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REVIEW

Evolution of LuxR solos in bacterial communication: receptors and signals

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Abstract Cell–cell communication in bacteria needs chemical signals and cognate receptors. Many Gram-negative bacteria use acyl-homoserine lactones (AHLs) and cognate LuxR-type receptors to regulate their quorum sensing (QS) systems. The signal synthase-receptor (LuxI–LuxR) pairs may have co-evolved together. However, many LuxR solo (orphan LuxR) regulators sense more signals than just AHLs, and expand the regulatory networks for inter-species and inter-kingdom communication. Moreover, there are also some QS regulators from the TetR family. LuxR solo regulators might have evolved by gene duplication and horizontal gene transfer. An increased understanding of the evolutionary roles of QS regulators would be helpful for engineering of cell–cell communication circuits in bacteria.

Keywords Cell–cell communication · LuxR solo · Multi-drug resistance · Quorum sensing · TetR family

Introduction

Bacteria communicate with each other for their adaption and survival in the complex environment (Mukherjee and Bassler 2019; Sperandio et al. 2003). The representative bacterial communication system is quorum sensing (QS), which involves signals and receptors (Waters and Bassler 2005; Whiteley et al. 2017). The dependency of QS relies on the signal concentration in a bacterial quorum, silent at low level and active at high level (Mukherjee and Bassler 2019; Waters and Bassler 2005). In the Gram-negative bacteria, acyl-homoserine lactones (AHLs) are widely used as signaling molecules (Camilli and Bassler 2006; Welsh and Blackwell 2016). AHLs are biosynthesized by LuxI-type synthases and sensed by cognate LuxR-type receptors, which may have co-evolved together as LuxI–LuxR pairs (Gray and Garey 2001; Lerat and Moran 2004). However, many LuxR solo (orphan LuxR) regulators, which are not associated with a synthase but sense more signals than just AHLs, have been characterized (Patankar and Gonzalez 2009; Subramoni et al. 2015; Subramoni and Venturi 2009; Venturi and Ahmer 2015).

AHL molecules and cognate LuxR receptors have been well summarized on the Quorum Sensing Site (www.nottingham.ac.uk/quorum/index.htm). The characterized LuxR solos and their roles in molecular regulation and evolution were also reviewed previously (Patankar and Gonzalez 2009; Subramoni and

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Venturi 2009). LuxR solos, which are widespread in the Proteobacteria, are major regulators in bacterial communication systems (Rajput and Kumar 2017; Subramoni et al. 2015). LuxR solos sense AHLs and other signals, and play diverse signaling roles in eavesdropping, intra-species, and inter-kingdom communication by extending bacterial regulatory networks (Patankar and Gonzalez 2009; Subramoni et al. 2015). The known diversity of signals and receptors in microbial communication is still expanding (Chen et al. 2002; Mukherjee and Bassler 2019). Here this mini-review mainly focuses on the evolution of LuxR solo regulator homologs in cell–cell communication.

Evolution of LuxR solo regulator homologs

In order to study the evolution of LuxR solo homologs, I conducted phylogenetic analyses by MEGA6 using the Neighbor-Joining method (Tamura et al. 2013). It indicates that many LuxR solos are clustered together with LuxR homologs from the same species (Fig. 1). For example, the LuxR solo TrlR and the AHL receptor TraR in *Agrobacterium tumefaciens*, the ExpR2 and ExpR in *Erwinia chrysanthemi*, the CarR and SmaR in *Serratia odorifera*, and the BisR and CinR in *Rhizobium leguminosarum*, etc (Supplementary Table 1). It's speculated that gene duplication and divergence might play a main role during the evolution of LuxR solo homologs (Lerat and Moran 2004; Patankar and Gonzalez 2009). Whereas in some bacteria, the LuxR homologs are relatively scattered in different clusters (Fig. 1); such as the LuxR solo QscR and the AHL receptors LasR and RhlR in *Pseudomonas aeruginosa*, and the LuxR solos AviR, AvhR and the AHL receptor AvsR in *Agrobacterium vitis*, etc (Chugani and Greenberg 2014; Hao and Burr 2006). This suggests that the horizontal gene transfer might be involved during their evolution process (Lerat and Moran 2004; Patankar and Gonzalez 2009; Subramoni et al. 2015).

Most ligands of these LuxR receptor homologs are AHLs with similar core structure and variable branches (Fig. 1). However, some functionally characterized LuxR solos have been reported to sense signals form other bacteria and even their host plants as well as endogenously produced non-AHLs (Brameyer and Heermann 2017; Gonzalez and Venturi 2013; Patel et al. 2013). For example, PauR (*Photorhabdus asymbiotica*) senses dialkylresorcinols (DARs), PluR (*Photorhabdus luminescens*) senses photopyrones (PPYs), and QscR (*P. aeruginosa*) detects AHLs produced by other bacterial species (Brachmann et al. 2013; Brameyer et al. 2015; Ha et al. 2012). On the phylogenetic tree, these LuxR solos are not clustered separately from the LuxR-type receptors that have a cognate synthase (Fig. 1). It seems that only these LuxR solos which might respond to plant compounds are clustered as a separate clade (Patel et al. 2013; Subramoni et al. 2011). For instance, XccR (*Xanthomonas campestris*), OryR and XocR from (*Xanthomonas oryzae*), and PsoR (*Pseudomonas fluorescens*) may play roles in the inter-kingdom communication with plants (Gonzalez et al. 2013; Subramoni et al. 2011; Xu et al. 2015; Zhang et al. 2007). It's speculated that these unknown ligands might be some plant hormones or signals mediating host-microbes interactions (Amin et al. 2015; Kabbara et al. 2018; Wang et al. 2017; Xu et al. 2018). Recently, PipR from *Pseudomonas* sp. GM79 was determined to sense an ethanolamine derivative, *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino) acetamide (HEHEAA) (Coutinho et al. 2018; Schaefer et al. 2016).

Structure-based multiple sequence alignment (MSA) analyses were assembled using MUSCLE and visualized using ESPript 3.0 server (Robert and Gouet 2014; Tamura et al. 2013). As shown in Supplementary Fig. 1, LuxR_Vfi (*Vibrio fischeri*) homologs have several conserved residues both in the ligand-binding domain (Y-W-Y-DP-W-A-G-G) and the DNA-binding domain (E-W-GK-I-V) (Bottomley et al. 2007; Patankar and Gonzalez 2009). Although LuxR solos sense other signals than AHLs, they still share relatively high ($\sim$ 50%) homology with these canonical LuxR-type receptors. Especially, the DNA-binding domain is highly conserved, while only the ligand-binding domain has local variations (Patankar and Gonzalez 2009). The variations in the ligand binding domain result in selective detection of cognate signals by LuxR-type receptors.

Protein structure modeling using evolutionary information was conducted by the SWISS-MODEL server (Biasini et al. 2014). The predicted structure of LuxR_Vfi is very similar to the reported LuxR family receptor homologs (Supplementary Fig. 2); such as

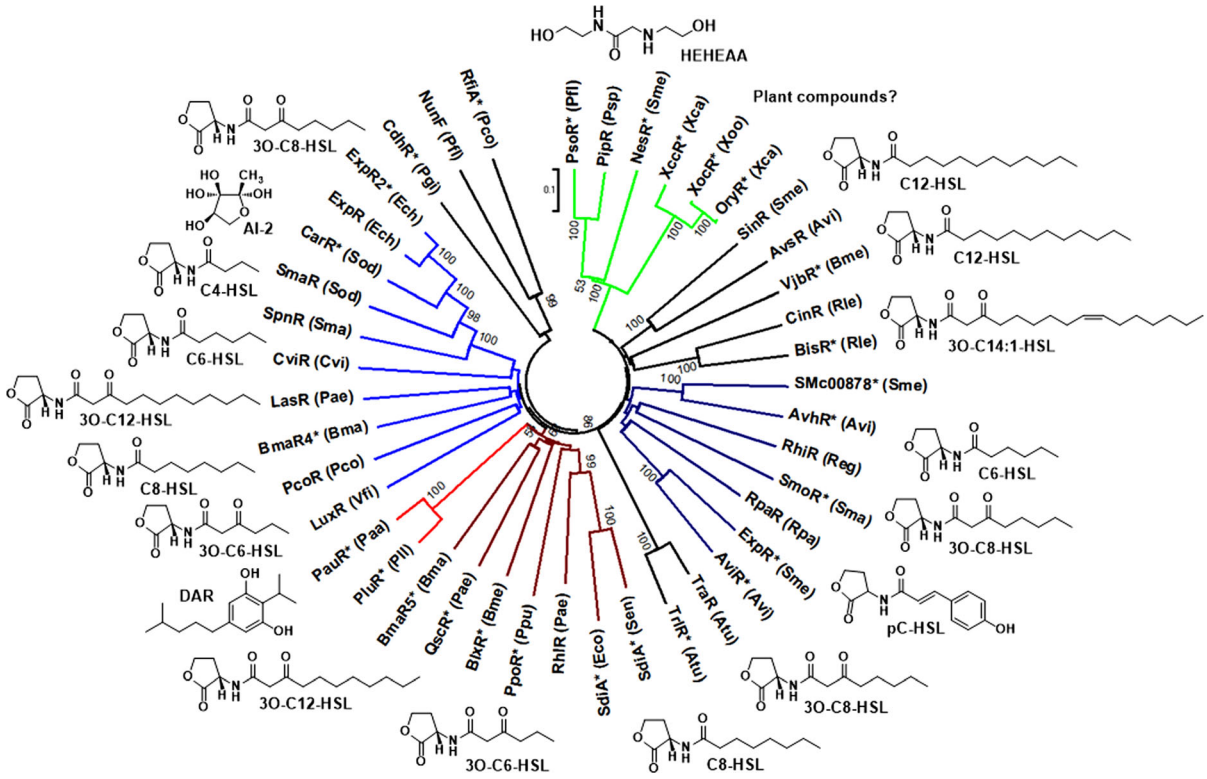

Fig. 1 Evolutionary relationships of the LuxR solo homologs in Gram-negative bacteria. Phylogenetic analyses were conducted in MEGA6 using the Neighbor-Joining method (Tamura et al. 2013). The evolutionary distances were computed using the p-distance method. Only bootstrap test (1000 replicates) values more than 50% are shown. Some specific ligands of these LuxR solo (*) and LuxR receptors are shown with chemical structures.

SidA, TraR, CviR, LasR and QscR, which is likely to indicate common folds (Bottomley et al. 2007; Chen et al. 2011; Lintz et al. 2011; Yao et al. 2006; Zhang et al. 2002). The evolutionary conservation in LuxR_Vfi homologs was also estimated by the ConSurf server (Ashkenazy et al. 2016). These conservations may ensure the specificity of the DNA-binding target and the recognized ligands (Supplementary Fig. 3). The ligand binding domain of LuxR-type receptors is relatively variable and it seems likely that this variability leads to signal specificity during microbial communication.

Evolution of QS regulators in the TetR family

Interestingly, not all QS regulators are from the LuxR family; the known diversity of cognate receptors is still growing (Mukherjee and Bassler 2019; Papenfort and Bassler 2016; Whiteley et al. 2017). Some QS regulators belong to the TetR family, such as LuxR_Vha (*Vibrio harveyi*), SmcR (*Vibrio vulnificus*) and HapR (*Vibrio cholerae*) (Ball et al. 2017; De Silva et al. 2007; Kim et al. 2010). These SmcR and HapR homologs also have some relationships to the multi-drug resistance (MDR) regulators (Fig. 2). As listed in Supplementary Table 2, the SmcR is closely related to HapR, QacR, TtgR, and EthR, etc (Ball et al. 2017; Kim et al. 2018). Although these proteins have very low ($\sim$ 30%) sequence conservation (De Silva et al. 2007), they all have the similar secondary structure, which has several α-helixes (Supplementary Fig. 4). The predicted structure of the LuxR_Vha is also very similar to the QS regulators SmcR and HapR (Supplementary Fig. 5). The *N*-terminal has characteristic HTH motif for the DNA binding, with relatively high conservation. The *C*-terminal is a regulatory domain, which contains several α-helixes, with relatively low conservation (Supplementary Fig. 6). However, it also shows some similarity to the MDR regulators, such as

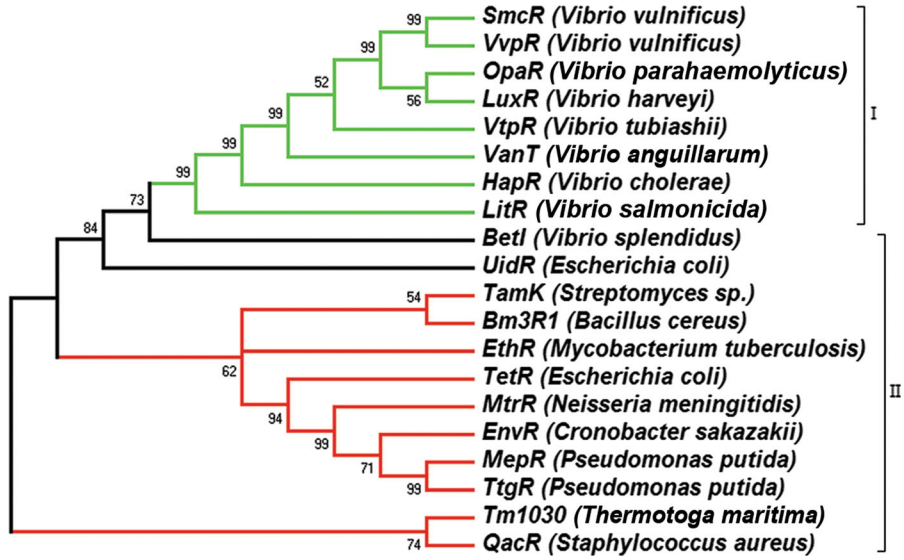

Fig. 2 Evolutionary relationships of the LuxR homologs (I) and the MDR regulators (II). Phylogenetic analyses were conducted in MEGA6 (Tamura et al. 2013) using the Minimum Evolution method with bootstrap test (1000 replicates). The 50% bootstrap consensus tree is shown.

QacR, TtgR and EthR (Alguel et al. 2007; Frenois et al. 2004; Schumacher et al. 2001). They all belong to the TetR family regulators, which have many other known biological functions. The highly similar overall structures suggest that these regulators might have some evolutionary relationships (Ball et al. 2017; Cuthbertson and Nodwell 2013; Yu et al. 2010).

Based on the reported crystal structures, these above mentioned QS regulators mainly divided into two types: the LuxR-family, like LuxR_Vfi, TraR, SidA, CviR, LasR, and QscR; and the TetR-family, such as LuxR_Vha, HapR, and SmcR, etc (Ball et al. 2017; Patankar and Gonzalez 2009). Although they have similar regulatory functions in the QS systems, the two types of regulators might have different evolutionary history (Gray and Garey 2001; Lerat and Moran 2004). Some of the ligand-binding domain is also relatively conserved, so that the AHL signal molecules they bind are very specific. Whereas, the ligand-binding pockets of the MDR regulators are relatively large enabling the recognition of various compounds with different structures (Cuthbertson and Nodwell 2013). Some TetR-like QS regulators, such as HapR, could also be regulated at the post-transcriptional level (De Silva et al. 2007). The higher structures of these regulatory proteins might determine the ligand-binding specificity for environmental adaption. Moreover, there are also the peptide-based systems, which widely distributed in Gram-positive bacteria, and the quinolone-based PQS system used by *Pseudomonas aeruginosa*, etc (Mukherjee and Bassler 2019; Waters and Bassler 2005).

In summary, LuxR solos might be obtained by gene duplication and horizontal gene transfer. They could sense endogenous and exogenous AHLs and signals other than AHLs. LuxR solos extend bacterial regulatory networks for more diverse communications, like eavesdropping, intra-species and inter-kingdom signaling. Moreover, there are also some QS regulators from the TetR family which are related to the MDR reguators. The chemical diversity of signals requires diverse cognate receptors for specific and efficient cell-cell communications. There are many roles for QS systems in biotechnology or bioengineering (Daniel et al. 2013; You et al. 2004). These new regulatory circuits could be useful tools for synthetic biology in the future (Biarnes-Carrera et al. 2015; Patankar and Gonzalez 2009).

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Supporting information Supplementary Table 1—Summary of characterized LuxR solo homologs in bacteria.

Supplementary Table 2—List of LuxR_Vha homologs in the TetR family.

Supplementary Fig. 1—Structure-based multiple sequence alignment (MSA) analysisof the LuxR_Vfi homologs.

Supplementary Fig. 2—Structure prediction and comparison of the LuxR_Vfihomologs.

Supplementary Fig. 3—The evolutionary conservation in

LuxR_Vfi homologsestimated and visualized by the ConSurf server (Ashkenazy et al. 2016).

Supplementary Fig. 4—Structure-based MSA analysis of the LuxR_Vha homologs.

Supplementary Fig. 5—Structure prediction of the LuxR_Vha and comparison withthe MDR regulators.

Supplementary Fig. 6—The evolutionary conservation in LuxR_Vha and MDRregulators estimated and visualized by the ConSurf server (Ashkenazy et al. 2016).

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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