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LuxR-type Quorum Sensing Regulators That Are Detached from Common Scents

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Abstract

The ability of LuxR-type proteins to regulate transcription is controlled by bacterial pheromones, N-acylhomoserine lactones (AHLs). Most LuxR-family proteins require their cognate AHLs for activity, and at least some of them require AHLs for folding and protease-resistance. However, a few members of this family are able to fold, dimerize, bind DNA, and regulate transcription in the absence of AHLs; moreover, these proteins are antagonized by their cognate AHLs. Complexes between some of these proteins and their DNA binding sites are disrupted by AHLs *in vitro*. All such proteins are fairly closely related within the larger LuxR family, indicating that they share a relatively recent common ancestor. The 3' ends of the genes encoding these receptors invariably overlap with the 3' ends of the cognate AHL synthase genes, suggesting additional antagonism at the level of mRNA synthesis, stability, or translation.

Bacteria are “scentient” beings. Collectively, they synthesize and detect a rich variety of diffusible chemical signals that impact diverse behaviours, including bioluminescence, horizontal DNA transfer, biofilm formation, pathogenesis, and the production of antimicrobials and other secondary metabolites (Whitehead *et al.*, 2001). New signals, signalling systems, and signal-controlled behaviours are continually being discovered. Chemical communication within populations of bacteria enable them to estimate their population density, a process sometimes referred to as quorum sensing. The ability of bacterial populations to coordinate their behaviour might reflect the difficulty of an individual bacterium to impact its environment. Bacteria give new poignancy to the aphorism “To be is to be perceived” (Berkeley, 1710).

Two general classes of diffusible signals, or pheromones, have been described in bacteria. Communication among Gram-positive bacteria often involves oligopeptides that are detected by membrane-spanning kinases (Dunny and Leonard, 1997) or by cytoplasmic receptors (Dunny, 2007; Perego and Brannigan, 2001). In contrast, signalling among Proteobacteria often involves N-acyl-homoserine lactones (AHLs), which have identical polar head groups and a variety of hydrophobic acyl groups that differ in length, oxidation, and desaturation. AHL signal molecules are often referred to as autoinducers. A number of other bacterial pheromones have also been described, including coumarylhomoserine lactone (Schaefer *et al.*, 2008), γ -butyrolactones (Chater and Horinouchi, 2003), unsaturated fatty acids (Ryan *et al.*, 2009; Vojnov *et al.*, 2001; Wang *et al.*, 2004), a fatty acid methyl ester (Flavier *et al.*, 1997), a quinolone (Dubern and Diggle, 2008), and a substituted alkane (Ng and Bassler, 2009). One signal, denoted autoinducer-2, is synthesized by many species of bacteria, and might therefore serve as an intergeneric signal, though this conclusion remains somewhat controversial (Ng and Bassler, 2009).

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This review focuses on members of the LuxR family of transcription factors. Virtually all known members of this family are controlled by AHLs, which are synthesized by LuxI-type AHL synthases. Most LuxR-type receptors require AHLs for function and in at least some cases, AHLs are required for protein folding and protease resistance (Zhu and Winans, 1999, 2001). This review focuses primarily on several atypical members of this family that are active only in the *absence* of cognate AHLs and whose activity they *inhibit*. We will briefly highlight recent biochemical and structural advances of three proteins that require AHLs for activity, LuxR, LasR, and TraR. We will then review in greater depth the genetic and biochemical literature on LuxR-type proteins that function only as apoproteins.

LuxR, LasR, and TraR: three representative AHL-dependent transcription factors

LuxR and LuxI are encoded by the bioluminescent bacterium *Vibrio fischeri* (Engebrecht *et al.*, 1983; Engebrecht and Silverman, 1984). LuxI synthesizes *N*-3-oxohexanoyl-L-homoserine lactone (OHHL) (Eberhard *et al.*, 1981) while LuxR is an OHHL sensor and an OHHL-dependent transcriptional activator of the luciferase operon (Engebrecht *et al.*, 1983; Engebrecht and Silverman, 1984, 1987). As a population of *V. fischeri* cells grows in density, the concentration of external OHHL increases. When the concentration of this signal reaches the nanomolar range, its passive efflux from the cells becomes balanced by an influx, so that it can interact with LuxR. LuxR-OHHL complexes bind the promoter of the *luxICDABEG* operon and activate its transcription (Urbanowski *et al.*, 2004) (Fig. 1).

LuxR has two domains, an N-terminal domain that binds pheromone, and a C-terminal domain that binds DNA (Choi and Greenberg, 1991; Hanzelka and Greenberg, 1995). A strain producing the N-terminal domain can sequester exogenous OHHL (Hanzelka and Greenberg, 1995). Such a fragment can also block the activity of full length LuxR by forming inactive heterodimers (Choi and Greenberg, 1992). Taken together, these studies suggest that AHLs stimulate dimerization of the N-terminal domains. A LuxR fragment containing only the C-terminal domain is constitutively active and unaffected by AHLs (Choi and Greenberg, 1991). This indicates that the C-terminal domain contains all the sites essential for LuxR-DNA and LuxR-RNA polymerase interactions. Three amino acids clustered in the C-terminal domain of LuxR are required for positive control of transcription, and are presumed to make direct contacts with RNA polymerase (Egland and Greenberg, 2001; Trott and Stevens, 2001). Experiments using RNA polymerase mutants suggest that LuxR contacts the alpha and sigma subunits (Finney *et al.*, 2002; Johnson *et al.*, 2003; Stevens *et al.*, 1999).

LuxR binds to a DNA binding site called a *lux* box, that is 20 nucleotides in length and centred 42.5 nucleotides upstream of the transcription start site (Devine *et al.*, 1989; Urbanowski *et al.*, 2004). This sequence has imperfect dyad symmetry, suggesting that the DNA binding domains are multimeric and have a corresponding two-fold rotational symmetry. Bases located 6–8 nucleotides away from the dyad axis were essential for wild type activity, while other bases were less critical (Antunes *et al.*, 2008).

LuxR was purified in a soluble, active form after high-level production of the protein in the presence of OHHL (Urbanowski *et al.*, 2004), and bound to *lux* box DNA, stimulating the functional binding of RNA polymerase to an adjacent promotor. An important property of LuxR that distinguishes it from LasR and TraR (see below) is that binding of OHHL is reversible (Urbanowski *et al.*, 2004). When LuxR was diluted to sub-micromolar concentrations for gel shift assays, it bound to *lux* box DNA only when additional OHHL was provided. LuxR bound OHHL non-cooperatively with a dissociation constant of 100 nM (Urbanowski *et al.*, 2004).

The LasR protein of *Pseudomonas aeruginosa* is a central component of a regulatory web that controls the expression of hundreds of genes, some of which play direct roles in disease. This organism produces the cognate AHL synthase LasI, which synthesizes 3-oxo-dodecanoyl-homoserine lactone (OdDHL), as well as another quorum sensing receptor, RhlR, and its cognate AHL synthase, RhlI (which synthesizes butanoyl-homoserine lactone (BHL), and the so-called orphan receptor QscR, which also detects OdDHL (Brint and Ohman, 1995; de Kievit and Iglewski, 2000; Parsek and Greenberg, 1999; Passador *et al.*, 1993). The regulons of these three receptors are partially overlapping. The LasR/LasI system stimulates production of the RhlI/RhlR system, causing the two *P. aeruginosa* quorum-sensing circuits to initiate sequentially. Genes that are controlled by LasR or RhlR have been identified by Tn5lac mutagenesis and by transcriptional profiling (Hentzer *et al.*, 2003; Schuster *et al.*, 2003; Wagner *et al.*, 2003; Whiteley *et al.*, 1999).

LasR was purified in a soluble form by producing it in the presence of OdDHL as described above for LuxR (Schuster *et al.*, 2004). LasR is dimeric in solution, and binds one molecule OdDHL per subunit. Unlike LuxR, LasR does not detectably release its AHL. It bound to six LasR-dependent promoters in EMSA experiments and by DNase I footprinting (Schuster *et al.*, 2004). It bound some promoters non-cooperatively, causing a DNase I footprint of approximately 30 nucleotides, and bound other promoters cooperatively, causing longer footprints. The former promoters were presumably bound by one dimer, while the latter were bound by two. Chromatin immunoprecipitation experiments revealed additional target genes including some encoding transcription factors and others encoding secreted proteins and secretion machinery (Gilbert *et al.*, 2009).

Two groups have described the crystal structure of the LasR N-terminal domain (Bottomley *et al.*, 2007; Zou and Nair, 2009). In the first study, the protein was stabilized and folded during synthesis by adding OdDHL. This fragment was dimeric and each subunit consisted of an alpha-beta-alpha sandwich with the AHL lying between the beta sheet and three alpha helices. OdDHL was entirely buried within the protein, with no contact to bulk solvent. The homoserine lactone moiety and the 1 oxo group made hydrogen bonds with a number of conserved amino acids, while the acyl chain made van der Waals contacts with hydrophobic residues. The 12-carbon acyl moiety of the AHL forms a compact S-shape, which is essential for it to be fully buried within the protein. The second study crystallized LasR with OdDHL or with each of three different triphenyl mimic compounds (Zou and Nair, 2009). Despite the lack of structural similarity between these compounds and AHLs, they aided in the folding and stabilization of LasR, and occupied the AHL binding site.

The TraR and TraI proteins are encoded by Ti plasmids of *Agrobacterium tumefaciens*. TraI synthesizes primarily 3-oxo-octanoyl homoserine lactone (OOHL), while TraR is an OOHL-dependent activator of genes required for vegetative replication and conjugative transfer of the Ti plasmid (Fuqua and Winans, 1994; Hwang *et al.*, 1994; Li and Farrand, 2000; Moré *et al.*, 1996; Pappas and Winans, 2003; Piper *et al.*, 1993). OOHL is essential for the folding of TraR *in vivo*, and for resistance to cellular proteases, as the half-life of TraR is increased over 20-fold by OOHL (Zhu and Winans, 1999, 2001). The protein binds to sequences called *tra* boxes that have dyad symmetry and are approximately 43 or 63 nucleotides upstream of the various transcription start sites (Pappas and Winans, 2003; Zhu and Winans, 1999). TraR binds these sites as a dimer and without cooperativity. Residues on both the N-terminal and C-terminal domains of TraR are essential for positive control and probably make direct contacts with RNA polymerase (Costa *et al.*, 2009; Qin *et al.*, 2009; White and Winans, 2005).

The structure of full-length TraR-OOHL-DNA complexes was solved by X-ray crystallography (Vannini *et al.*, 2002; Zhang *et al.*, 2002). TraR is dimeric and contains one

molecule of OOHL per subunit. OOHL is fully embedded within the N-terminal domain of the protein, just as OdDHL is embedded within LasR. The C-terminal domain of TraR contains a bundle of four helices per subunit that binds to half of a *tra* box (Vannini *et al.*, 2002; Zhang *et al.*, 2002). The N-terminal and C-terminal domains of TraR are connected by a flexible twelve amino acid linker that, if fully extended, would allow the N-terminal domains and C-terminal domains to be separated by over 40 angstroms.

There are several surprising structural differences between LasR and TraR. First, although the 3-oxo groups of both AHLs make water-mediated hydrogen bonds to their respective receptor proteins, these 3-oxo groups and their bound water molecules are highly displaced in TraR relative to LasR (Fig. 2a and 2b). In TraR, the water is hydrogen-bonded by Thr129, while in LasR the water is bonded by Arg61, located on the opposite face of the binding pocket. The remainder of the acyl moiety is also oriented quite differently in LasR compared to TraR (Fig. 2c and 2d). Another surprising difference between LasR and TraR is in their subunit interfaces. The entire N-terminal domains are rotated by 90 degrees in TraR relative to those in LasR. Both proteins have long helices at the C-terminal end of these domains. In TraR, these helices are almost parallel, forming a coiled coil that is the primary dimerization determinant. In contrast, the corresponding helices of LasR are perpendicular and make fewer contributions to dimerization. In TraR, the C-terminal residues of the two NTDs are 10 angstroms apart, while their counterparts in LasR are separated by 30 angstroms.

The structure of a third LuxR homolog was reported in 2006, when the ligand-binding domain of SdiA of *E. coli* was solved using NMR (Yao *et al.*, 2006). The solubility of the SdiA fragment was enhanced by providing an AHL (octanoyl-HSL) during protein synthesis, as was done for LasR and TraR. The resolution of this structure was insufficient to identify protein-AHL contacts. This fragment was reported to be monomeric in solution, possibly due to the harsh conditions that were required to maintain solubility.

EsaR of *Pantoea stewartii* is inhibited by its cognate AHL

As described above, a small number of LuxR homologues appear to function only as apoproteins, in that they are fully active in the absence of any AHL, and their activity is blocked by cognate AHLs. Among the best characterized of these systems is the EsaR/EsaI system of *Pantoea stewartii* (formerly *Erwinia stewartii*), a pathogen of maize. In 1995, von Bodman and Farrand reported that *P. stewartii* produced OHHL, the same AHL as made by *V. fischeri*, and isolated the *esal* and *esaR* genes from a cosmid library (Beck von Bodman and Farrand, 1995). The two genes are transcribed convergently and their reading frames overlap by 21 nucleotides at their 3' ends (Fig. 3). EsaR represses transcription of its own gene, but does not affect expression of *esal*. Disruption of *esal* caused a sharp decrease in exopolysaccharide accumulation, and production was restored by adding OHHL (Beck von Bodman and Farrand, 1995). In a subsequent study, it was reported that EsaR mutants *overproduce* the same exopolysaccharide (von Bodman *et al.*, 1998), indicating that null mutations in *esaR* and *esal* have *opposite* phenotypes. Apparently, whatever EsaR does to regulate EPS was *antagonized* by OHHL. An *esaR*, *esal* double mutant had the same phenotype as an *esaR* mutant.

It was later discovered that apo-EsaR directly represses the promoter of *rcsA*, a gene required for exopolysaccharide synthesis (Minogue *et al.*, 2005). The fact that EsaR repressed *rcsA* and *esaR* was unusual among LuxR proteins, which generally activate transcription. However, many transcription factors can activate some promoters while repressing others, often depending on whether they bind upstream of the target promoter, or within or downstream of the promoter. Both LuxR and TraR have been converted to repressors simply by moving their binding sites, even though they are not known to repress

any natural promoters (Egland and Greenberg, 2000; Luo and Farrand, 1999). The fact that EsaR was a repressor could therefore be explained easily. In contrast, the fact that OHHL antagonized EsaR function was completely unprecedented, and in stark contrast to the properties of LuxR, LasR, TraR, and most related proteins. This finding shows that EsaR can fold and bind DNA in the absence of any AHL. It is not clear yet whether fully folded, active apo-EsaR can bind its AHL and if so, whether binding is reversible, as it is for LuxR, or irreversible, as it is for LasR and TraR.

EsaR negative autoregulation was reconstituted in *E. coli*, where OHHL again blocked repression (Minogue *et al.*, 2002), providing more evidence for a direct interaction between EsaR and the *esaR* promoter. As might be predicted, EsaR does not require any AHL to remain soluble. Purified EsaR bound to the *esaR* and *rcsA* promoters fragments in gel shift assays (Minogue *et al.*, 2002; Minogue *et al.*, 2005). At the *esaR* promoter, EsaR bound to a DNA sequence that contained an imperfect rotational symmetry. Strangely, the binding site in the *rcsA* promoter showed little or no symmetry and little or no similarity to the EsaR binding site in the *esaR* promoter. EsaR bound the *esaR* promoter as a dimer and without cooperativity (Minogue *et al.*, 2002). Surprisingly, OHHL did not have any effect on binding affinity in gel shift assays, though it did inhibit EsaR binding to its binding site in assays using surface plasmon resonance (Minogue *et al.*, 2002). OHHL also altered intrinsic fluorescence of EsaR tryptophan residues and increased EsaR thermostability, providing additional evidence for a direct interaction (Minogue *et al.*, 2002).

As described above, EsaR was first characterized as a repressor. However data published in 2003 showed that EsaR can also serve as a transcriptional activator, albeit in an artificially reconstituted system. EsaR was produced in an *E. coli* strain that also expressed the *luxICDABEG* operon of *V. fischeri*. EsaR activated transcription of this operon, but only in the absence of OHHL (von Bodman *et al.*, 2003). The related protein ExpR of *Pectobacterium carotovorum* (see below) had similar properties in this assay. Activation of the *lux* operon was further enhanced by replacing the LuxR binding site with an EsaR binding site. Although this system was artificial, it proved that EsaR can in principle recruit RNA polymerase to a promoter, which would seem highly unlikely unless it had evolved to do so. A native promoter of *P. stewartii* was recently discovered that is activated by EsaR (Schu *et al.*, 2009). This promoter expresses a small, noncoding RNA that is divergent from the *esaR* gene. OHHL blocked the activation of this gene by EsaR. An EsaR binding site located between *esaR* and *esaS* is required for repression of the former gene and is thought to be required for activation of the latter. This binding site is centred 60 nucleotides upstream of a possible transcription start site. Therefore, as described above, EsaR can activate or repress gene expression, depending largely on the position of the binding site, but in all cases, OHHL antagonizes EsaR function. The properties of EsaR and related proteins (see below) are summarized in Table 1.

The exoenzyme regulators ExpR of *Pectobacterium carotovorum* and *Erwinia chrysanthemi* are inhibited by OHHL and OOHL

P. carotovorum (formerly *Erwinia carotovora*) and *E. chrysanthemi* are plant pathogens that macerate plant tissues by releasing enzymes capable of degrading pectate, cellulose, and protein. Expression of the corresponding genes is controlled by several master regulatory proteins. Mutations in the gene for one such regulator, ExpI, abolished expression of all such genes (Pirhonen *et al.*, 1993). ExpI is related to members of the LuxI family. This gene is linked to *expR*, and two are expressed convergently, and overlap at their 3' ends (Andersson *et al.*, 2000), reminiscent of *esaR* and *esaI* (Fig. 3). Null mutations in *expR* cause a modest increase in plant tissue maceration and pectate lyase activity, while over-production of ExpR caused a decrease (Andersson *et al.*, 2000).

Three groups identified a second possible copy of *expR* in the genome sequence of a related bacterium, and used this sequence to identify the orthologous gene in *P. carotovorum* (Burr *et al.*, 2006; Cui *et al.*, 2005; Sjoblom *et al.*, 2006). In one study, mutants lacking just one of these two receptors still produced exoenzymes at slightly elevated levels. Expression of target genes increased when AHL was added. A strain lacking both receptor proteins produced exoenzymes at high constitutive levels (Sjoblom *et al.*, 2006). This suggests that there are two receptor proteins with overlapping functions, and that both are able to repress transcription of exoenzyme genes. The newly identified receptor (ExpR2) detected a broader variety of AHLs than ExpR1. In a different study using a different strain of *P. carotovorum*, this second receptor (referred to as VirR) was solely responsible for exoenzyme production, in that a *virR* mutation fully restored exoenzyme production in an *expI* mutant. An *expR*, *virR*, *expI* triple mutant was a phenocopy of the *virR*, *expI* double mutant, suggesting that ExpR does not play any role in regulating these genes. Surprisingly, exoenzyme production in a *virR*, *expI* mutant was still induced at high cell density, rather than constitutive. This observation was unexplained.

E. chrysanthemi has orthologous *expI* and *expR* genes, once again transcribed convergently and overlapping at their 3' ends (Nasser *et al.*, 1998). Mutation of *expI* abolished production of two AHLs, but did not affect the production of a third one, suggesting the existence of at least one more AHL synthase gene. Mutations of *expI* and *expR* had little effect on pectate lyase synthesis, which remained quorum-regulated. This provided additional evidence for a second quorum sensing system. Despite this genetic evidence that ExpR does not (at least solely) regulate pectate lyase genes, the protein was tested for binding to five different *pel* gene promoters. Apo-ExpR caused a gel shift at all five promoters. For the one promoter tested, OHHL blocked DNA binding in gel shift assays. DNase I protection assays were performed using three of these promoters. In the absence of OHHL, footprints were detected at two promoters. Inexplicably (in light of the gel shift data), addition of OHHL caused far longer and more distinct footprints at these promoters.

In a subsequent study, apo-ExpR was shown to autorepress its synthesis, while OHHL almost fully blocked autorepression (Castang *et al.*, 2006). Purified apo-ExpR bound the *expR* promoter without cooperativity, forming two high-affinity complexes (containing one or two ExpR dimers, respectively) and four additional slow migrating, low affinity complexes, possibly due to protein aggregation. OHHL largely disrupted these complexes. ExpR protected a region extending from -40 to +6 of the *expR* promoter from DNase I digestion. This 46 nucleotide region is sufficiently long to account for the two complexes observed in gel shift assays. Similar to the pectate lyase promoters described above, protection was more pronounced in the presence of OHHL than in its absence. This finding is extremely difficult to reconcile with the gel shift data. RNA polymerase bound an overlapping but longer region, probably displacing ExpR, although the authors of that study concluded that both proteins bound simultaneously. Apo-ExpR blocked open complex formation at the *expR* promoter, while OHHL restored open complex formation. Similar results were obtained using runoff transcription assays (Castang *et al.*, 2006).

Although ExpR of these species represses most known target genes, it can also activate at least one gene, *rsmA* (orthologous to *csrA* of *E. coli*), whose product binds mRNAs and accelerates their degradation (Chatterjee *et al.*, 2005; Cui *et al.*, 2005). Even here, ExpR functions only as an apoprotein, as OHHL blocks activation *in vivo* and disrupts ExpR-DNA complexes *in vitro*. Both copies of ExpR are able to activate this promoter (Cui *et al.*, 2005).

SmaR and SmaI act antagonistically to regulate antibiotic biosynthesis

SmaR of *Serratia* sp. 39006 is yet another example of a LuxR-type protein whose activity is blocked by the cognate AHL. The *smaR* gene and the cognate *smaI* gene are expressed convergently (Fig. 3). SmaI synthesizes predominantly butanoyl-HSL (BHL) and smaller amounts of hexanoyl-HSL (HHL). A *smaI* mutation abolishes the synthesis of the antibiotic carbapenem, the pigment prodigiosin, and several hydrolytic enzymes (Thomson *et al.*, 2000), while a *smaR smaI* double mutant restores their production (Slater *et al.*, 2003). Apo-SmaR is thought to bind to the promoters of the *carR* gene and the *car* (carbapenem) biosynthetic operon and repress their expression, and binding is blocked by BHL or HHL (Fineran *et al.*, 2005; Slater *et al.*, 2003). CarR is also a LuxR homolog and directly activates the *car* operon. CarR is rather closely related to members of the EsaR family (Fig. S1), which might suggest that its activity could be blocked by cognate AHLs. CarR is often referred to as “AHL-independent,” as it was able to activate the *car* operon of *P. carotovorum* in a strain lacking AHLs (Barnard and Salmond, 2007). CarR is essential for transcription of the *Serratia car* operon and functions perfectly well in an AHL-defective strain. It still seems possible that CarR could be antagonized by AHLs, although it functions in strains that produce BHL. The question is complicated by some rather intricate regulatory circuits. It would be interesting to express CarR constitutively in a SmaI SmaR double mutant of *Serratia* and measure the expression of the *carR* or *carA* promoters in the presence and absence of exogenous BHL. What makes this question even more fascinating is that a close relative of CarR clearly requires AHLs for activity. The CarR protein of *P. carotovorum* requires OHHL (synthesized by ExpI) to activate that organism's *car* operon (McGowan *et al.*, 1995). The AHL binding domains of the two CarR proteins are 57% identical.

Serratia marcescens strain SS-1 is similar to *Serratia* sp. 39006 in that its SpnI/SpnR system controls production of prodigiosin, endonuclease, and a surfactant that affects motility (this strain does not synthesize carbapenem) (Hornig *et al.*, 2002). SpnR is thought to directly repress target promoters, while the AHL synthesized by SpnI antagonizes SpnR. SpnI and SpnR are not closely related to their counterparts in *Serratia* sp. 39006 (SmaI and SmaR), and instead are more closely related to EsaR and EsaI of *P. stewartii*. SpnI (like EsaI) synthesizes primarily OHHL, while SpnR is inhibited primarily by the same signal. SpnR activates the *spnR* promoter, providing positive autoregulation. Surprisingly, it was reported that SpnR does so more effectively in the presence of OHHL than in its absence. It is not terribly surprising that SpnR could activate some promoters and repress others, as several EsaR-type proteins can do both (see above). However, the finding that OHHL would block SpnR function at one promoter and enhance it at another is unprecedented and counterintuitive. No explanation was offered (Hornig *et al.*, 2002). The *spnR/I* genes are located on a mobile genetic element, and SpnR represses transcription of the Tn3-type transposase of this element (Wei *et al.*, 2006). The idea that a quorum of bacteria would stimulate transposition provides an interesting example of Lamarckian evolution.

Yersinia spp and *Pseudomonas syringae*

At least two other gammaproteobacteria encode proteins that fall into the EsaR clade. *Yersinia enterocolitica* encodes YenR and YenI as well as one orphan LuxR homolog, while *Y. pestis* and *Y. pseudotuberculosis* (which have extremely similar chromosomes and differ largely in their plasmid content) each encode two LuxR/LuxI systems. The former organism encodes YpeRI and YspRI, while the latter encodes YpsRI and YtbRI. YenRI, YpeRI, and YpsRI are quite similar and are presumed to be orthologous (Fig. S1) while YtbRI and YspRI are also orthologous. All five pairs of genes are transcribed convergently and overlap at their 3' ends. Unpublished work from the authors' laboratory indicates that YenR and

YenI are similar to other members of the EsaR clade. Apo-YenR activates transcription of a non-coding RNA gene, *yenS*, which is divergent from *yenR*. Activation is blocked by OHHL. Purified apo-YenR binds to two sites between the two genes, and binding is reversed by addition of OHHL (Tsai and Winans, manuscript in preparation). Given that YpeR and YpsR are extremely similar in sequence to YenR, it is plausible that they have similar properties. The published literature on YpsRI and YtbRI is difficult to interpret, as the two systems may have redundant or overlapping functions and so the phenotypes of single mutants may be masked. It would be interesting to delete all four genes, and individually express YpsR and YtbR from constitutive promoters in the presence or absence of cognate AHLs, measuring expression of a target gene such as the small RNA homologous to *yenS*.

Many or perhaps all isolates of the plant pathogen *Pseudomonas syringae* encode members of the EsaR clade. The protein pairs are designated PsyRI in some strains and AhlRI in other strains. In all cases, the pairs of proteins are transcribed convergently and overlap at their 3' ends. The AHL synthases of this group synthesize predominantly OHHL. Although a number of papers have appeared that describe these proteins, no published study shows whether or not the receptor proteins are antagonized by their cognate AHLs.

Members of this group form a monophyletic clade

A phylogenetic dendrogram of LuxR homologs shows that all the proteins whose activities are blocked by AHLs (indicated using an asterisk) form a single clade (Fig. S1). The closest relative that is known to require AHLs for function is CarR of *E. carotovora* (McGowan *et al.*, 1995).

Members of the EsaR clade have several similarities in addition to their being antagonized by cognate AHLs. First, all confirmed members are found only within the Enterobacteriales, which are themselves within the Gammaproteobacteriales. Second, all but one of these proteins preferentially bind OHHL. The exception is SmaR of *P. carotovorum*, which preferentially binds BHL. Third, while most LuxR-type proteins activate expression of their cognate AHL synthase genes, members of the EsaR subfamily generally neither activate nor repress these genes. The activation of AHL synthase genes is thought to play a role in the tendency of these systems to remain stably in one of the two possible states in the face of subthreshold variations in AHL concentrations, a phenomenon referred to as hysteresis. EsaR-type proteins might have other mechanisms to achieve this (see below).

A fourth similarity between these proteins is they can function as repressors (though some can also activate certain promoters), while most LuxR type proteins are thought to act solely as activators. Target genes that are repressed by a member of the EsaR subfamily would be induced at high population density, just as they are in more conventional quorum sensing systems, as repression is blocked by AHLs, whose concentration increases at high cell densities (Fig. 1, middle panel). On the other hand, genes that are *activated* by such proteins would be expressed preferentially at low cell densities, where quorum signals are limited (Fig. 1, bottom panel).

A fifth similarity between members of this family is that they are all encoded by genes that are adjacent to their cognate AHL synthase genes, and each pair of genes are convergently transcribed (Fig. 3). In every case, the coding sequences of these gene pairs overlap by a few nucleotides at their 3' ends. Although the translation overlap is short, the transcription overlap would certainly be longer, possibly encompassing the entire length of the two genes. These genes lack any obvious transcriptional stop signals.

The convergent arrangement of these gene pairs suggests that expression of one gene might antagonize expression of the other. First, RNA polymerases transcribing the two genes could collide, possibly causing one to be dislodged and the other to continue transcription. Similarly, elongating RNA polymerases might dislodge convergently oriented RNA polymerases bound at the opposite promoter. This mechanism of transcriptional dominance, called “sitting duck” inhibition, has been reported previously (Callen *et al.*, 2004). Third, the mRNAs of cognate receptor and synthase genes could form RNA-RNA duplexes. Such duplexes might inhibit the translation of one or both RNAs, or might enhance their degradation, or both (Waters and Storz, 2009). If all duplexed molecules are degraded, then only the more abundant mRNA will persist. Such a contest between the two genes or their mRNAs could contribute to hysteresis. If under some conditions the promoter of a synthase gene were more active than that of a receptor gene, these mechanisms could accentuate the imbalance, locking the system in the high cell density state. Conversely, if the receptor promoter were more active than the synthase promoter, these mechanisms would exacerbate the imbalance, locking the cells in the low cell density state. Given the antagonism between these receptor proteins and their cognate pheromones, it seems striking that the expression of the two convergent genes might also be antagonistic.

How are EsaR-type proteins inactivated by their cognate AHL pheromones?

Mechanistic explanations for inhibition by AHLs are only beginning to emerge. The ability of several EsaR-like proteins to bind DNA is blocked by AHLs (Castang *et al.*, 2006; Cui *et al.*, 2005; Minogue *et al.*, 2002; Minogue *et al.*, 2005). The most obvious way for AHLs to block DNA binding would be to inhibit dimerization of the NTDs, which in turn would inhibit dimerization of the CTDs by cooperative effects. Alternatively, AHLs might cause the two NTDs of a dimer to bind their respective CTDs in such a way that prevents DNA binding. It seems far less likely that a conformational change caused by AHL binding could be propagated through each subunit to its DNA binding domain, especially in light of the long, flexible linkers between the two domains. The predicted linkers of EsaR-type proteins are even longer than those of other LuxR-type proteins (Fig. S2), and probably impart some degree of functional autonomy to the CTDs. It would be extremely useful to determine experimentally whether AHLs can inhibit NTD dimerization. *In vivo*, AHLs could also alter the rate of proteolysis of these proteins, analogous to the decrease in proteolysis of TraR caused by OOHL (Zhu and Winans, 1999, 2001).

It is tempting to attempt to model the structure of the EsaR NTD, using the crystal structures of TraR and LasR, and to look for differences that could explain the antagonistic effects of AHLs. All members of the EsaR clade contain all the residues that are predicted to make hydrogen bonds with the homoserine lactone group of the pheromone (Fig. S2). Unfortunately, proteins in the EsaR subfamily are only distantly related to LasR and to TraR, and even the latter two proteins have surprising structural differences (see above). Therefore, it would be exceedingly difficult to make any meaningful predictions about the structures of these proteins. This fascinating group of proteins is therefore ripe for further genetic, biochemical, and structural studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Andersson RA, Eriksson AR, Heikinheimo R, Mae A, Pirhonen M, Koiv V, Hyytiäinen H, Tuikkala A, Palva ET. Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. *carotovora*: the role of *expR*(Ecc). *Mol Plant Microbe Interact*. 2000; 13:384–393. [PubMed: 10755301]
- Antunes LC, Ferreira RB, Lostroh CP, Greenberg EP. A mutational analysis defines *Vibrio fischeri* LuxR binding sites. *J Bacteriol*. 2008; 190:4392–4397. [PubMed: 18083819]
- Barnard AM, Salmond GP. Quorum sensing in *Erwinia* species. *Anal Bioanal Chem*. 2007; 387:415–423. [PubMed: 16943991]
- Beck von Bodman S, Farrand SK. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an N-acylhomoserine lactone autoinducer. *J Bacteriol*. 1995; 177:5000–5008. [PubMed: 7665477]
- Berkeley, G. A Treatise Concerning the Principles of Human Knowledge. Dublin: Aaron Rhames; 1710.
- Bottomley MJ, Muraglia E, Bazzo R, Carfi A. Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer. *J Biol Chem*. 2007; 282:13592–13600. [PubMed: 17363368]
- Brint JM, Ohman DE. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J Bacteriol*. 1995; 177:7155–7163. [PubMed: 8522523]
- Burr T, Barnard AM, Corbett MJ, Pemberton CL, Simpson NJ, Salmond GP. Identification of the central quorum sensing regulator of virulence in the enteric phytopathogen, *Erwinia carotovora*: the VirR repressor. *Mol Microbiol*. 2006; 59 :113–125. [PubMed: 16359322]
- Callen BP, Shearwin KE, Egan JB. Transcriptional interference between convergent promoters caused by elongation over the promoter. *Mol Cell*. 2004; 14 :647–656. [PubMed: 15175159]
- Castang S, Reverchon S, Gouet P, Nasser W. Direct evidence for the modulation of the activity of the *Erwinia chrysanthemi* quorum-sensing regulator ExpR by acylhomoserine lactone pheromone. *J Biol Chem*. 2006; 281:29972–29987. [PubMed: 16831870]
- Chater KF, Horinouchi S. Signalling early developmental events in two highly diverged *Streptomyces* species. *Mol Microbiol*. 2003; 48:9–15. [PubMed: 12657041]
- Chatterjee A, Cui Y, Hasegawa H, Leigh N, Dixit V, Chatterjee AK. Comparative analysis of two classes of quorum-sensing signaling systems that control production of extracellular proteins and secondary metabolites in *Erwinia carotovora* subspecies. *J Bacteriol*. 2005; 187:8026–8038. [PubMed: 16291676]
- Choi SH, Greenberg EP. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent lux gene activating domain. *Proc Natl Acad Sci U S A*. 1991; 88:11115–11119. [PubMed: 1763027]
- Choi SH, Greenberg EP. Genetic dissection of DNA binding and luminescence gene activation by the *Vibrio fischeri* LuxR protein. *J Bacteriol*. 1992; 174:4064–4069. [PubMed: 1597420]
- Costa ED, Cho H, Winans SC. Identification of amino acid residues of the pheromone-binding domain of the transcription factor TraR that are required for positive control. *Mol Microbiol*. 2009; 73:341–351. [PubMed: 19602141]
- Cui Y, Chatterjee A, Hasegawa H, Dixit V, Leigh N, Chatterjee AK. ExpR, a LuxR homolog of *Erwinia carotovora* subsp. *carotovora*, activates transcription of *rsmA*, which specifies a global regulatory RNA-binding protein. *J Bacteriol*. 2005; 187:4792–4803. [PubMed: 15995194]
- de Kievit TR, Iglewski BH. Bacterial quorum sensing in pathogenic relationships. *Infect Immun*. 2000; 68:4839–4849. [PubMed: 10948095]
- Devine JH, Shadel GS, Baldwin TO. Identification of the operator of the lux regulon from the *Vibrio fischeri* strain ATCC7744. *Proc Natl Acad Sci U S A*. 1989; 86 :5688–5692. [PubMed: 2762291]

- Dubern JF, Diggle SP. Quorum sensing by 2-alkyl-4-quinolones in *Pseudomonas aeruginosa* and other bacterial species. *Mol Biosyst.* 2008; 4:882–888. [PubMed: 18704225]
- Dunny GM, Leonard BA. Cell-cell communication in gram-positive bacteria. *Annu Rev Microbiol.* 1997; 51:527–564. [PubMed: 9343359]
- Dunny GM. The peptide pheromone-inducible conjugation system of *Enterococcus faecalis* plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. *Philos Trans R Soc Lond B Biol Sci.* 2007; 362:1185–1193. [PubMed: 17360276]
- Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry.* 1981; 20:2444–2449. [PubMed: 7236614]
- Egland KA, Greenberg EP. Conversion of the *Vibrio fischeri* transcriptional activator, LuxR, to a repressor. *J Bacteriol.* 2000; 182:805–811. [PubMed: 10633117]
- Egland KA, Greenberg EP. Quorum sensing in *Vibrio fischeri*: analysis of the LuxR DNA binding region by alanine-scanning mutagenesis. *J Bacteriol.* 2001; 183:382–386. [PubMed: 11114939]
- Engelbrecht J, Nealson K, Silverman M. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell.* 1983; 32:773–781. [PubMed: 6831560]
- Engelbrecht J, Silverman M. Identification of genes and gene products necessary for bacterial bioluminescence. *Proc Natl Acad Sci U S A.* 1984; 81:4154–4158. [PubMed: 6377310]
- Engelbrecht J, Silverman M. Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. *Nucleic Acids Res.* 1987; 15 :10455–10467. [PubMed: 3697093]
- Fineran PC, Slater H, Everson L, Hughes K, Salmond GP. Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol Microbiol.* 2005; 56:1495–1517. [PubMed: 15916601]
- Finney AH, Blick RJ, Murakami K, Ishihama A, Stevens AM. Role of the C-terminal domain of the alpha subunit of RNA polymerase in LuxR-dependent transcriptional activation of the lux operon during quorum sensing. *J Bacteriol.* 2002; 184:4520–4528. [PubMed: 12142422]
- Flavier AB, Clough SJ, Schell MA, Denny TP. Identification of 3- hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol Microbiol.* 1997; 26:251–259. [PubMed: 9383151]
- Fuqua WC, Winans SC. A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J Bacteriol.* 1994; 176:2796–2806. [PubMed: 8188582]
- Gilbert KB, Kim TH, Gupta R, Greenberg EP, Schuster M. Global position analysis of the *Pseudomonas aeruginosa* quorum-sensing transcription factor LasR. *Mol Microbiol.* 2009; 73:1072–1085. [PubMed: 19682264]
- Hanzelka BL, Greenberg EP. Evidence that the N-terminal region of the *Vibrio fischeri* LuxR protein constitutes an autoinducer-binding domain. *J Bacteriol.* 1995; 177:815–817. [PubMed: 7836318]
- Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Hoiby N, Givskov M. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *Embo J.* 2003; 22:3803–3815. [PubMed: 12881415]
- Horng YT, Deng SC, Daykin M, Soo PC, Wei JR, Luh KT, Ho SW, Swift S, Lai HC, Williams P. The LuxR family protein SpnR functions as a negative regulator of N-acylhomoserine lactone-dependent quorum sensing in *Serratia marcescens*. *Mol Microbiol.* 2002; 45:1655–1671. [PubMed: 12354232]
- Hwang I, Li PL, Zhang L, Piper KR, Cook DM, Tate ME, Farrand SK. TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid N-acylhomoserine lactone autoinducer. *Proc Natl Acad Sci U S A.* 1994; 91:4639–4643. [PubMed: 8197112]
- Johnson DC, Ishihama A, Stevens AM. Involvement of region 4 of the sigma70 subunit of RNA polymerase in transcriptional activation of the lux operon during quorum sensing. *FEMS Microbiol Lett.* 2003; 228:193–201. [PubMed: 14638424]

- Li PL, Farrand SK. The replicator of the nopaline-type Ti plasmid pTiC58 is a member of the *repABC* family and is influenced by the TraR-dependent quorum-sensing regulatory system. *J Bacteriol.* 2000; 182:179–188. [PubMed: 10613878]
- Luo ZQ, Farrand SK. Signal-dependent DNA binding and functional domains of the quorum-sensing activator TraR as identified by repressor activity. *Proc Natl Acad Sci U S A.* 1999; 96:9009–9014. [PubMed: 10430886]
- McGowan S, Sebaihia M, Jones S, Yu B, Bainton N, Chan PF, Bycroft B, Stewart GS, Williams P, Salmond GP. Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. *Microbiology.* 1995; 141 (Pt 3):541–550. [PubMed: 7711893]
- Minogue TD, Wehland-von Trebra M, Bernhard F, von Bodman SB. The autoregulatory role of EsaR, a quorum-sensing regulator in *Pantoea stewartii* ssp. *stewartii*: evidence for a repressor function. *Mol Microbiol.* 2002; 44:1625–1635. [PubMed: 12067349]
- Minogue TD, Carlier AL, Koutsoudis MD, von Bodman SB. The cell density-dependent expression of stewartan exopolysaccharide in *Pantoea stewartii* ssp. *stewartii* is a function of EsaR-mediated repression of the *rcaA* gene. *Mol Microbiol.* 2005; 56:189–203. [PubMed: 15773989]
- Moré MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC. Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science.* 1996; 272:1655–1658. [PubMed: 8658141]
- Nasser W, Bouillant ML, Salmond G, Reverchon S. Characterization of the *Erwinia chrysanthemi* *expI-expR* locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. *Mol Microbiol.* 1998; 29:1391–1405. [PubMed: 9781877]
- Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. *Annu Rev Genet.* 2009; 43:197–222. [PubMed: 19686078]
- Pappas KM, Winans SC. A LuxR-type regulator from *Agrobacterium tumefaciens* elevates Ti plasmid copy number by activating transcription of plasmid replication genes. *Mol Microbiol.* 2003; 48:1059–1073. [PubMed: 12753196]
- Parsek MR, Greenberg EP. Quorum sensing signals in development of *Pseudomonas aeruginosa* biofilms. *Methods Enzymol.* 1999; 310:43–55. [PubMed: 10547781]
- Passador L, Cook JM, Gambello MJ, Rust L, Iglewski BH. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science.* 1993; 260:1127–1130. [PubMed: 8493556]
- Perego M, Brannigan JA. Pentapeptide regulation of aspartyl-phosphate phosphatases. *Peptides.* 2001; 22:1541–1547. [PubMed: 11587783]
- Piper KR, Beck von Bodman S, Farrand SK. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature.* 1993; 362:448–450. [PubMed: 8464476]
- Pirhonen M, Flego D, Heikinheimo R, Palva ET. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *Embo J.* 1993; 12:2467–2476. [PubMed: 8508772]
- Qin Y, Keenan C, Farrand SK. N- and C-terminal regions of the quorum-sensing activator TraR cooperate in interactions with the alpha and sigma-70 components of RNA polymerase. *Mol Microbiol.* 2009; 74:330–346. [PubMed: 19732344]
- Ryan RP, McCarthy Y, Watt SA, Niehaus K, Dow JM. Intraspecies signaling involving the diffusible signal factor BDSF (cis-2-dodecenoic acid) influences virulence in *Burkholderia cenocepacia*. *J Bacteriol.* 2009; 191:5013–5019. [PubMed: 19482924]
- Schaefer AL, Greenberg EP, Oliver CM, Oda Y, Huang JJ, Bittan-Banin G, Peres CM, Schmidt S, Juhaszova K, Sufrin JR, Harwood CS. A new class of homoserine lactone quorum-sensing signals. *Nature.* 2008; 454:595–599. [PubMed: 18563084]
- Schu DJ, Carlier AL, Jamison KP, von Bodman S, Stevens AM. Structure/function analysis of the *Pantoea stewartii* quorum-sensing regulator EsaR as an activator of transcription. *J Bacteriol.* 2009; 191:7402–7409. [PubMed: 19820098]
- Schuster M, Lostroh CP, Ogi T, Greenberg EP. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J Bacteriol.* 2003; 185:2066–2079. [PubMed: 12644476]

- Schuster M, Urbanowski ML, Greenberg EP. Promoter specificity in *Pseudomonas aeruginosa* quorum sensing revealed by DNA binding of purified LasR. *Proc Natl Acad Sci U S A*. 2004; 101:15833–15839. [PubMed: 15505212]
- Sjoblom S, Brader G, Koch G, Palva ET. Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen *Erwinia carotovora*. *Mol Microbiol*. 2006; 60:1474–1489. [PubMed: 16796682]
- Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol Microbiol*. 2000; 38:986–1003. [PubMed: 11123673]
- Slater H, Crow M, Everson L, Salmond GP. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol Microbiol*. 2003; 47 :303–320. [PubMed: 12519208]
- Stevens AM, Fujita N, Ishihama A, Greenberg EP. Involvement of the RNA polymerase alpha-subunit C-terminal domain in LuxR-dependent activation of the *Vibrio fischeri* luminescence genes. *J Bacteriol*. 1999; 181:4704–4707. [PubMed: 10419977]
- Thomson NR, Crow MA, McGowan SJ, Cox A, Salmond GP. Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol Microbiol*. 2000; 36:539–556. [PubMed: 10844645]
- Trott AE, Stevens AM. Amino acid residues in LuxR critical for its mechanism of transcriptional activation during quorum sensing in *Vibrio fischeri*. *J Bacteriol*. 2001; 183:387–392. [PubMed: 11114940]
- Urbanowski ML, PLC, Greenberg EP. Reversible acyl-homoserine lactone binding to purified *Vibrio fischeri* LuxR protein. *J Bacteriol*. 2004; 186:631–637. [PubMed: 14729687]
- Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, De Francesco R, Neddermann P, Marco SD. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *EMBO J*. 2002; 21:4393–4401. [PubMed: 12198141]
- Vojnov AA, Slater H, Newman MA, Daniels MJ, Dow JM. Regulation of the synthesis of cyclic glucan in *Xanthomonas campestris* by a diffusible signal molecule. *Arch Microbiol*. 2001; 176:415–420. [PubMed: 11734884]
- von Bodman SB, Majerczak DR, Coplin DL. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. *Proc Natl Acad Sci U S A*. 1998; 95:7687–7692. [PubMed: 9636211]
- von Bodman SB, Ball JK, Faini MA, Herrera CM, Minogue TD, Urbanowski ML, Stevens AM. The quorum sensing negative regulators EsaR and ExpR(Ecc), homologues within the LuxR family, retain the ability to function as activators of transcription. *J Bacteriol*. 2003; 185:7001–7007. [PubMed: 14617666]
- Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J Bacteriol*. 2003; 185:2080–2095. [PubMed: 12644477]
- Wang LH, He Y, Gao Y, Wu JE, Dong YH, He C, Wang SX, Weng LX, Xu JL, Tay L, Fang RX, Zhang LH. A bacterial cell-cell communication signal with crosskingdom homologs. *Mol Microbiol*. 2004; 51:903–912. [PubMed: 14731288]
- Waters LS, Storz G. Regulatory RNAs in bacteria. *Cell*. 2009; 136:615–628. [PubMed: 19239884]
- Wei JR, Tsai YH, Horng YT, Soo PC, Hsieh SC, Hsueh PR, Horng JT, Williams P, Lai HC. A mobile quorum-sensing system in *Serratia marcescens*. *J Bacteriol*. 2006; 188:1518–1525. [PubMed: 16452435]
- White CE, Winans SC. Identification of amino acid residues of the *Agrobacterium tumefaciens* quorum-sensing regulator TraR that are critical for positive control of transcription. *Mol Microbiol*. 2005; 55:1473–1486. [PubMed: 15720554]
- Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol Rev*. 2001; 25:365–404. [PubMed: 11524130]
- Whiteley M, Lee KM, Greenberg EP. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A*. 1999; 96:13904–13909. [PubMed: 10570171]

- Yao Y, Martinez-Yamout MA, Dickerson TJ, Brogan AP, Wright PE, Dyson HJ. Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. *J Mol Biol.* 2006; 355:262–273. [PubMed: 16307757]
- Zhang RG, Pappas T, Brace JL, Miller PC, Oulmassov T, Molyneaux JM, Anderson JC, Bashkin JK, Winans SC, Joachimiak A. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature.* 2002; 417:971–974. [PubMed: 12087407]
- Zhu J, Winans SC. Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters *in vitro* and decreases TraR turnover rates in whole cells. *Proc Natl Acad Sci U S A.* 1999; 96:4832–4837. [PubMed: 10220379]
- Zhu J, Winans SC. The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc Natl Acad Sci U S A.* 2001; 98:1507–1512. [PubMed: 11171981]
- Zou Y, Nair SK. Molecular basis for the recognition of structurally distinct autoinducer mimics by the *Pseudomonas aeruginosa* LasR quorum-sensing signaling receptor. *Chem Biol.* 2009; 16:961–970. [PubMed: 19778724]

Fig. 1. Gene induction at high cell density can be achieved via a diffusible signal that activates a transcription activator (top panel, for example *LuxR*, *LasR*, and *TraR*) or that inhibits a repressor (middle panel, for example, *EasR*, *ExpR1*, *ExpR2*, *SmaR*). Gene activation at low cell density can be achieved via a signal that activates a repressor (not shown) or by a signal that inhibits an activator (bottom panel, *EasR* family proteins).

Fig. 2. Structural comparisons of LasR and TraR N-terminal domains

A and B. The conformation of OdDHL bound to LasR (A) and OOHl bound to TraR (B). The homoserine lactone group and residue W60 (LasR) and W57 (TraR) are aligned for reference. The 3-oxo group of OdDHL makes a water-mediated hydrogen bond to residue R61 of LasR, while that of OOHl makes a water mediated hydrogen bond to residue T129 of TraR. The remainders of the two acyl chains are located in different parts of the two proteins.

C and D. Dimerization of the two N-terminal domains of LasR (C) and of TraR (D). The left subunits of each dimer are aligned for reference. The C-terminal helices of the two proteins are in red, and are oriented approximately 90° apart in TraR relative to LasR (black arrows).

Fig. 3. The *esaR* and *esaI* genes are expressed convergently (A) and overlap by eight codons (B). The *expR* and *expI* genes, the *smaR* and *smaI* genes, and the *yenR* and *yenI* genes have similar orientations and overlaps (B). This convergent and overlapping arrangement of all four gene pairs suggests that the expression of one member of a gene pair might be antagonized by the expression of the other member, either via RNA polymerase collisions, or by hybridization of the two complementary mRNAs.

Table 1

Functions of the EsaR clade of LuxR receptors

Protein	Organism	Cognate AHL	Repressed Genes/Functions	Activated Genes/Functions	References
EsaR	<i>P. stewartii</i>	OHHL	<i>esaR</i> , <i>rcsA</i>	<i>esaS</i> , <i>lux</i>	(Beck von Bodman and Farrand, 1995; Minogue <i>et al.</i> , 2005; Schu <i>et al.</i> , 2009; von Bodman <i>et al.</i> , 2003)
ExpR	<i>P. carotovora</i>	OHHL, OOHHL	hydrolytic exoenzymes, <i>pelA</i> , <i>pelC</i> , <i>celV</i>	<i>rmsA</i>	(Andersson <i>et al.</i> , 2000; Cui <i>et al.</i> , 2005; Pirhonen <i>et al.</i> , 1993)
ExpR	<i>E. chrysanthemi</i>	OHHL	<i>expR</i>	<i>rmsA</i>	(Chatterjee <i>et al.</i> , 2005; Cui <i>et al.</i> , 2005)
VirR (ExpR2)	<i>P. carotovora</i>	OHHL, OOHHL	<i>pelA</i> , <i>pelC</i> , <i>celV</i>		(Burr <i>et al.</i> , 2006; Cui <i>et al.</i> , 2005; Sjöblom <i>et al.</i> , 2006)
SmaR	<i>Serratia</i> sp 39006	BHL	<i>carR</i> , <i>carA</i> , <i>smaR</i>		(Fineran <i>et al.</i> , 2005; Slater <i>et al.</i> , 2000; Thomson <i>et al.</i> , 2000)
SpnR	<i>S. marcescens</i>	OHHL	<i>pig</i> , <i>nucA</i> , surfactant		(Hong <i>et al.</i> , 2002)
YenR	<i>Y. enterocolitica</i>	OHHL		<i>yenR</i> , <i>yenS</i>	Tsai and Winans, unpublished