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REVIEW

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New insights into the regulatory mechanisms of the LuxR family of quorum sensing regulators

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Abstract Bacteria use small signal molecules, referred to as autoinducers, in order to monitor their population density and coordinate gene expression in a process named quorum sensing. In Gram-negative bacteria, acylated homoserine lactones are the most common autoinducer used for cell-to-cell communication. Increasing evidence that many different functions are controlled by acylated homoserine lactone quorum sensing has stimulated intensive investigations into the physiology, molecular biology and biochemistry that underlie this process. Here we review our current understanding of the molecular mechanisms used by the transcriptional regulators responsive to acylated homoserine lactone autoinducers to control gene expression and the structural modifications induced by acylated homoserine lactones binding specifically on these regulators.

Keywords Quorum sensing · Cell population density · Bacterial autoinducer · Acylated homoserine lactone · LuxR · Transcriptional regulation · Enzymes · Pharmaceuticals · Nucleic acids (DNA|RNA)

Introduction

Many bacteria appear to use chemical hormone-like signals called *autoinducers* to regulate various physiological processes, including virulence, secondary metabolism and bioluminescence in response to fluctuations in cell population density [1, 2]. The concentration of the signal molecule increases concomitantly with bacterial cell density and, upon reaching a threshold concentration, the bacteria detect and respond to this signal by altering their gene expression. This phenomenon, named quorum sensing (QS), allows a population of bacteria to collectively control gene expression and synchronize group behaviour when the minimal number, or *quorum*, of bacteria has been reached. Bacteria use a wide variety of autoinducer molecules: oligopeptide signalling molecules are used primarily by Gram-positive bacteria [3]; acylated homoserine lactones (AHLs) are the most common signal used for cell-to-cell communication in Gram-negative bacteria [1]; and AI-2 molecules, derived from *S*-4,5-dihydroxy-2,3-pentanedione, are consideredto be universal signalling molecules used for interspecies communication [4].

This review will focus on AHL-dependent QS systems and will explore our current understanding of the molecular mechanisms used by the transcriptional regulators responsive to AHLs in the control of gene expression. Particular emphasis will be placed on the structural modifications induced by AHL binding on these regulators.

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Quorum sensing systems mediated by acylated homoserine lactones

Quorum sensing was first described in the LuxI/LuxR system of the Gram-negative bacteria *Vibrio fischeri*, a symbiotic species that provides its marine eukaryotic hosts with light [5]. This bacterium produces AHL molecules that were shown to be freely diffusible across the bacterial membranes and accumulate in the cell environment [6]. In Gram-negative bacteria, these AHL molecules act as cell density cues. The basic molecular system of AHL QS is mediated by two proteins: the enzyme responsible for AHL synthesis and a transcriptional regulator, the activity of which is modulated by AHL [1]. When bacteria proliferate, the concentration of AHL molecules increases intra- and extracellularly and, upon reaching a critical concentration, AHL interacts with the transcriptional regulator that in turn modulates the expression of the target genes. Many Gram-negative bacterial species have been reported to produce

AHLs differing in the length of the acyl-chain moiety and substitution at position C3, which is either unmodified or carries an oxo- or hydroxyl group (Fig. 1a). These structural differences in AHL molecules guarantee a good degree of selectivity for their specific recognition by their cognate regulators. This specificity of interaction is essential for bacteria to distinguish the AHLs produced by their own species from the AHLs that are produced by other species.

The AHL synthases

Three different AHL synthase families have been reported: the LuxI family, the AinS family and the HdtS family. The LuxI-type proteins are the major class of enzymes responsible for AHL synthesis, and they are present in more than 50 species, including α-, β- and γ-proteobacteria [7]. These enzymes use *S*-adenosyl-methionine (SAM) to synthesize the homoserine lactone ring, whereas the acyl chains come from lipid metabolism, carried by various acyl-carrier proteins (ACP) [8, 9]. The reaction is initiated by SAM binding to the synthase followed by the acylated-ACP, such that amide bond formation occurs and the holo-ACP is released prior to the lactonization and liberation of the AHL signal molecule [10]. Structural studies of two LuxI-type proteins, EsaI and LasI, which catalyse 3-oxo-C6-homoserine lactone (3OC6-HL) and 3-oxo-C12-homoserine lactone (3OC12-HL), respectively (Fig. 1b), revealed that in LasI, the acyl-chain binding pocket is a tunnel which permits the end of the acyl-chain to protrude, whereas in EsaI the acyl-chain binding pocket is closed. The residues in EsaI that occlude the pocket are larger than those in the same position in LasI, thus limiting the acyl-chain size to a C6 acyl chain [11, 12]. Therefore, the selectivity of the acyl-chain length seems to be correlated to the hydrophobic pocket size restriction. The second class of AHL synthases is present only in *Vibrio* species, and includes AinS from *V. fischeri* [13], LuxM from *V. harveyi* [14] and VanM from *V. anguillarum* [15]. Unlike LuxI-type proteins, AinS catalyzes AHL synthesis from SAM and from either acyl-ACP or acyl-CoA [16]. The third class of AHL synthases is represented by the HdtS protein from *Pseudomonas fluorescens*, which is related to the lysophosphatidic acid acyltransferase family [17]. This enzyme is supposed to transfer acyl chains onto a substrate such as SAM to generate AHL.

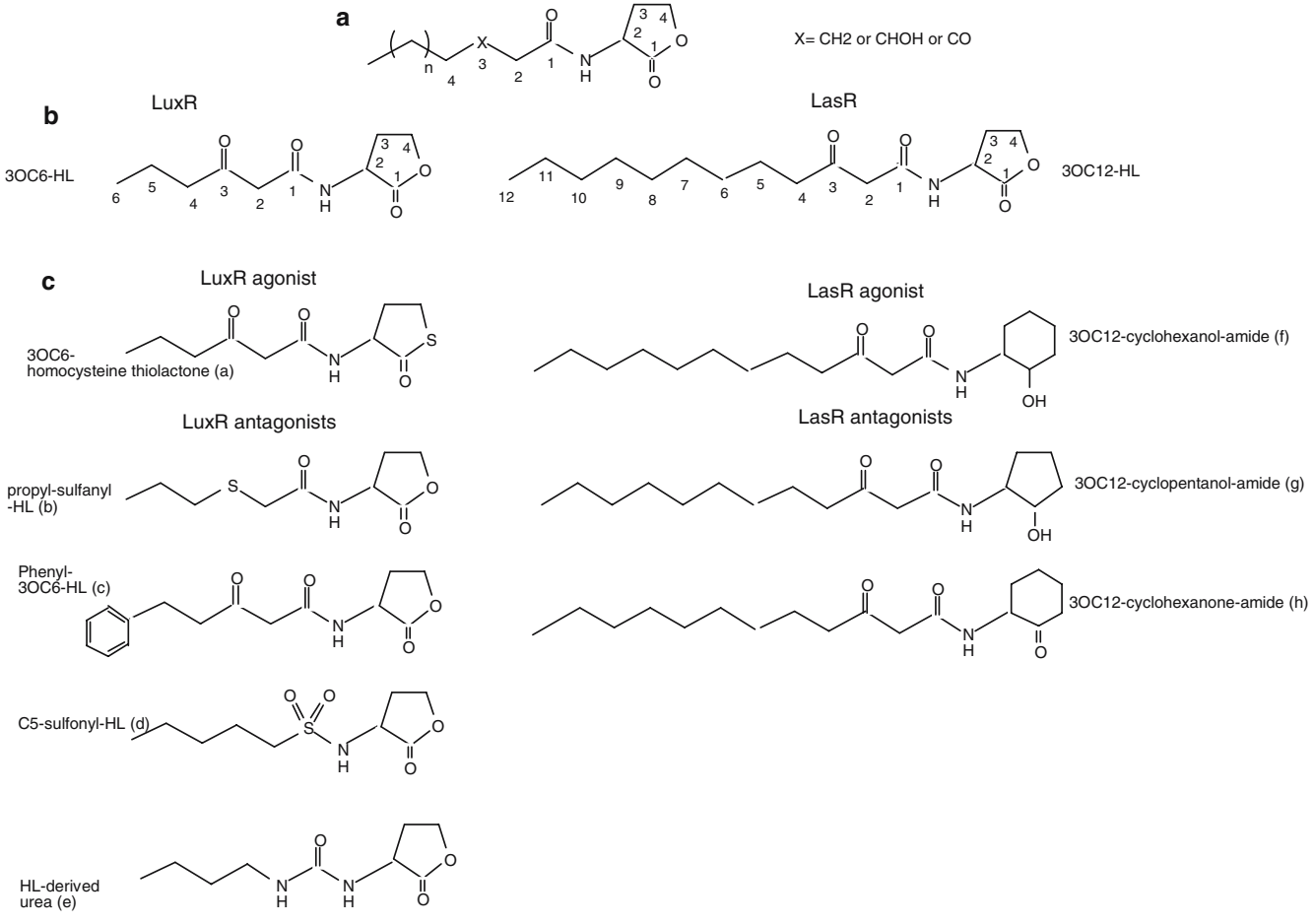

Fig. 1 **a** Basic structure of the AHLs. **b** AHL used as signal molecules by LuxR (3OC6-HL) from *Vibrio fischeri* and by LasR (3OC12-HL) from *Pseudomonas aeruginosa*. **c** Structures of analogues displaying either antagonist or agonist activities

Transcriptional regulators responsive to AHL

All of the regulators responsive to AHL are LuxR-type proteins. These proteins, which are about 250 amino acids in length, are composed of two functional domains: an amino-terminal domain involved in AHL-binding and a carboxy-terminal transcription regulation domain, which includes a helix-turn-helix (HTH) DNA-binding motif. The LuxR-type proteins interact with DNA in a dimeric state and specifically recognise a dyad symmetric sequence located in the regulatory regions of the target genes. Most of them are activators and the R protein/DNA interaction induces the recruitment of the RNA polymerase to the promoter and, therefore, the transcription of the target genes. In these cases, the association of AHL with R protein is a key prerequisite step enabling the activator to bind DNA [18–22]. However, several LuxR-type proteins act as transcriptional repressors whose DNA-binding activity is reduced by the interaction with AHL [23–26].

Phylogenetic analyses of the LuxI/R systems show that they are clearly subdivided into two different families of homologous genes with virtually no homology between them. Proteins of the first family (family A) belong to proteobacteria from different divisions (α, β and γ), whereas proteins of family B belong only to γ-proteobacteria [7]. Interestingly, proteins of family A are usually activators, whereas proteins acting as repressors are mainly members of family B, namely EsaR from *Pantoea stewartii* [23], YpsR from *Yersinia pseudotuberculosis* [27], SpnR from *Serratia marcescens* [24], and ExpR and VirR from pectinolytic *Erwinia* species [28, 29]. This observation reinforces the idea that proteins from families A and B have evolved from two distinct ancestors, which have activator and repressor functions, respectively.

Despite their wide distribution, only a few AHL QS systems have been studied at the molecular level. Among the most closely studied systems are the *V. fischeri* LuxI/R system controlling bioluminescence, the *Agrobacterium tumefaciens* TraI/R system controlling Ti plasmid conjugation, the *Pseudomonas aeruginosa* LasI/R and RhlI/R systems controlling biofilm formation and virulence factor production, and the *Pantoea stewartii* EsaI/EsaR system which controls the synthesis of virulence factors [30, 31]. Hence, in the following section, we mainly report the experimental results that have been obtained with these four systems.

Regulatory mechanisms of the LuxR members

Specific recognition of AHL molecules by their cognate receptor proteins

Biochemical analysis revealed that all of the LuxR-type proteins bind their cognate AHL with a stoichiometric ratio of one molecule of protein to one molecule of AHL [20, 21, 32]. However, some qualitative differences were observed in terms of the affinity of the regulator for its ligand, as well as in the stability of the ligand–regulator complex. For example, 3OC8-HL molecules were shown to tightly bind TraR, forming an irreversible holo–TraR complex [20]. In contrast, the LuxR–3OC6-HL complex can be reversibly dissociated by dilution, supporting the idea that AHL is not so tightly bound to LuxR [19]. An intermediate situation was observed for LasR, which bound 3OC12-HL more tightly than was observed for LuxR with 3OC6-HL, but this was partially reversible [21]. Since TraR, LuxR and LasR all respond to a decrease in signal concentrations or to a rapid drop in cell population density, the physiological relevance of the qualitative differences in the binding of AHL by the activators of the LuxR members remains to be clearly elucidated.

Structural studies of two LuxR-type proteins, TraR and SdiA from *Escherichia coli*, led to the characterization of the AHL-binding cavity [33–35]. This cavity consists of a five-stranded antiparallel β-sheet packed against three α-helices on each side (Fig. 2a). The cavity is formed by a cluster of hydrophobic and aromatic residues, which are highly conserved among the LuxR family members (Fig. 2b). In the crystal structure of TraR, the homoserine lactone moiety of the autoinducer is stabilized in the binding-pocket by four hydrogen bonds (Fig. 2c). One is between the keto ring group of AHL and the residue Trp57, and another is between residue Asp70 and the imino group. These two residues, Trp57 and Asp70, are strictly conserved among LuxR-type proteins (Fig. 3). A third hydrogen bond connects residue Tyr53 to the 1-keto group and another hydrogen bond is formed between the 3-keto group of the autoinducer and a water molecule that is stabilized by hydrogen bonding to residues Thr129 and Ala 38. The acyl side chain of the autoinducer runs parallel to the β-sheet surface and is stabilized further by hydrophobic interactions with Leu40, Thr51, Tyr53, Tyr61, Phe62, Val72, Trp85 and Ile110. Among the residues interacting with the acyl moiety, only Tyr61 and Trp85 are strictly conserved, according to the variability of the length of the acyl group and the diversity in LuxR-type proteins. In TraR, Phe62 is responsible for the occlusion of the cavity at the extremity of the acyl side chain of the ligand (Fig. 2b). This occlusion explains why AHL is irreversibly bound to TraR. This Phe62 residue is not conserved in other LuxR-type proteins and residues at the corresponding position are generally smaller than Phe (Fig. 3) and leave the AHL-binding site open so that the AHL-R protein interaction can be reversible [34]. Thus, it appears that the nature of the acyl side chain of AHL and the amino acid composition of the AHL cavity play an important role in the affinity/specificity of the regulator for its ligand, as well as in the stability of the ligand–regulator complex. In support of this idea are studies performed on TraR derivatives modified at residues that had been predicted to contribute to AHL-binding and selectivity [36]. Modifications of T129 (T129Y and T129A) or T115 (T115I) perturb the water-mediated hydrogen bond with the 3-oxo group of 3OC8-HL and abolish the discrimination of the 3-oxo group from 3OC8-HL, whereas modifications of the residue A49 (A49I or A49M) or Q58 (Q58L), believed to contact the C8 carbon of the acyl chain, result in proteins which preferentially

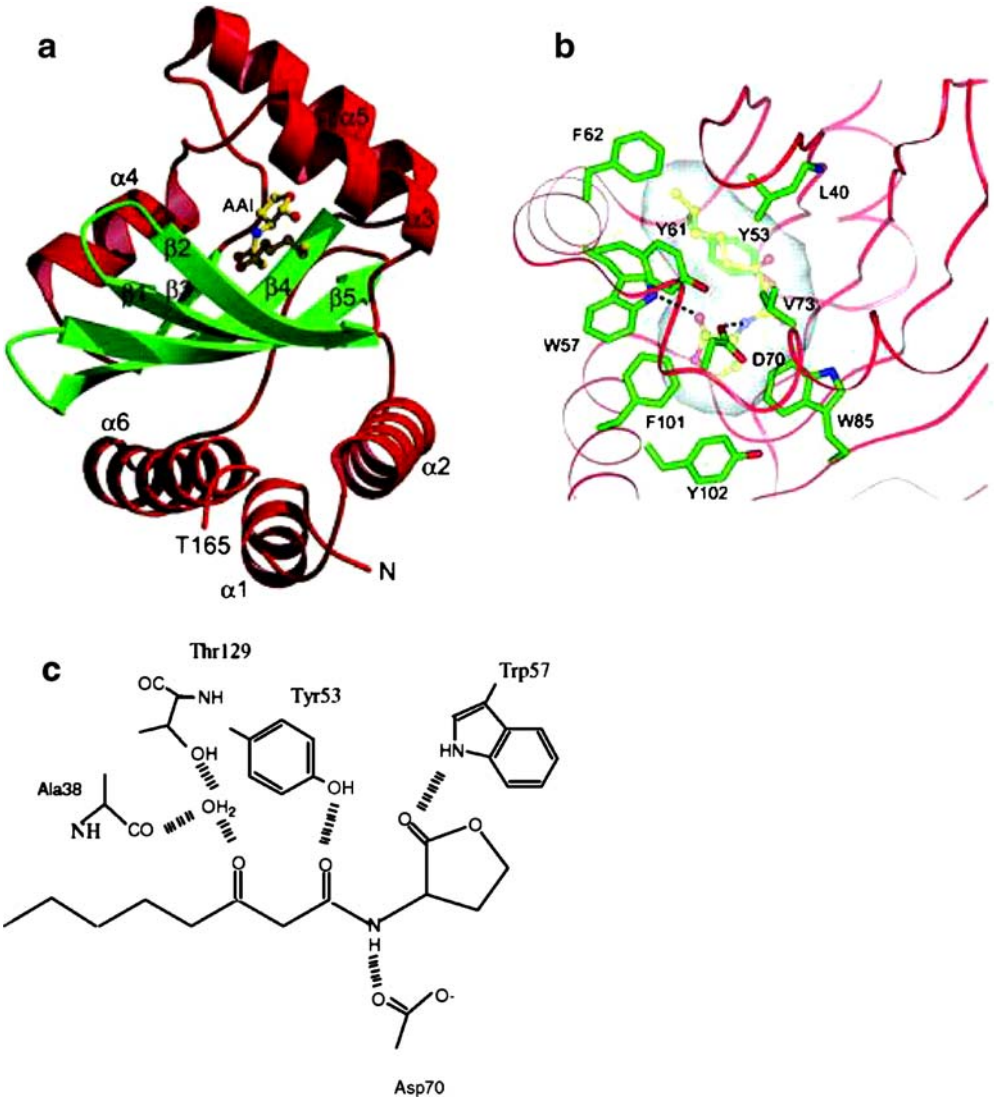

Fig. 2a–c Organization of the TraR ligand-binding domain. **a** Ribbon diagram of the N-terminal receiver domain; the α-helices are shown in *red* and the β-strands in *green*; the AHL ligand is shown in *bold and stick representations*. **b** Details of the AHL cavity. **c** Schematic overview of the active stir displaying the hydrogen bond network with AHL. Parts a and b are reproduced from [34]

detect shorter AHLs. In addition to TraR, mutagenesis of amino acids delimiting the AHL cavity have been performed on various regulators, including LuxR, LasR and RhlR, and these gave rise to protein derivatives that partially or totally lost their capacity to respond to their cognate AHL autoinducer [22, 37–40]. Therefore, this leads to the prediction of an overall structural conservation of the ligand-binding sites among the whole LuxR family.

Synthetic AHL analogues were also used to investigate which moiety of the AHL molecule is important for specific recognition by its cognate regulator. Basically, three types of modifications have been introduced in AHL molecules: alterations in the lactone ring, substitution in the acyl side chain and modifications in the carboxamide function that connect the homoserine lactone ring to the acyl side chain. The resulting analogues have been evaluated for both agonist and antagonist activities, mainly with LuxR, TraR and LasR as the receptor probes which naturally recognize 3OC6-HL, 3OC8-HL, and 3OC12-HL, respectively. The results obtained for agonist activity have identified clear structure/activity relationships, with the length of the *N*-linked acyl chain being a key structural feature. Indeed, there is a minimum side chain of five carbons required for binding to LuxR and the optimal acyl side chain lengths are of six or eight carbons [41, 42]. TraR tolerates acyl groups that are one carbon shorter or up to four carbons longer than the natural cognate 3OC8-HL autoinducer [43]. Similarly, increasing or decreasing the length of the acyl chain by two carbons compared to the natural 3OC12-HL autoinducer results in compounds which exhibit a strongly decreased affinity to LasR [44]. The presence of the 3-oxo moiety was reported to improve the binding efficiency for LuxR and LasR [42, 44]. Consistently, replacing the 3-oxo group with sulfur yielded analogues with strong antagonist activities in both the LuxR and LasR systems (Fig. 1c, compound b) [45]. Modifications of the ring size have different repercussions in LuxR and LasR. For LuxR, AHL analogues carrying one or more additional carbon atoms in the lactone ring lose their activity [42]. Therefore, the head group preference in

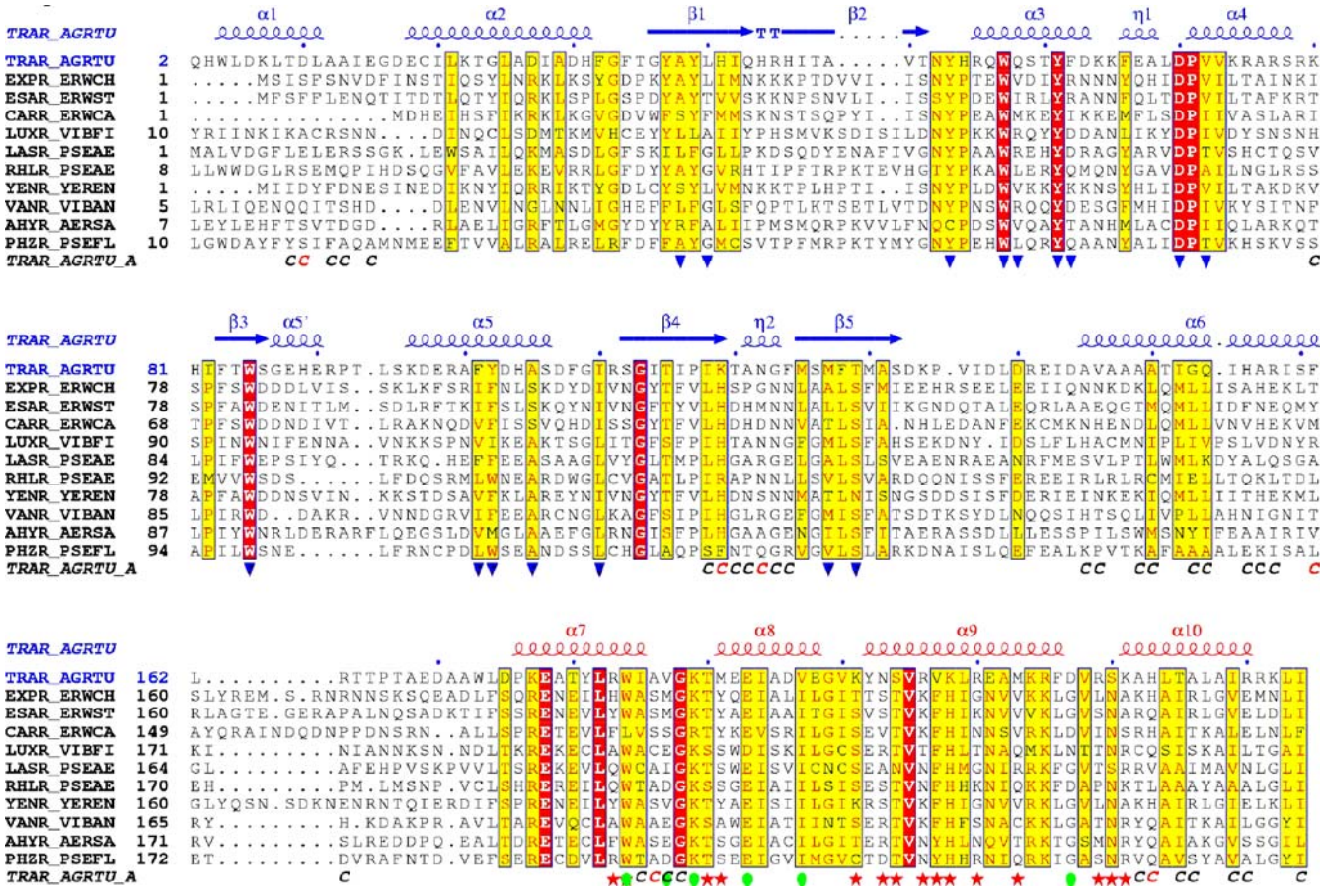

Fig. 3 Multiple sequence alignment of eleven LuxR homologues (TraR from *Agrobacterium tumefaciens*, ExpR from *Erwinia chrysanthemi*, EsaR from *Pantoea stewartii*, CarR from *Erwinia carotovora*, LuxR from *Vibrio fischeri*, LasR and RhlR from *Pseudomonas aeruginosa*, YenR from *Yersinia enterocolitica*, VanR from *Vibrio anguillarum*, AhyR from *Aeromonas salmonicida*, PhzR from *Pseudomonas fluorescens*). Identities are *boxed in red*. Similarities are *boxed in yellow* according to physicochemical properties. Secondary structural elements have been calculated from the 3-D structure of TraR (PDB code 1L3L). They are displayed on the top of the sequence blocks. Alpha and 3_{10} helices are represented by *squiggles* labelled α and η respectively. Strands are represented by *arrows*. Secondary structural elements are coloured in blue for the N-terminal receiver domain and in *red* for the C-terminal regulator domain. TraR residues interacting with the bound 3OC8HL are marked with *blue solid triangles*, residues interacting with the DNA fragment are marked with *red stars*. TraR residues interacting with RNA polymerase are indicated by *green dots*. Residues in contact at the dimer interface are marked with a letter "c", which is in *red* if the distance is less than 3.2 Å and in *black* if the distance is within the range 3.2–4 Å. The sequence alignment was drawn with ESPript [73]. The two N-terminal domains dimerize through the α6 helix; the HTH motif is composed of the scaffold α8 helix and the recognition α9 helix. The α10 helices from the two monomers mediate dimerization of the C-terminal regulatory domain

LuxR is clearly for a five-membered ring. The same conclusions were drawn with CarR from *Erwinia carotovora*, which controls carbapenem antibiotic production [46, 47]. In contrast, with LasR, conversion of the five-membered homoserine lactone ring into the six-membered cyclohexanol ring yields a compound (Fig. 1c, compound f) with strong agonist activity, whereas the cyclopentanol derivative (Fig. 1c, compound g) possesses an antagonist activity [48]. Therefore, in LasR the HSL ring could be agonized by a six-membered ring system. Using enantiomerically pure cyclohexanol and cyclopropanol derivatives, Jog et al. [49] demonstrated that, depending on the ring structure, an appropriate combination of absolute and relative stereochemistries of the amide and hydroxyl groups dictates the exhibition of agonist activity. Surprisingly, replacing the cyclohexanol ring by a cyclohexanone ring resulted in compounds that lost their agonist activity but gained antagonist activity in the LasR system (Fig. 1c, compound h) [48]. Opposite results were obtained with RhlR, the second *Pseudomonas* QS regulator, which can accommodate a cyclohexanone ring but cannot accommodate a cyclopentanol ring [48]. In the crystal structure of the TraR–3OC8-HL complex, the ring keto group participates in an H-bond with residue Trp57 [33] (Fig. 2c). An alcohol substitution is supposed to maintain the H-bond, but less efficient binding would be expected. The opposite results obtained with LasR suggest that the microenvironment of the protein–AHL interface differs between LasR, RhlR and TraR. Modifications of the ring hetero atom have also been examined in the LuxR and LasR systems [42, 44]. While the ring oxygen can be replaced by sulfur (Fig. 1c, compound a), more radical substitutions such as a replacement with nitrogen atom resulted in the abolition of inducing activity. Likewise, if

extra moieties are placed on the C-4 ring atom, the resulting analogues lose their autoinducer function [50]. Introduction of aryl substituents at the end of the acyl side chain of the AHL molecule gave rise to antagonist compounds in the LuxR system (Fig. 1c, compound c) [51]. Modelling of the LuxR AHL cavity and docking of the aryl-AHL analogues in this cavity suggest that the aryl compounds interact with either the aromatic residues Tyr62 (equivalent to Tyr53 in TraR) or Tyr70 (equivalent to Tyr61 in TraR) of LuxR, depending on the distance between the lactone and the aryl group. Based on the crystal structure of the TraR–3OC8-HL complex, the acyl side chain extremity is fixed near the interface separating the regulator and receiver domains in one monomer. It is thought that aryl groups at the extremity of the side chain perturb this interface and probably inhibit the structural rearrangements that normally lead to regulator domain activation. Modification of the junction between the acyl side chain and the lactone ring gave rise to analogues showing a strong antagonist activity [52, 53]. Such analogues include the *N*-sulfonyl homoserine lactones (Fig. 1c, compound d) [52] and the AHL-derived ureas (Fig. 1c, compound e) [53]. Docking of these two types of antagonists in the model of the LuxR AHL cavity suggests that a carboxamide to sulfonamide substitution provokes a new hydrogen bonding with Ser137 (equivalent to Thr129 in TraR) in the core of the LuxR receiver domain, whereas the urea function permits the formation of an additional hydrogen bond with Asp79 (equivalent to Asp70 in TraR), apart from the one already present with the amide function of AHLs. It was proposed that, in the two cases, the additional intermolecular contact blocks LuxR in an inactive conformation by inhibiting the dimerization of the two N-terminal receiver domains. Inhibition of dimerization was confirmed with urea analogues [53] (see later). So far, all of the QS antagonists have been evaluated in activator systems, and it would be interesting to analyse the effects of such antagonists in repressor systems, such as EsaR or ExpR.

Binding of AHL induces multimerization of the activators

It is clear that AHL binding is associated with large conformational changes, which result in a modification of the transcriptional activity of the regulators [20, 32]. For most of the activators of the LuxR family, AHL binding induces dimerization of the protein, which is a prerequisite for the activation of transcription. Indeed, TraR, LuxR, RhlR and LasR bind to their target DNA only after dimerization and in the presence of their cognate autoinducer [19–21, 40].

The crystal structure of TraR reveals that the two N-terminal domains dimerize chiefly through the α6 helix of each protomer, which is the longest α helix in the protein (Fig. 3). Although the N-terminal domains are sufficient for dimer formation, the C-terminal parts also have a dimerization interface which involves the α10 helix (Fig. 3). This C-terminal dimerization interface is not as extensive as the N-terminal dimerization interface. Mutagenesis experiments performed on LuxR, RhlR and LasR confirmed the involvement of both the N- and the C-terminal domains in the dimerization of the proteins, similar to that observed for TraR [22, 40]. However, while these activators possess many similar conserved amino acid residues in the autoinducer binding site and the transcriptional activation site, it seems that the amino acids that participate in multimerization are not strictly homologous between these proteins [40]. These observations suggest that the structural modifications induced by ligand binding on the activators of the LuxR family might be variable. This assertion is strengthened by the situation reported for CarR, which can exist as a stable preformed dimer, able to specifically interact with its DNA binding site in the absence of AHL. In this case, AHL binding causes the dimers to form higher order multimers and results in the activation of target gene expression [32]. Whether the multimerization induced by ligand binding is a prerequisite for the activation of transcription initiation of the target genes by CarR remains to be demonstrated by direct in vitro experiments.

Deletion of the LuxR N-terminal domain results in a derivative that is able to interact with DNA and able to activate the transcription of target genes, even in the absence of AHL [54]. This observation has led to the suggestion that in the native LuxR protein, in the absence of AHL, the N-terminal portion reduces the DNA binding affinity of the C-terminal domain [55]. Further investigations conducted on RhlR have reinforced the concept that, in the activators of the LuxR family, the N-terminal domain masks the C-terminal DNA binding domain in the absence of AHL and thus interferes with DNA binding. One simple model for dimer activation proposes that binding of the appropriate ligand to the activator induced conformational changes, which led to the exposure of the DNA binding site (Fig. 4). Point mutations that result in the activation of LuxR in the absence of the cognate autoinducer have been identified throughout the whole protein [56, 57]. Of particular interest is the residue Ala221 for which a Val substitution renders LuxR capable of activating the expression of target genes independent of the presence of AHL. Since the resulting mutant Ala221-Val was not affected in the autoinducer binding, Poellinger et al. [58] postulated that this residue is involved in the interaction between the two domains that enables the N-terminal domain to impair the activity of the C-terminal domain (Fig. 4). The corresponding residue in TraR (Ala212) is located in the α9 recognition helix of the HTH-motif (Fig. 3) and a potential interaction between this residue and an amino acid located in the N-terminal domain could be responsible for the maintenance of the protein in an inactive form. Binding of the appropriate autoinducer may help to dissociate these amino acid interactions.

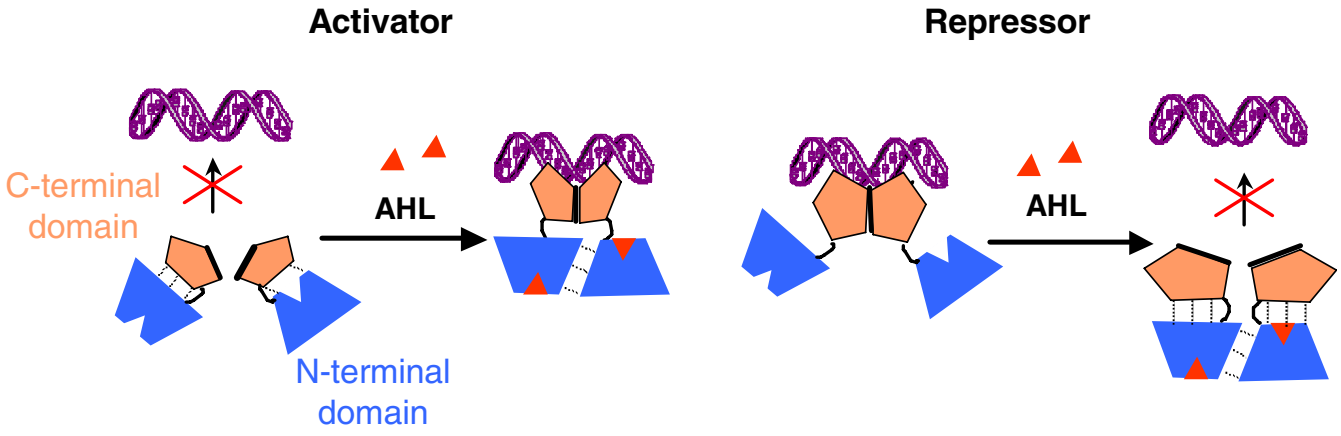

Fig. 4 Schematic representation of the structural modifications induced by AHL ligand binding on the activators and repressors of the LuxR family. For the activators: in the absence of AHL, the N-terminal domain masks the C-terminal DNA binding domain and thus interferes with the DNA binding properties of the protein; the binding of the appropriate ligand to the activator induces conformational changes which lead to the exposure of the DNA binding domain. This gives rise to a formation of the activator–DNA complex. For the repressors: in the absence of AHL, the C-terminal activating region is fully folded and is in a dimeric form, which leads to the formation of a repressor–DNA complex; the binding of the ligand to the N-terminal domain results in its dimerization that interferes with the appropriate folding of the C-terminal region. This gives rise to a dissociation of the repressor–DNA complex

LuxR members activate transcription of target genes through different mechanisms

The C-terminal domain of the LuxR members contains a HTH-motif which is particularly well-adapted for recognition of a DNA segment containing a dyad structure. The binding site, the first described for LuxR, consists of a 20-bp inverted repeat sequence, called a *lux* box [59]. *lux* box-type sequences have been further identified in the binding sites of many other LuxR members, including TraR and LasR [55, 60]. While the binding sites of the LuxR members are similar, they contain some subtle discriminative variabilities, which introduce a specificity into the interaction of the regulators with their target genes. In support of this idea are studies performed on LasR and QscR, two members of the LuxR family in *P. aeruginosa*. Although the binding sites of these two regulators contain 20 bp, of which 15 bp are identical, the binding of LasR and QscR onto their target genes are not interchangeable [61].

In vitro DNA–protein interaction studies revealed different modes of binding of regulators onto their target genes. While LuxR and TraR bind DNA in a noncooperative fashion [19, 62], CepR from *Burkholderia cenocepacia* exhibits cooperative binding properties [63]. For LasR, both cooperative and noncooperative binding were observed on different target genes [21]. Usually noncooperative binding occurs in a DNA target containing a well-defined *lux*-type sequence, whereas cooperative binding takes place on relatively degenerated sequences [21]. However, the significance of the optional cooperative binding in the modulation of target gene expression remains to be elucidated. On the other hand, while LuxR activates target gene expression on a linear template [19], TraR only significantly stimulates promoter expression on a supercoiled template [62].

Another important clue to the function of a regulator is the position of the binding site relative to the transcription initiation site of the target genes, because it determines the interaction of the regulatory protein with RNA polymerase. The relationship between binding site structure and function has been questioned for LuxR and TraR [37, 38, 59].

Egland and Greenberg [59] created *lacZ* reporter constructs that contain a *lux* box at different positions relative to the *luxI* transcription initiation site and they demonstrated that only the wild-type position centred to −42.5 is suitable for operon activation by LuxR. Thus, a CRP class II-type promoter (binding site of the regulator centred around −42) [64] appears to be critical for LuxR activity [59]. In addition, since both half-sites of the *lux* box have been shown to be required for LuxR-dependent activation of *luxI* transcription, LuxR was predicted to be an ambidextrous activator [59]. Such activators not only make contact with the α-subunit C-terminal domain (αCTD) of RNA polymerase upstream of their binding site, but they also make contact with other regions of RNA polymerase downstream of their binding site [65]. Further mutagenesis experiments revealed that the αCTD as well as region 4 of the σ^{70} factor make contact with LuxR [39, 66–68]. Single amino acid substitution mutants of LuxR that bind DNA but are deficient in activating transcription have led to the definition of a putative contacting domain with RNA polymerase in the activator C-terminal region. This domain includes the residues Trp 201, Ile 206 and Lys 198, which are predicted to be of surface-exposed residues [69].

For TraR, two types of RNA polymerase recruitment have been proposed, based on the location of the *tra* box relative to the target gene transcription initiation sites. Indeed, TraR activates target gene expression by binding either to a *tra* box located on a class II-type promoter or to a

tra box located further upstream (class I promoters) [37, 38]. In this last case, it is supposed that regulatory proteins make contact only with the αCTD of RNA polymerase [64]. Consistent with these observations, TraR can make contact either with the αCTD only or with both the αCTD and the σ^{70} subunit of RNA polymerase from *A. tumefaciens* [70]. Similarly to what was observed for LuxR, a putative contacting domain with RNA polymerase has been identified in the C-terminal region of TraR [38, 70]. This domain, which contains the surface-exposed residues Trp184, Val187, Lys189, Glu193 and Val197, has been assimilated to an "activating region" (AR) (Fig. 3), usually encountered in the activators containing an HTH motif [64]. The AR identified in TraR is supposed to make contact with the αCTD of RNA polymerase at both class II and class I promoters [38]. By contrast, the AR of the LuxR C-terminal domain, which overlaps that of TraR, is predicted to interact specifically with the σ-subunit [38, 69]. Thus LuxR and TraR might use different mechanisms to recruit RNA polymerase at the class II-type promoter of target genes. It then appears that LuxR members activate target gene expression via various mechanisms. Further investigations should however identify the residues of TraR and LuxR that establish contact with RNA polymerase regions neighbouring the core promoter and with the αCTD at the class II-type promoter, respectively. It would also be interesting to clarify how the other activators of the LuxR family recruit RNA polymerase and activate target gene expression.

Repressors of the LuxR family are active in the absence of the cognate autoinducer

Some LuxR homologues adopt the dimeric active conformation and function as repressors in the absence of the cognate ligand [2, 71]. Examples of such repressors are EsaR from *Pantoea stewartii* [23], ExpR from *Erwinia chrysanthemi* [25] and VirR from *E. carotovora* [29], among others. The EsaR protein is the best characterized of the repressor systems. EsaR forms a functional homodimer that could repress the transcription of its own gene (*esaR*) in the absence of AHL. The DNA binding site of EsaR is located in the *esaR* promoter such that it is thought to sterically block transcription initiation by RNA polymerase. The interaction of EsaR with AHL abrogates the regulatory function of the repressor, resulting in an increase in the expression of *esaR*. EsaR represses the expression of genes involved in the production of stewartan, an exopolysaccharide that contributes to the virulence of this bacterium. However, this control results from an indirect mechanism [72]. Indeed, EsaR directly represses the expression of *rcsA*, encoding an essential activator for the transcriptional activation of virulence gene expression. The AHL signal inactivates EsaR, releasing it from its target DNA, in turn elevating *rcsA* transcription which activates virulence gene expression. However, as for most of the activators, no direct evidence of an EsaR action on RNA polymerase activity has been observed. This point was recently addressed in studies on ExpR from *E. chrysanthemi*. By using various in vitro experiments, including potassium permanganate footprinting and in vitro transcription, Castang et al. [28] have shown that, in the absence of AHL, ExpR prevents transcription initiation by RNA polymerase at the *expR* promoter. The presence of an appropriate ligand renders the promoter accessible to RNA polymerase and results in an increase in transcription initiation. Thus, repressors of the LuxR family appear to modulate target gene expression by directly acting at the level of transcription initiation.

The fact that the repressors of the LuxR family can exist in an active conformation only in the absence of AHL led to the idea that, contrary to the activators, the C-terminal region of the repressor is fully folded and in a dimeric form in a ligand-free state and that interaction of the N-terminal domain with the ligand might induce conformational changes that interfere with the appropriate folding of the C-terminal region (Fig. 4). The structural modification on the N-terminal domain induced by ligand binding is supported by recent data obtained by Frezza et al. [53], which showed that the overproduced N-terminal domain of ExpR exists as a monomer in a ligand-free state and that interaction of the domain with an appropriate ligand results in a dimerization of ExpR-N. Thus, as reported for the activators, ligand binding to the N-terminal domain of the repressor induces conformational changes which result in its multimerization. How these conformational changes are transduced to the C-terminal domain is still to be established. One logical deduction of these observations is that the dimeric status of the full-length repressor, observed in the absence of ligand, is mainly due to interactions between residues located in the C-terminal domain.

Conclusion and perspective

Over the past decade, we have gained an in-depth understanding of the mechanisms by which bacteria communicate with each other. The AHL QS is one of the best studied, and we now understand how AHL is detected and how bacteria respond to the conversation. Solving the crystal structure of the TraR activator, as well as its biochemical characterisation, has increased our understanding of the structure–function relationship of the LuxR proteins, but since the structural studies performed on TraR were restricted to the ternary complex TraR–3OC8-HL-DNA, they were not relevant for providing substantial and precise information concerning the structural changes induced by the ligand binding on the protein. The structure of a LuxR-type activator, with and without binding to the appropriate ligand, needs to be determined in order to clearly establish how the structural changes induced by the ligand binding on the N-terminal domain are transduced to the C-terminal activating domain. Moreover, structural investigations and improved functional characterisation studies on the repressors of the LuxR family need to be carried out on the basis of the structure–function relation-

ship directing the regulatory function of these proteins. Ultimately, such investigations will lead to a fully better understanding of the differences that exist between the activators and the repressors of the LuxR-type proteins.

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