuxR homologs lacking a cognate LuxI synthase. These LuxR homologs are designated as LuxR 'orphans' (Patankar and González 2009) or LuxR 'solos' (Subramoni and Venturi 2009). They can exist in AHL-producing bacteria besides an entire LuxI/LuxR system or in bacteria that do not produce any AHLs (Fig. 1).

In general, a LuxR-type receptor is composed of two functional domains, a N-terminal signal-binding domain (SBD) and a C-terminal DNA-binding domain (DBD) (Nasser and Reverchon 2006). The DBD contains the conserved "HTH LUXR" helix-turn-helix motif, which is typical for LuxR-type proteins. Furthermore, LuxR-type regulators are usually transcriptional activators. The N-terminal SBD is important for signal binding, binding specificity, and shaping of

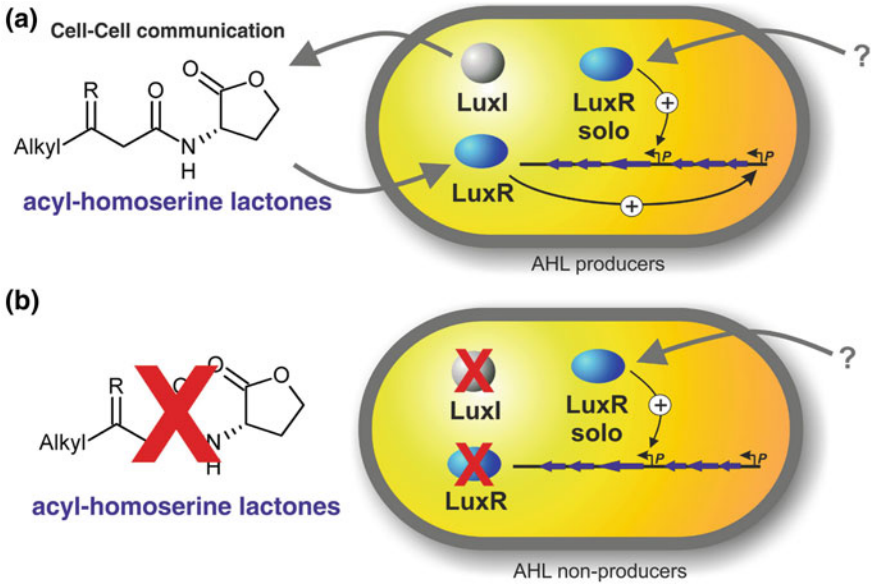

Fig. 1 Quorum sensing and LuxR solos in Gram-negative bacteria. The prototypical quorum sensing system in Gram-negative bacteria is the LuxI/LuxR system, which uses acyl-homoserine lactones (AHLs) for signalling that are produced by LuxI and sensed by the cognate LuxR-type receptor. However, many proteobacteria contain LuxR-type receptors that are not paired with a LuxI-type AHL-synthase. Those so called LuxR orphans or solos occur in AHL-producing (**a**) or AHL non-producing bacteria (**b**). The LuxR-type receptors are drawn in *blue*. R = H/H, H/OH, O

the ligand-binding pocket. Additionally, LuxR-type receptors share a low protein sequence identity (18–25 %). However, nine amino acids are highly conserved within this protein family. The DBD contains three conserved amino acids (E178, L182 and G188, with respect to the LuxR-type receptor TraR of *Agrobacterium tumefaciens*), which are important for DNA-binding. The SBD harbours six conserved amino acids (W57, Y61, D70, P71, W85 and G113, with respect to TraR) that are important for AHL-binding (Fuqua et al. 1996; Patankar and González 2009).

Besides the LuxR-type receptor, the LuxI-type synthase is part of a classical QS system, which synthesises the signalling molecule. LuxI-type synthases are able to synthesise distinct AHLs depending on the precursors, hence the lengths of the acyl moieties of the AHLs can vary between 4 and 18 C-atoms. The third C-atom in the acyl chain can be either a carbonyl group, a hydroxy group or a methylene moiety (Whitehead et al. 2001). These structural differences among the AHLs play a crucial role for signalling specificity of QS LuxR-type receptors in different bacterial species (Kim et al. 2014). Moreover, the signalling molecule is termed autoinducer if transcription of *luxI* is positively regulated via the cognate LuxR-type regulator since this process further boosts AHL synthesis and therefore QS response (Fuqua and Winans 1994). The basic chemical structure of each AHL is identical, but the different AHLs side chains make up certain specificity for the cognate LuxR-type receptor. For that reason, AHLs have been supposed to be one chemical bacterial language, whereas the various derivatives can be treated as different bacterial 'dialects' (Brameyer et al. 2015a).

Furthermore, QS regulatory systems can be more complex as several distinct QS circuits can be present in one bacterium that are inter-connected to each other. For example, *Pseudomonas aeruginosa* harbours three QS systems, the two systems LasI/LasR and RhlI/RhlR respond to AHLs and the third system is dependent on the *Pseudomonas* quinolone signal (PQS) sensed by PqsR and primary synthesised by the *pqsABCD* gene products (Gallagher et al. 2002). Deletion of genes encoding the QS system components reduced *P. aeruginosa* virulence in mice. Moreover, this QS regulatory network controls the expression of different virulence determinants in a hierarchical manner (Martínez 2014; Lee and Zhang 2015). Assuming that a single QS system is sufficient to perceive cell density, a complex QS circuit additionally allows social exploitation and kin recognition (Even-Tov et al. 2016).

2 LuxR Solos

Many proteobacteria posses LuxR 'solos' (Subramoni and Venturi 2009), meaning LuxR-type receptors that are not paired with a cognate LuxI synthase. LuxR solos are supposed to respond to different signals, like exogenous or endogenous AHLs, non-AHLs or eukaryotic signals, and thereby expand the signalling network and influence different cellular processes (Subramoni and Venturi 2009). LuxR-type proteins are mainly restricted to proteobacteria, however recently also few

non-proteobacterial sequenced genomes were also found to carry genes that encode LuxR-type proteins. The majority of bacteria contain one or more LuxR solos, either with or without a classical entire QS system (Subramoni et al. 2015). Moreover, only about 26 % of the 265 proteobacterial genomes analysed yet contain genes encoding a complete classical QS circuit (Case et al. 2008). It is therefore obvious that the majority of bacteria use additional and different, yet unexplored, QS systems.

In AHL-producing bacteria, LuxR solos can sense endogenous or exogenous AHLs, and therefore extend the QS regulon to additional target genes. Furthermore, they can respond with different affinities towards specific AHLs and also recognise ligands produced by the surrounding bacterial community. Well-characterised LuxR solos in AHL-producing bacteria are BisR of *Rhizobium leguminosarum* bv. *viciae*, ExpR of *Sinorhizobium meliloti* and QscR of *P. aeruginosa*, which are functional integrated in a resident AHL-mediated QS system (Subramoni and Venturi 2009).

In AHL non-producing bacteria LuxR solos are able to bind exogenous signalling molecules, either AHLs or even other signals produced by eukaryotes, like hormones. These LuxR solos enable bacteria to 'listen' to their bacterial neighbours and benefit from this information to adapt their behaviour according to their environment (Subramoni and Venturi 2009; Hudaiberdiev et al. 2015). In plant-associated bacteria, a new subfamily of LuxR solos was recently described to respond to low molecular weight plant compounds. These LuxR solos are present in beneficial and pathogenic plant-associated bacteria, including members of xanthomonads, rhizobia, agrobacteria and pseudomonads, and are important for plant–bacteria interactions (González and Venturi 2013).

All three *Photorhabdus* species, *Photorhabdus luminescens*, *Photorhabdus temperata* and *Photorhabdus asymbiotica*, contain an unusually high number of genes that encode potential LuxR solos and are therefore assumed to have a huge capacity for cell–cell and/or inter-kingdom communication (Heermann and Fuchs 2008; Wilkinson et al. 2009). *Photorhabdus*-specific LuxR solos share the conserved C-terminal HTH LuxR motif of LuxR-type regulators and are classified based on their distinct N-terminal SBD (Fig. 2). In summary, the presence of the very high number of LuxR solos in all three *Photorhabdus* species probably enable the bacteria to sense diverse signals during their life cycle, and the overall number might reflect the diversity of invertebrate or vertebrate hosts they can infect (Brameyer et al. 2014).

2.1 AHL-LuxR Solos

LuxR solos are not necessarily involved in QS processes and may alternatively collect information about the surrounding area (Subramoni and Venturi 2009). The LuxR-type receptor SdiA (Suppressor of cell division inhibitor A) is one prominent example of a LuxR solo that exists in bacteria that do not have a *luxI* gene and

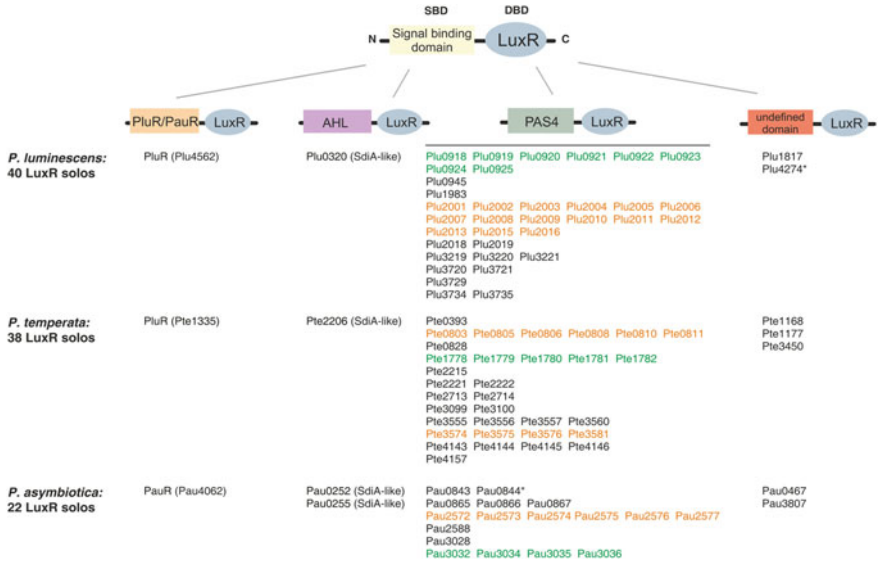

Fig. 2 Domain structure of the several LuxR solos in *P. luminescens, P. asymbiotica* and *P. temperata.* The three types of different LuxR solos and their homologues in *P. luminescens* TT01, *P. temperata* NC19 *and P. asymbiotica* ATCC43949 are shown. The "HTH LUXR" motif (SMART00421) is indicated by a *sphere*, the "Autoind_bind"-domain (PFAM03472) and the "PAS_4"-domain (PFAM08448) by *boxes shaped* in different colours. LuxR solos marked with an asterisk additionally have a predicted N-terminal transmembrane domain (not illustrated). PAS4-LuxR receptors encoded by homologous gene clusters are marked with similar colours (*orange* and *green*) (Brameyer et al. 2014)

therefore do not produce any AHLs, like *Escherichia coli* or *Salmonella enterica* (Ahmer 2004). SdiA homologs are known to detect 'exogenous' AHLs, in other words those produced by neighbouring bacteria enabling these organisms to sense and respond to mixed microbial communities (Michael et al. 2001). In *S. enterica*, SdiA regulates expression of the *rck* and the *srgE* operon in presence of AHLs that were produced by other bacteria (Ahmer et al. 1998; Michael et al. 2001). Since a temperature of 37 °C is essential for recognition of AHLs in *S. enterica*, it is assumed that SdiA is important for sensing the surrounding bacteria when passing the mammalian gut (Ahmer 2004). The SdiA-mediated gene expression in *E. coli* drastically differs from that in *S. enterica*, among others in the temperature optimum. SdiA activates expression of the *ftsQAZ* operon in *E. coli*, which encodes essential cell division proteins (Wang et al. 1991). Furthermore, SdiA is involved in regulating the expression of several genes that are responsible for diverse functions such as metabolism, motility, virulence, survival and defence mechanisms in the presence of AHLs (Kim et al. 2014). Therefore, it is assumed that SdiA-mediated gene expression in *E. coli* is important when changing hosts, especially when the bacteria are exposed to nutrient limitation and other stress outside of a mammalian host (Houdt et al. 2006).

All three *Photorhabdus* species contain at least one AHL-LuxR solo that is homologous to SdiA (Fig. 2). While *P. luminescens* and *P. temperata* each have one SdiA homologue, Plu0320 and Pte2206, respectively, the human pathogen *P. asymbiotica* has two SdiA homologues, Pau0252 and Pau0255 (Brameyer et al. 2014). All SdiA homologues in *Photorhabdus* species have a N-terminal AHL-domain, which is typical for AHL-sensors. Similar to SdiA, the four LuxR solos, Plu0320, Pau0252, Pau0255 and Pte2206 are assumed to detect AHLs as well. Since no *luxI* gene is present in any of the *Photorhabdus* genomes, it is most likely that these LuxR solos respond to exogenously produced AHLs, probably produced by bacteria of the insect gut, which are then sensed by *Photorhabdus* species during the infection process. As the human pathogen *P. asymbiotica* has two putative AHL-sensors, it is most likely that AHL-sensing plays an even more important role for vertebrate than invertebrate infection, putatively by sensing bacteria of the human skin surface. All *Photorhabdus* species have a huge potential to degrade AHLs and therefore to interfere with the QS of other bacteria (Brameyer et al. 2014). They all have several copies of lactonases and acylases, enzymes that hydrolyse the lactone ring and cleave the amide bond of AHLs, respectively. The expression of these different AHL-lactonases and/or AHL-acylases encoding genes could be activated via the respective SdiA homologues in *P. luminescens*, *P. temperata*, *and P. asymbiotica*, respectively, after sensing external AHLs. Overall, the defence of the dead insect host, which is exposed to competitors in the soil, is crucial for the survival of both *Photorhabdus* bacteria as well as their nematode symbionts and vectors. Thus, 'muzzling' the ambient mixed microbial community of the host and its communication might be an important step for a successful infection process and reproduction. It may also protect the dead insect cadaver from invading saprophytic bacteria as well. It has been proposed that the presence of a second SdiA-homologue in *P. asymbiotica* might be an important step for the successful infection of humans by the otherwise insect pathogen (Brameyer et al. 2014).

2.2 PluR/PauR-LuxR Solos

The three LuxR solos PluR (Plu4562) of *P. luminescens*, PauR (Pau4062) of *P. asymbiotica*, and PluR (Pte1335) of *P. temperata* have been previously predicted to be AHL-LuxR solos. However, these LuxR homologs do not sense AHLs, but instead sense photopyrones (PPYs) or dialkylresorcinols (DARs), respectively (Brachmann et al. 2013; Brameyer et al. 2015b). This altered ligand specificity is caused by a modified conserved amino acid motif within the SBD in PluR and PauR (Fig. 3b), which is intact in the SdiA homologues (Figs. 2 and 3a). This altered motif within PluR and PauR is essential for sensing the cognate signalling molecule (Brameyer and Heermann 2015). Amino acid Y61 of AHL-sensing LuxR-type receptors is also conserved in the PluR/PauR motif. This amino acid is known to be involved in binding of the acyl chain of the signalling molecule via hydrophobic interactions, for example in TraR (Churchill and Chen 2011) or LuxR

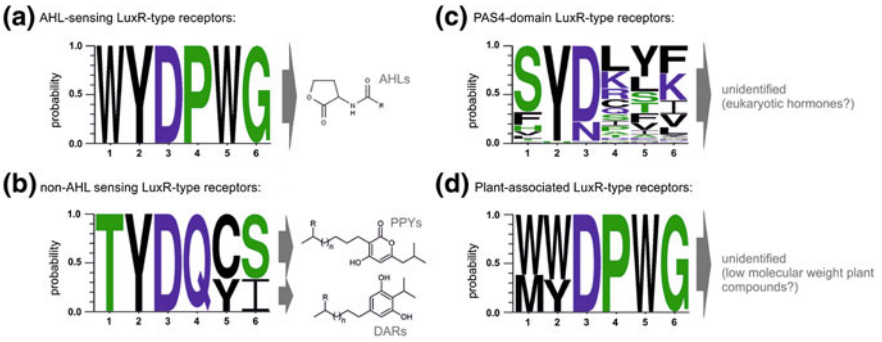

Fig. 3 Conserved amino acid motifs within the signal-binding domain of LuxR-type receptors and their corresponding signalling molecules. **a** Motif of the six conserved amino acid positions in AHL-sensors. Protein sequences of LuxR from *V. fischeri*, TraR from *A. tumefaciens*, SdiA from *Escherichia coli*, QscR and LasR from *Pseudomonas aeruginosa* were used to generate the alignment. **b** Motif of the six conserved amino acids positions of PluR (Plu4562) from *P. luminescens*, PluR (Pte1335) from *P. temperata* and PauR (Pau4062) from *P. asymbiotica* (Brameyer and Heermann 2015). PluR from *P. luminescens* and *P. temperata* sense photopyrones (PPYs) as signalling molecule and PauR senses dialkylresorcinols (DARs) (Brachmann et al. 2013; Brameyer et al. 2015b). **c** Motif of the six conserved amino acid positions of the overall 80 PAS4-LuxR solos in all three *Photorhabdus* species, whereas the corresponding signal molecules are yet unknown, but are possibly eukaryotic hormones (Brameyer and Heermann 2015). **d** Motif of the six conserved amino acids positions of LuxR solos from a subset of plant-associated bacteria possibly sensing low molecular weight plant compounds (Patel et al. 2013)

(Nasser and Reverchon 2006), and is also proposed to be involved in pyrone and dialkylresorcinol binding of PluR and PauR, respectively (Brachmann et al. 2013; Brameyer et al. 2015b). Furthermore, amino acid D70 of AHL-sensors like TraR is known to be important for binding the amide group of *N*-3-oxooctanoyl-L-homoserine lactone (Churchill and Chen 2011). Likewise, D75 (D70 with respect to TraR) of PluR and PauR is deduced to form a hydrogen bond to the hydroxy group attached to the pyrone and the DAR-hydroxy group, respectively (Brachmann et al. 2013; Brameyer et al. 2015b). Therefore, substitution of the conserved amino acid D75 of each PluR and PauR highly decreased recognition of the cognate signalling molecule. Certainly in PluR and PauR, the size and charge of amino acid at position D75 mediates correct signalling molecule binding since substitution against glutamic acid impaired conformation and substitution against asparagine affects binding of PPYD or DAR, respectively. The LuxR solos PluR and PauR are part of novel types of quorum-sensing systems (Brachmann et al. 2013; Brameyer et al. 2015b), which are described below in more detail.

2.3 PAS4-LuxR Solos

The majority of the LuxR solos in *Photorhabdus* contain a N-terminal PAS4 signal-binding domain (Fig. 2). Commonly, PAS (Per-ARNT-Sim) domains are ubiquitous, they are present in archaea, eubacteria and eukarya and are involved in binding of a diverse set of small regulatory molecules either covalent or non-covalent (Hefti et al. 2004). In the fruit fly *Drosophila melangonaster* PAS3 domains have been proven as insect juvenile hormone (JH) receptors (Dubrovsky 2005). The homologous PAS4 domains in *Photorhabdus* are assumed to bind hormone-like molecules and are therefore supposed to be major players in inter-kingdom signalling via the detection of hormone-specific signals from the eukaryotic hosts (Heermann and Fuchs 2008). However, LuxR solos with PAS4 domains of *Photorhabdus* species are assumed to be adapted to signals from the invertebrate hosts (insects and nematodes) or in case of *P. asymbiotica* additionally to vertebrate hosts, especially humans (Brameyer et al. 2014; Wilkinson et al. 2009). The majority of the genes encoding the PAS4-LuxR solos in *Photorhabdus* species are organised in the large gene clusters *plu0918-0925*, *plu2001-2016*, *pau2572-2577*, *pau3032-3036*, *pte0803-0811*, *pte1778-1782* and *pte3574-3581*. The function of this redundancy of PAS4-LuxR solos is still unclear. However, they might respond to a diverse set of eukaryotic signals displaying the wide range of insect hosts they can infect to specifically adapt toxin production and therefore pathogenicity. For that reason, the high redundancy of PAS4-LuxR solos might be a co-evolutional result by adaptation of the bacteria to a broad diversity of insect hosts. This idea is underlined by the fact that several LuxR solos from plant-associated bacteria are known to respond to plant signalling molecules and are therefore assumed to have undergone a co-evolution with the related host plant (Covaceuszach et al. 2013; González and Venturi 2013). *Photorhabdus*-specific LuxR solos with a N-terminal PAS4-domain show diverse variations in the WYDPWG-motif of AHL-sensing LuxR-type regulators, possibly reflecting a higher variety of signals they might sense (Fig. 3c). However, the precise signals that are sensed by PAS4-LuxR solos are yet unknown. The huge diversity of the SBD motifs in PAS4-LuxR solos of *Photorhabdus* and the variations in the conserved amino acid motifs probably gives the bacteria the capacity to respond to a broad range of signals that occur in the different environments. High variability within the WYDPWG-motif in the SBD also occurs in LuxR solos of plant-associated bacteria, which would allow the perception of a broad range of low molecular weight plant compounds rather than AHLs (Fig. 3d). Remarkably, the highly conserved amino acid Y61 of AHL-sensing LuxR-type regulators (with respect to TraR) is present in 97 % of the LuxR solos of *Photorhabdus* species (Brameyer et al. 2014), whereas it is altered in the majority of the LuxR solos from plant-associated bacteria (Fig. 3). This lends support to the idea that different classes of signals can be detected which are specific to either invertebrates or plants.

3 Quorum Sensing in *Photorhabdus*

Photorhabdus bacteria have the capacity to switch readily between different hosts, for example they can colonise either different insects or (in the case of *P. asymbiotica*) humans following their release from their vector *Heterorhabditis* nematodes. Therefore, *Photorhabdus* bacteria constantly need to monitor the local environment and their current host (nematode, insect or man) to appropriately regulate gene expression. Moreover, the behaviour of the whole population needs to be adapted and communicated to be either symbiotic (with its nematode vector) or pathogenic (to either insects or men). Since *Photorhabdus* species do not harbour a LuxI synthase homologue and are therefore unable to produce AHLs, cell–cell communication must be mediated via different signalling molecules. Recently, two LuxR solos sensing non-AHL signalling molecules were described that are used for QS-mediated communication in *Photorhabdus* species both contributing to pathogenicity. One is the LuxR solo PluR from *P. luminescens* and *P. temperata* and the other the LuxR solo PauR from *P. asymbiotica* (Brachmann et al. 2013; Brameyer et al. 2015b). These LuxR solos are not paired with a LuxI synthase.

Notably, *P. luminescens* harbours a LuxS-synthase homolog and has been shown to produce autoinducer-2 (AI-2), which is a furanosyl-borate diesther molecule (Schauder et al. 2001), and acts as a QS molecule as well (Joyce et al. 2011). However, this compound has also been suggested to act as a universal QS signalling molecule in all bacteria (Winzer et al. 2002). LuxS has been demonstrated to influence the carbapenem antibiotic synthesis in *P. luminescens* (Derzelle et al. 2002; Coulthurst et al. 2005). Overall, more than 300 genes have been identified to be under control of LuxS. These genes are involved in metabolism, regulation and general stress response, as well as in pathogenicity. A deletion of *luxS* in *P. luminescens* resulted in reduced biofilm formation and reduced pathogenicity against insects (Krin et al. 2006) However, the detailed molecular mechanisms of LuxS-mediated signalling in *P. luminescens* are still unclear.

3.1 The PpyS/PluR Quorum-Sensing System of P. luminescens *and* P. temperata

The LuxR solo PluR was formerly annotated to contain a putative AHL-binding domain in the N-terminal signal-binding domain (SBD). However, it has been found that this regulator does not recognise AHLs signalling molecules but α-pyrones named ‘photopyrones’ (PPYs) (Brachmann et al. 2013). The ketosynthase enzyme PpyS is necessary and sufficient for the synthesis of eight different PPYs, depending on the precursors from the fatty acid metabolism. A two-chain biosynthesis step has been described for the synthesis of photopyrones where thioester-activated 9-methyldecanoic acid is first covalently bound to an active site cysteine, and then deprotonated (Kresovic et al. 2015). This results in the formation

of a nucleophile, which subsequently attacks the carbonyl carbon of a 5-methyl-3-oxohexanoyl thioester that is formed by the BkdABC pathway (Brachmann et al. 2012) to form a new carbon–carbon (C–C) bond. After an additional deprotonation of the bound intermediate the α-pyrone ring is formed and the respective PPY is released from PpyS (Kresovic et al. 2015). Due to the variability of the first substrate regarding chain length and starting unit, different PPYs are produced. PPYs are produced in standard complex laboratory media, as well as in insect larvae. Furthermore, production of PPYs is dependent on the cell density of a *Photorhabdus* population. Eight α-pyrones (PPYA-PPYH) were isolated from *P. temeperata* spp. *thracensis*, whereas only three (PPYA, PPYB and PPYD) are produced by *P. luminescens*. These three PPYs are all sensed with altered specificity. PluR most specifically senses PPYD with the highest sensitivity in a concentration as low as 3.5 nM, but is also able to sense the other PPYs but only at higher concentrations. It has been proposed that, similar to AHLs, PPY-sensing can be seen as a novel kind of bacterial 'language', whereas the different PPYs can be compared to different bacterial 'dialects' (Brameyer et al. 2015a).

Upon PPY-binding, PluR activates expression of the adjacent *pcfABCDEF* operon. The *pcf* operon encodes a synthesis pathway for a 'clumping' factor named PCF (*Photorhabdus* Clumping Factor) as induction of the operon caused intense cell clumping (Fig. 4a). Insecticidal bioassays using normally harmless *E. coli* cells over-expressing *pcfABCDEF* resulted in high mortality of Greater waxmoth, *Galleria mellonella*, larvae. This led to the conclusion that PCF is a virulence factor that contributes to the high pathogenicity of the bacteria (Brachmann et al. 2013). The entire QS system comprising PluR, the PluR-target operon *pcf*, and PpyS was functionally reconstituted in *E. coli* confirming the nature of this novel cell-cell communication circuit. However, deletion of *pluR* in *P. luminescens* did not result in decreased pathogenicity of the bacteria against insects (Brachmann et al. 2013), but in a decreased symbiotic association with entomopathogenic *Heterorhabditis* nematodes, revealing that cell clumping and therefore QS must also be important for symbiosis with the nematodes (our own unpublished data).

The class of α-pyrones is not unique to *Photorhabdus*, but were correlated to cell–cell communication for the first time with these bacteria. α-pyrones are well-known secondary metabolites in species like pseudomonads (Chu et al. 2002; Kong et al. 2005), fungi (Elbandy et al. 2009) and streptomycetes (Chemler et al. 2012). In streptomycetes pyrones are involved in spore germination (Aoki et al. 2011). Thus, these organisms might use pyrones as signalling molecules beside their other known functions as well.

3.2 *The DarABC/PauR Quorum Sensing System of* P. asymbiotica

The insect and human pathogen *P. asymbiotica* harbours a *pcf* regulon including the LuxR solo PauR that is highly homologous to PluR of *P. luminescens*. However,

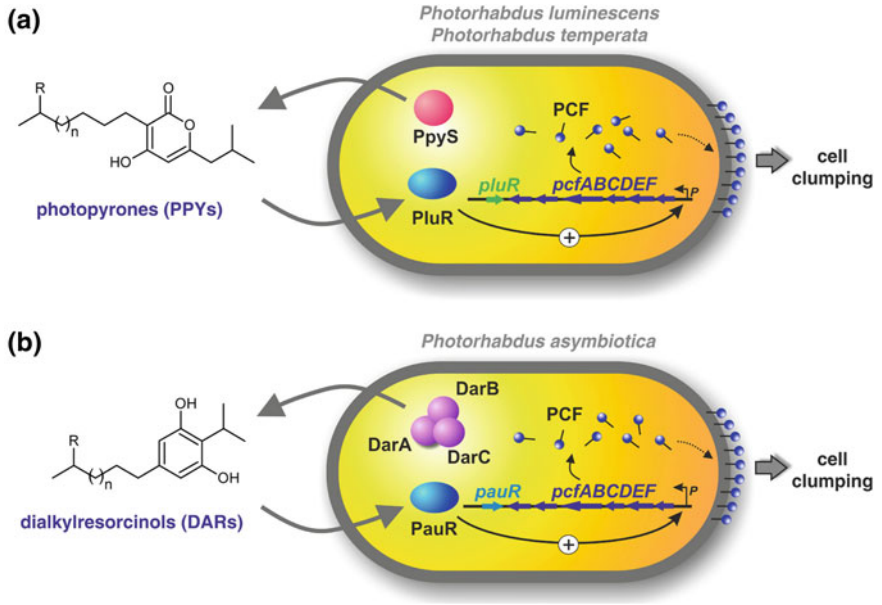

Fig. 4 Quorum sensing systems in *Photorhabdus* species. **a** In *P. luminescens* and *P. temperata* QS to control cell clumping is mediated via photopyrones (PPYs) as signalling molecules, which are produced by the photopyrone synthase PpyS and sensed by the LuxR solo PluR (Brachmann et al. 2013). **b** *P. asymbiotica* uses dialkylresorcinols (DARs) as signalling molecules to control cell clumping, which are synthesised by the DarA/DarB/DarC pathway and sensed by the LuxR solo PauR (Brameyer et al. 2015). The LuxR solos PluR and PauR are both drawn in *blue*. R = H or Me, n = 1–4

P. asymbiotica contains neither a PpyS nor a LuxI homologue and does therefore not produce PPYs or AHLs. The signalling molecules sensed by PauR have been identified to belong to the class of dialkylresorcinols (DARs). Upon binding of 2,5-dialkylresorcinol (DAR) to PauR, cell clumping is induced due to expression of the *pcf* operon (Fig. 3b) (Brameyer et al. 2015b). DARs and its biochemical precursors cyclohexanediones (CHDs) are synthesised by enzymes encoded by the *darABC* operon. However, *P. asymbiotica* produces a specific subset of DARs and CHDs depending on the precursors derived from two fatty acids (Fuchs et al. 2013). PauR most specifically recognises DAR at concentrations as low as 5 nM (Brameyer et al. 2015b). Plenty of bacteria possess DarABC homologs, several of them being pathogenic to animals, plants or humans, like various *Neisseria* strains for example (Fuchs et al. 2013). Therefore, the presence of the *darABC* operon and the respective metabolites might be linked to virulence. DAR derivatives have also been described as antibiotics (Joyce et al. 2008), cytotoxins (Kronenwerth et al. 2014), free radical scavengers (Kato et al. 1993), and growth-stimulating factors (Imai et al. 1993). Furthermore, DARs produced by *Pseudomonas* sp. can have antimicrobial properties (Pohanka et al. 2006). Therefore, it might be possible that

DAR-dependent regulation of virulence in general might be more important for colonisation of vertebrates than for invertebrates. It has been suggested that DARs might also be used as QS molecules in many more bacteria, even when they co-occur with LuxR solos. Similar to the PCF of *P. luminescens*, heterologous expression of the *P. asymbiotica pcf* operon in *E. coli* also lead to insect pathogenicity and clumping of normally non-pathogenic *E. coli* cells. In contrast to *pluR* in *P. luminescens*, deletion of *pauR* in *P. asymbiotica* resulted in highly decreased pathogenicity towards *G. mellonella* insect larvae (Brameyer et al. 2015b). For that reason, DAR-mediated QS systems might be a useful and specific target for novel antimicrobials in human pathogenic bacteria.

4 Outlook and Future Perspectives

In the past 10 years several signalling molecules beside AHLs have been implicated in QS-regulated processes, like aryl-HSLs from *Bradyrhizobium* and *Rhodopseudomonas* (Schaefer et al. 2008), photopyrones from *P. luminescens* and *P. temperata* (Brachmann et al. 2013) and dialkylresorcinols from *P. asymbiotica* (Brameyer et al. 2015b). Besides different AHLs, *P. aeruginosa* also uses 2-heptyl-3-hydroxy-4-quinolone (PQS) and the newly identified 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) as languages (Lee et al. 2013). Hence, signalling molecules that are not AHLs appear to play an important role in bacterial communication circuits that have been identified so far, and probably many more await discovery. Moreover, LuxR solos emerge to be more and more important players in cell–cell communication or inter-kingdom signalling as they offer possibilities to use alternative communication molecules to AHLs (Venturi and Ahmer 2015). All three *Photorhabdus* species have an extraordinary high number of LuxR solos, which makes them to optimal model organisms for studying the function of LuxR solos in bacteria. *Photorhabdus*-specific LuxR solos contain different SBDs, which include diverse amino acid motifs at conserved positions compared to AHL-sensing LuxR-type regulators. The diversity of these motifs gives rise to the speculation that signal-binding of all these LuxR solos goes far beyond AHL-signalling as it has been demonstrated for PluR and PauR (Brachmann et al. 2013; Brameyer and Heermann 2015; Brameyer et al. 2015b). Thereby, regulation via LuxR solos is probably important at different steps in the *Photorhabdus* life and infection cycle (Fig. 5). One can only guess the variety of signals perceived by all these LuxR solos and their function in cell–cell communication and inter-kingdom signalling. It will be the goal of future research to unravel the various signalling molecules and correlate them to the specific LuxR solos or amino acid motifs in the signal-binding domain of these proteins. The presence of all those different types of LuxR solos gains first insight into the complexity of the communication network between bacteria among each other and with their hosts. Since most of the LuxR solos that have been investigated so far are involved in regulation of pathogenicity, the homologous receptors or the related

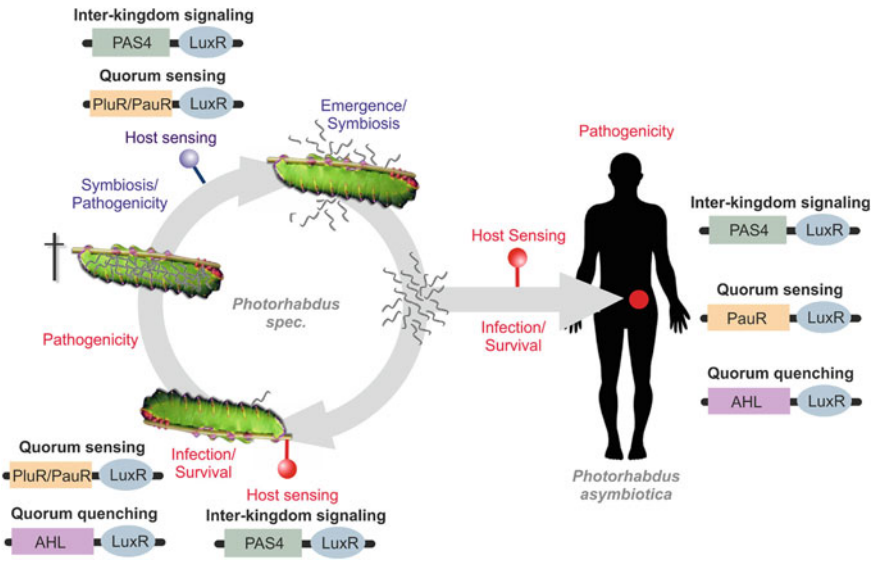

Fig. 5 Overview of the possible role of LuxR solos during life and infection cycle of *Photorhabdus* species. The bacteria colonise the upper gut of heterorhabditis nematodes that invade insect larvae. After release into the insect's hemolymph the bacteria produce several toxins that rapidly kill the prey. After death of the insect host, the bacteria degrade the cadaver and additionally support nematode development. When the cadaver is depleted from nutrients, bacteria and nematode re-associate and leave the carcass in search for a new victim. *P. asymbiotica* can additionally infect humans by inducing systemic and soft tissue infections (*right panel*). The *red* (pathogenic) or *blue* (symbiotic) tags indicate the points within the life cycle where the bacteria switch hosts. Putative involvement of the different LuxR solos (*same colour* code as in Fig. 2) at steps of the infection process or host sensing is indicated at the respective position of the life cycle (see text for details). The figure is modified after Brameyer et al. (2014)

signalling molecules in human pathogens are promising specific drug targets of novel antimicrobials.

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