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The Quorum Sensing Negative Regulators EsaR and ExpR$_{Ecc}$, Homologues within the LuxR Family, Retain the Ability To Function as Activators of Transcription

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Most LuxR homologues function as activators of transcription during the process of quorum sensing, but a few, including EsaR and ExpR$_{Ecc}$, negatively impact gene expression. The LuxR-activated *luxI* promoter and LuxR binding site, the *lux* box, were used in artificial contexts to assess the potential for transcriptional activation and DNA binding by EsaR and ExpR$_{Ecc}$. Although the acyl-homoserine lactone responsiveness of both proteins is the opposite of that shown by most LuxR family members, EsaR and ExpR$_{Ecc}$ have preserved the ability to interact with RNA polymerase and activate transcription despite their low affinity for the *lux* box DNA.

The quorum sensing regulator LuxR of *Vibrio fischeri* and most homologous proteins found in various proteobacteria function as acyl-homoserine lactone (AHL)-dependent transcriptional activators (reviewed in references 10, 17, 26, and 28) (Fig. 1, top). However, a few LuxR-type transcription factors function as negative regulators of gene expression. The quorum sensing regulators EsaR and ExpR$_{Ecc}$ fall into this latter category.

EsaR of *Pantoea stewartii* subsp. *stewartii* normally governs the expression of specific target genes by repression and AHL-dependent derepression (3, 18). *P. stewartii* subsp. *stewartii* causes Stewart's wilt disease in maize, in part, through the synthesis of an exo/capsular polysaccharide (EPS) virulence factor that clogs the xylem of the plant host (4). EPS synthesis is tightly controlled by a multitiered regulatory cascade (2, 24). The dominant level of control is mediated by EsaR and the AHL produced by the cognate AHL synthase, EsaI (3, 18). The role of EsaR as a repressor of quorum sensing has been demonstrated genetically and biochemically (3, 18). The key to EsaR repressor activity is twofold. First, EsaR exists as a dimer and binds target promoters in the absence of AHL (18, 21). Second, the placement of the *esaR* box DNA binding site is positioned to block the transcriptional activity of RNA polymerase (2) (Fig. 1, bottom).

The ExpR proteins studied in isolates of *Erwinia carotovora* (ExpR$_{Ecc}$) and *Erwinia chrysanthemi* (ExpR$_{Ech}$) also have characteristics of a repressor-like activity, although their precise regulatory role related to exoenzyme production is unclear (1, 19, 27). However, it was shown that an *expR* mutant strain of *E. carotovora* strain SCC3193 exhibited a slight increase in pectinase production, suggesting a weak repressor role, possibly by sequestering AHL to limit the activation activity of an unidentified alternate AHL-responsive activator (1).

Overall, there exists only 18 to 25% amino acid sequence identity between the members of the LuxR protein family (26). EsaR and ExpR$_{Ecc}$ exhibit 24% and 23% amino acid identity with LuxR, respectively (1, 2), but are 47% identical to each other (1). Interestingly, all three proteins recognize the same AHL signaling molecule, the L isomer of 3-oxo-hexanoyl-homoserine (3-oxo-C6-HL). The binding site for AHL in the LuxR family is predicted to be within the N-terminal domain (15, 16, 29). Repressor activity during quorum sensing requires binding of the C-terminal domain of the protein to the target site (7), while activation requires, in addition to DNA binding, appropriate surfaces with which to establish a productive interaction with RNA polymerase (RNAP) (9, 23). The goal of this study was to measure the ability of EsaR and ExpR$_{Ecc}$ to bind to the *lux* box DNA and activate the *lux* operon and, thereby, further examine the level of structural and functional conservation among LuxR-type regulators.

Construction of derivatives of pBAD22 expressing *luxR*, *esaR*, and *expR*. *Escherichia coli* DH5α (14) was used as the host organism for recombinant DNA manipulations. The *luxR*, *esaR*, and *expR* genes were amplified via PCR using the templates pJE202 (8), pSVB5-18 (2), and pSAO18 (1), respectively, with the appropriate primers listed in Table 1. In all three cases, the forward primer contained an *Eco*RI site and was designed to maintain optimal spacing between the Shine-Dalgarno sequence and start codon, while the reverse primer contained either a *Sma*I or *Xba*I site and two stop codons.

The three PCR products were first cloned into pGEM-T Easy (Promega, Madison, Wis.) and were then subcloned into pBAD22 (13) using primer or vector-derived *Eco*RI-*Sma*I sites for *luxR* and *Eco*RI-*Eco*RI sites for *esaR* and *expR*. The pBAD22 constructs were named pBAD-LuxR, pBAD-EsaR, and pBAD-ExpR (Table 1). Sequencing reactions performed at the Core Sequencing Facility at the Virginia Bioinformatics

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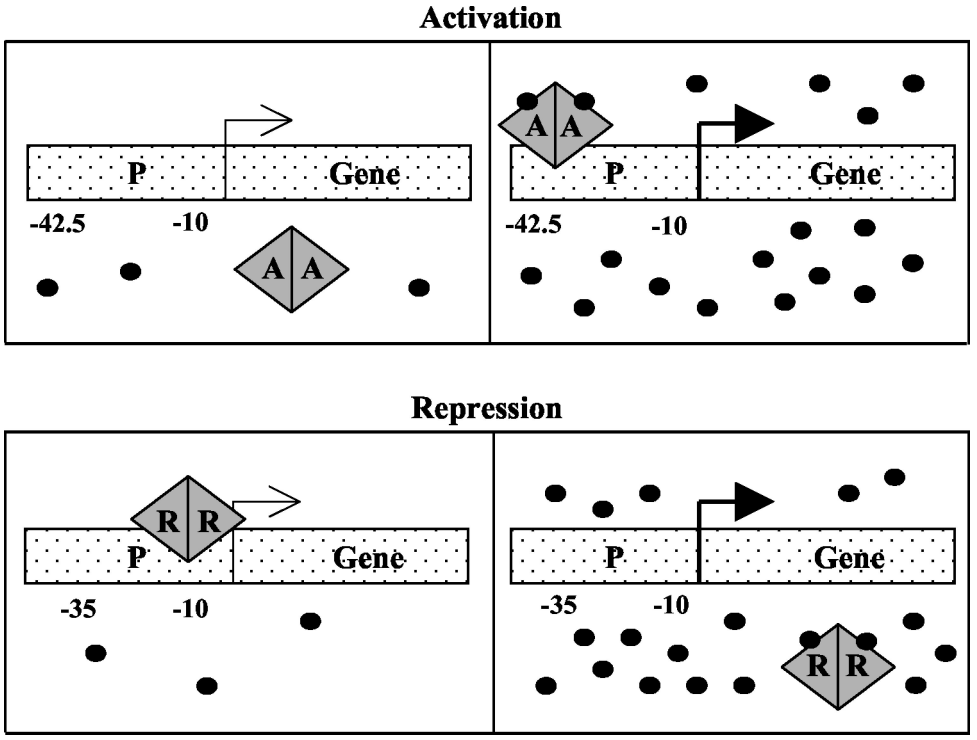

FIG. 1. Cartoon model of quorum sensing regulation by an activator (A) or a repressor (R). (Top) An activator binds to the DNA at a position around −42.5 and initiates transcription only in the presence of a high concentration of AHL, represented by the small black circles. (Bottom) A repressor remains bound to the DNA near −10 until a high enough concentration of AHL is reached to neutralize the repressor activity.

Institute, Virginia Tech, with primers annealing to pBAD22 (Table 1) verified the fidelity of the DNA sequence for *luxR* and *esaR*; the *expR* construct does contain a single silent nucleotide change from the wild-type sequence. LuxR and EsaR protein expression from pBAD-LuxR and pBAD-EsaR was confirmed via Western immunoblotting, since polyclonal antisera against these two proteins were readily available (data not shown).

Development of a lambda-based reporter system. The *luxI-lacZ* translational gene fusion was constructed by PCR generation of a *luxI* promoter fragment from the template pKE555 (6), carrying the *luxICDABE* genes from *V. fischeri* strain MJ1, using the appropriate primers (Table 1). After *Eco*RI-*Bam*HI digestion, the resulting 396-bp fragment was cloned into the *Eco*RI and *Bam*HI sites of the *lacZYA* reporter pMC1403 (5). In the resulting plasmid, p*luxI-lacZ*, the *Bam*HI site fuses the 19th codon of *luxI* to the 8th codon of *lacZ*. Plasmid p*luxI-lacZ* was digested with *Eco*RI and *Mfe*I, and the 5.8-kbp fragment carrying the *luxI-lacZYA* gene fusion was ligated into the *Eco*RI site of phage λgt2 (20). The ligated DNA was packaged into phage particles using the Gigapack λ packaging system (Stratagene, La Jolla, Calif.), and the packaging mixture was used to infect *E. coli* cells. A λ*luxI-lacZ* fusion phage was isolated and used to lysogenize *E. coli* strain GS162. The lysogen GS162λ*luxI-lacZ* (Table 1) was tested for a single copy of λ (22).

Phage λ35LB10 (Table 1) is derived from p35LB10 (7) and carries the LuxR-repressible artificial 35LB10-*lacZ* fusion. It was constructed using an intermediate plasmid, pMU100, in which the two *E. coli gcvB* transcription terminators t_1 and t_2 from p*gcvB-lacZ*$^{+251}$ (25) were inserted upstream of the *lacZ* gene in pMC1403 (5). Plasmid p35LB10 was digested with *Hin*dIII and *Sac*I, and a 2.2-kbp fragment carrying the 35LB10-*lacZ* fusion was used to replace the *Hin*dIII-*Sac*I *lacZ* fragment in pMU100. The 5.7-kbp fragment carrying the t_1 and t_2 dual terminators followed by the 35LB10-*lacZYA* fusion was then excised from this intermediate plasmid with *Mfe*I, cloned into the *Eco*RI site of phage λgt2 (20), packaged, and used to lysogenize *E. coli* GS162 as described above. The lysogen GS162λ35LB10 was also tested for a single copy of λ (22).

The λ*luxI-lacZ* and λ35LB10 constructs were subsequently transduced into *E. coli* Top 10 (Δ*araBAD*) (11) for use in the in vivo expression assays. The resulting Top 10λ*luxI-lacZ* and λ35LB10 strains were chosen as single lysogens by comparison of their β-galactosidase levels to those of the confirmed single lysogens of *E. coli* GS162 described above.

Activation of the *lux* promoter in vivo by EsaR and ExpR$_{Ecc}$. The *E. coli* λ*luxI-lacZ* strains separately containing pBAD22, pBAD-LuxR, pBAD-EsaR, and pBAD-ExpR were grown overnight at 30°C in RM minimal medium (2% Casamino Acids, 1× M9 salts [12.8 g of $Na_2HPO_4 \cdot 7H_2O$, 3 g of KH_2PO_4, 0.5 g of NaCl, and 1 g of NH_4Cl per liter], 0.4% glucose, and 1 mM $MgCl_2$) containing 100 μg of ampicillin ml^{-1} to an optical density at 600 nm (OD_{600}) of 0.2 to 0.5. The strains were then subcultured by inoculating to an OD_{600} of 0.005 into six sets of RM minimal medium with ampicillin (100 μg ml^{-1}) broth supplemented either with or without the P_{BAD} inducer 0.2% L-(+)-arabinose, in the absence or presence of a 1 or 100 μM concentration of a D,L-racemic mixture of 3-oxo-C6-HL (Fig. 2). At an OD_{600} of 0.5 (mid-exponential log-phase

TABLE 1. Strains, plasmids, phages, and primers

Strain, plasmid, phage, or primer	Relevant information	Source or reference
E. coli strains		
DH5α	F$^-$ ϕ80d *lac*ZΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsd17 phoA supE44 thi-1 gyrA96 relA1*	14
K-12 GS162	MC4100 *pheA905 thi* Δ(*argF-lac*)*U169 araD129 rpsL150 relA1 deoC1 fibB5301 ptsF25 rbsR*	G. Stauffer
Top10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lac*ZΔM15 Δ*lac*X74 *deoR recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL*(Str^r) *endA1 nupG*	11
Plasmids		
pGEM-T Easy	Cloning vector, f1 ori, Ap^r, used as an intermediate cloning vector	Promega
pBAD22	Arabinose inducible vector, Ap^r	13
pBAD-LuxR	*luxR* ligated into *Eco*RI and *Sma*I sites in pBAD22	This study
pBAD-EsaR	*esaR* ligated into *Eco*RI sites in pBAD22, 15-bp carryover of pGEM vector	This study
pBAD-ExpR	*expR* ligated into *Eco*RI sites in pBAD22, 15-bp carryover of pGEM vector, single silent mutation creates a *Rsa*I site	This study
pKE555	*luxICDABE*, Cm^r	6
pMC1403	translational *lacZ* fusion vector, Ap^r	5
p35LB10	Artificial LuxR-repressible *lac* promoter, Ap^r	7
p*gcvB-lacZ*$^{+251}$	Source of transcription terminators, Ap^r	25
pMU100	Dual terminators in pMC1403, Ap^r	This study
p*luxI-lacZ*	*luxI-lacZYA* fusion, Ap^r	This study
Phages		
λgt2	λ cloning vector, *cI*857 repressor	20
λ*luxI-lacZ*	*luxI-lacZYA* fusion in λgt2	This study
λ35LB10	35LB10-*lacZYA* fusion in λgt2	This study
Primers		
luxR forward	5′-CCGGAATTCACCATGAAAAACATAAATGCCGACGAC	This study
luxR reverse	5′-TCCCCCGGGCTATTAATTTTTAAAGTATGGGCA	This study
esaR forward	5′-GGAATTCACCATGTTTTCTTTTTTCCTTG	This study
esaR reverse	5′-CTCTAGATCACTACCTGGCCGCTGAC	This study
expR forward	5′-GGAATTCACCATGTCGCAGTTATTCTACAAC	This study
expR reverse	5′-CTCTAGATCACTATGACTGAACCGGTCGG	This study
pBAD forward	5′-TCGCAACTCTCTACTGTTTC	This study
pBAD reverse	5′-CTTCTCTCATCCGCCAAAAC	This study
P_{luxI} forward	5′-AAGAATTCACAATGTACCATTTTAGTCATATCAG	This study
P_{luxI} reverse	5′-AAGGATCCTTATACTCCTCCGATGGAATTGCC	This study
lux box upper[a]	5′-TCTTACCTGTAGGATCGTACAGGT	This study
lux box lower[a]	5′-CTTAACCTGTACGATCCTACAGGT	This study
esaR box upper[a]	5′-TCTTGCCTGTACTATAGTGCAGGT	This study
esaR box lower[a]	5′-CTTAACCTGCACTATAGTACAGGC	This study

[a] The protein binding site is underlined.

growth), 5 μl of cells was diluted 1:200 in Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$) with 400 μM dithiothreitol and lysed with 50 μl of chloroform. Chemiluminescent β-galactosidase assays (Tropix, Bedford, Mass.) were performed on 10-μl aliquots of the cell lysate in a Lucy 1 microplate luminometer (Rosys Anthos, Wals, Austria) over a 20-s integration time as previously described (7). Each sample was tested in triplicate, and light output was measured in relative light units.

In this system, *E. coli* Top10λ*luxI-lacZ* transformed with pBAD22 constitutes the negative control and indicates the basal level of expression of the reporter in the absence of any regulators. Addition of arabinose, 3-oxo-C6-HL, or both had no impact on the level of β-galactosidase produced by this strain (Fig. 2). As expected, the positive control reporter strain *E. coli* Top10λ*luxI-lacZ* transformed with pBAD-LuxR was stimulated to express *lacZ* at levels roughly 16- or 14-fold above the negative control in the presence of arabinose plus 1 or 100 μM 3-oxo-C6-HL, respectively (Fig. 2).

The *E. coli* Top10λ*luxI-lacZ* strain expressing EsaR was able to activate transcription of the *luxI-lacZ* fusion at levels about fourfold above the background in the presence of arabinose only, suggesting that EsaR can enter into a productive interaction with RNAP at the *luxI* promoter. Addition of 1 μM 3-oxo-C6-HL inhibited EsaR-mediated *luxI-lacZ* expression about twofold, and a concentration of 100 μM 3-oxo-C6-HL was needed to more fully neutralize EsaR activation. This is consistent with previous in vivo and surface plasmon resonance analyses that indicated the ability of EsaR to bind to its DNA recognition site is antagonized by the presence of 3-oxo-C6-HL (18). Finally, $ExpR_{Ecc}$ could only activate the *luxI-lacZ* reporter at levels about twofold above the background and also

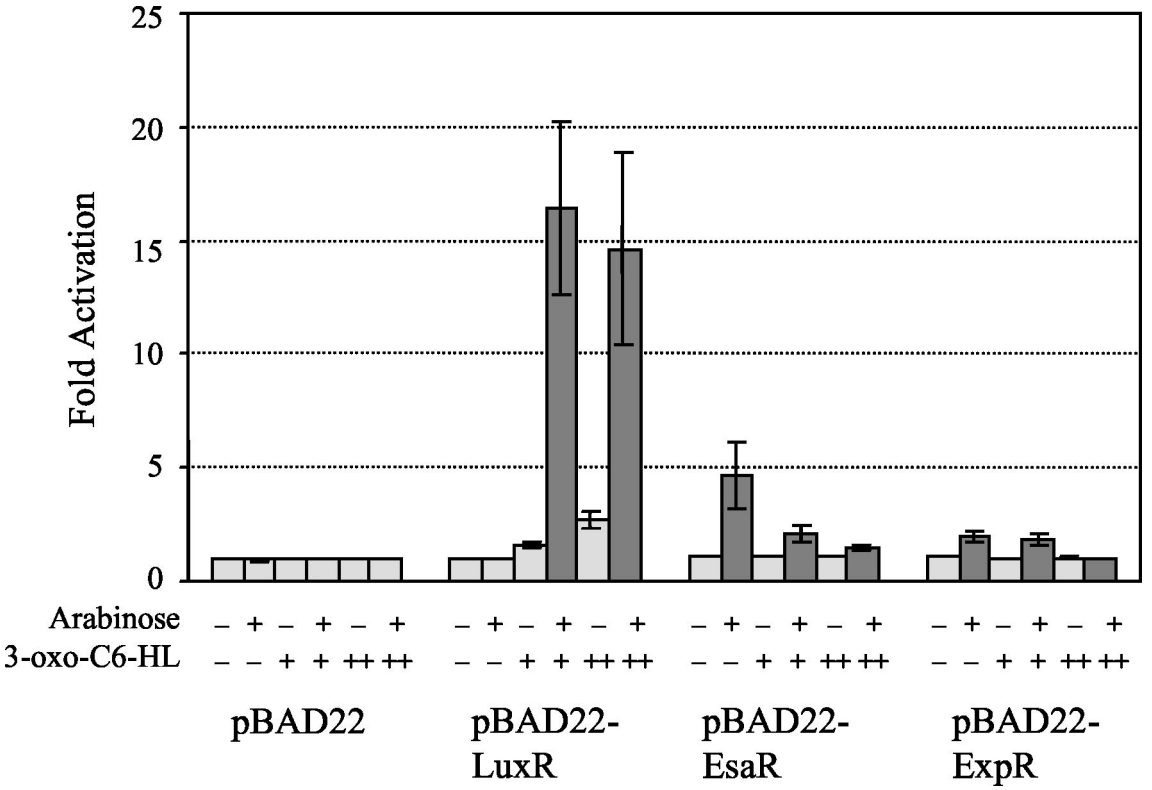

FIG. 2. Activation assays: β-galactosidase assays using the *E. coli* Top10λ*luxI* strain transformed with pBAD22, pBAD-LuxR, pBAD-EsaR, or pBAD-ExpR. The cells were grown in six sets of RM minimal medium with 100 μg of ampicillin ml^{-1} either without (−) or with (+) 0.2% L-(+)-arabinose and either without (−) or with a 1 μM (+) or 100 μM (++) concentration of a D,L-racemic mixture of 3-oxo-C6-HL. Samples from two independent trials were tested in triplicate. Error bars represent the range of each experiment from the mean. The negative control (pBAD22, no supplements) was set at 1 for each experiment, with the actual average value being equivalent to 3.04 ± 0.364 relative light units. Results discussed in the text are highlighted in dark grey.

required 100 μM 3-oxo-C6-HL for complete inhibition of β-galactosidase expression.

These findings suggest that EsaR and $ExpR_{Ecc}$ have retained the ability to function as activators of transcription when bound in the appropriate manner to promoter DNA, creating a functional transcription initiation complex with RNAP. The efficiency of activation of the *luxI* promoter by EsaR and $ExpR_{Ecc}$ is four- and eightfold lower, respectively, than that with LuxR. In order to determine if this difference is due to inefficient recognition of the heterologous target DNA, protein-protein interactions, or both, we measured the ability of EsaR and $ExpR_{Ecc}$ to bind to the *lux* box by using an in vivo transcriptional repression assay with an artificial promoter construct.

In vivo analysis of the binding of EsaR and ExpR to the *lux* box. Strains of *E. coli* Top10λ35LB10 containing the pBAD series plasmids were grown and assayed for β-galactosidase production (Fig. 3) under the same conditions described above for the activation assays. In this assay, if LuxR, EsaR, or $ExpR_{Ecc}$ binds to the *lux* box, it should repress transcription from the artificial 35LB10-*lacZ* promoter fusion, resulting in a decrease in β-galactosidase levels (7). Thus, the degree to which transcription is repressed will reflect the relative affinity of the proteins for the *lux* box DNA target. The strain expressing LuxR demonstrated roughly eightfold repression in the presence of arabinose and 1 μM 3-oxo-C6-HL in comparison to the pBAD22 control. The ability of LuxR to repress β-galactosidase expression was improved to about 15-fold below the negative control with arabinose and 100 μM 3-oxo-C6-HL in the medium.

In this assay system, EsaR was able to repress *lacZ* expression roughly twofold in the presence of arabinose alone. The ability of EsaR to bind to the *lux* box and repress the 35LB10 promoter was decreased by the addition of 1 μM 3-oxo-C6-HL and abolished by the addition of 100 μM 3-oxo-C6-HL. No repression of the 35LB10 promoter was seen with $ExpR_{Ecc}$. These data suggest that the lower levels of activation observed in the λ*luxI-lacZ* reporter assay are due at least in part to a diminished affinity of EsaR and $ExpR_{Ecc}$ for the nonnative *lux* box DNA. Since EsaR appeared to retain some ability to bind to and recognize the *lux* box, a direct in vitro DNA binding assay was used to further examine this activity.

EMSAs measuring the ability of EsaR to recognize DNA targets. EsaR protein was partially purified from *E. coli* Top10 pBAD-EsaR induced with 0.02% arabinose as previously described (18). Essentially, the cells were resuspended in buffer (50 mM Tris [pH 7.5], 10% glycerol) and lysed under pressure (20,000 lb/in^2) using a French press. After centrifugation (30,000 × *g* for 30 min), the soluble lysate was fractionated by heparin column chromatography and NaCl (400 to 800 mM)

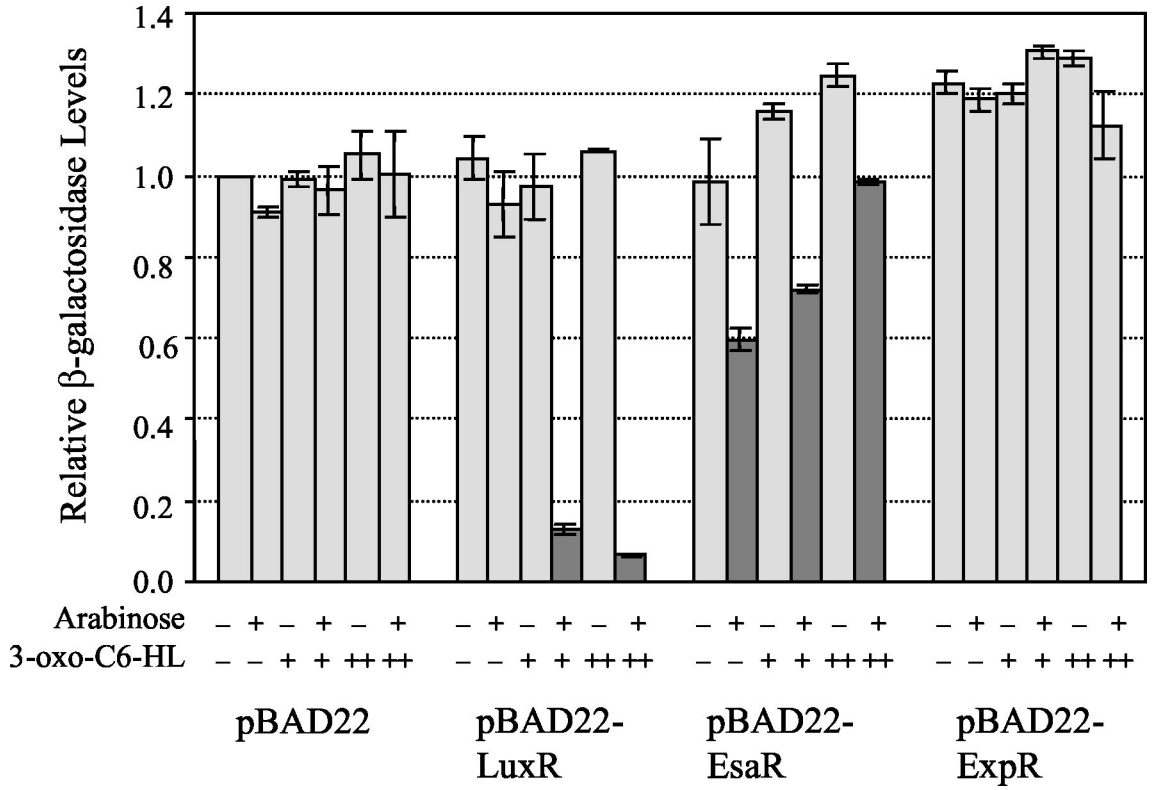

FIG. 3. Repression assays: β-galactosidase assays using the *E. coli* Top10λ35LB10 strain transformed with pBAD22, pBAD-LuxR, pBAD-EsaR, or pBAD-ExpR. The cells were grown in six sets of RM minimal medium with 100 μg of ampicillin ml^{-1} either without (−) or with (+) 0.2% L-(+)-arabinose and either without (−) or with a 1 μM (+) or 100 μM (++) concentration of a D,L-racemic mixture of 3-oxo-C6-HL. Samples from two independent trials were tested in triplicate. Error bars represent the range of each experiment from the mean. The negative control (pBAD22, no supplements) was set at 1 for each experiment, with the actual average value being equivalent to 168 ± 14.0 relative light units. Results discussed in the text are highlighted in dark grey.

gradient elution. Fractions containing EsaR were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and pooled. This EsaR-enriched preparation, estimated by SDS-PAGE analysis to be 50% pure, was used in gel electromobility shift assays (EMSAs) along with ^{32}P-end-labeled DNA probes generated by annealing oligonucleotides containing either the *lux* box or *esaR* box (Table 1). The *lux* box and *esaR* box are identical at 15 out of 20 positions.

EMSAs were performed essentially as described by Minogue et al. (18). Assays included partially purified EsaR protein as indicated in Fig. 4 and either 1.6 μM *lux* box or *esaR* box oligonucleotides in binding buffer [20 mM HEPES (pH 7.6), 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, 1 mM dithiothreitol, 0.2% Tween 20, 30 mM KCl, 50 mg of lambda DNA ml^{-1}, and 150 mg of bovine serum albumin ml^{-1}]. Reactions were incubated at 28°C for 30 min. Addition of excess (250 μM) unlabeled competitor DNA substrate was used to demonstrate the binding specificity of EsaR for the oligomeric substrates (Fig. 4, lanes 5 and 10). This unlabeled competitor DNA also annealed with radiolabeled single-stranded oligonucleotides, resulting in the enhanced radiolabeled double-stranded DNA band seen in the EMSA reactions containing competitor DNAs (Fig. 4, lanes 5 and 10). Reaction mixtures were resolved on a nondenaturing 6% polyacrylamide gel in Tris-borate-EDTA buffer (Fisher, Pittsburgh, Pa.) at 4°C, 200 V, for 40 min. The probes were imaged with a Molecular Imager FX system (Bio-Rad) and quantified with the Quantity One software (Bio-Rad).

The relative binding efficiency of EsaR for the separate target DNAs was calculated as the percent total radiolabeled double-stranded DNA present in the protein-DNA complex. EsaR protein at concentrations of 2, 20, and 200 nM was found to bind the *lux* box with efficiencies of 0.6, 2, and 13%, respectively, while the same concentrations of EsaR bound the native *esaR* box with efficiencies of 2, 11, and 52% (Fig. 4). Thus, the *esaR* box DNA is recognized by EsaR about fivefold more efficiently than is *lux* box DNA. This difference in binding affinity corresponds roughly to the degree of difference measured in the in vivo activation assays and suggests that the primary reason for the decreased activation of the *luxI-lacZ* fusion exhibited by EsaR in vivo is its inability to bind the *lux* box as efficiently as LuxR.

Conclusions. The LuxR homologue EsaR, previously shown to function as a repressor, retains an ability to function as an activator of transcription by RNAP. For most LuxR family members, AHL binding by the apo-activator is thought to facilitate formation of the active DNA binding conformation. In contrast, EsaR DNA binding is neutralized by 3-oxo-C6-HL, as shown indirectly in the in vivo β-galactosidase activation and repression assays and directly in the in vitro EMSAs, consistent with previous studies (3). Moreover, both the in vivo and in

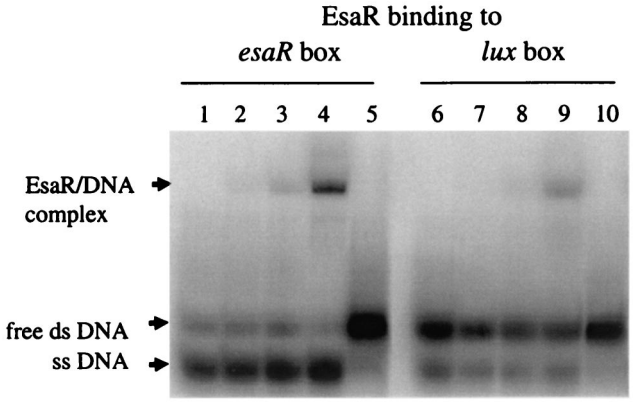

FIG. 4. EsaR EMSAs. Synthetic 28-bp radiolabeled oligonucleotides (1.6 μM) specifying either the *esaR* box (lanes 1 to 5) or the *lux* box (lanes 6 to 10) were incubated with semipure EsaR at concentrations of 0 (lanes 1 and 6), 2 nM (lanes 2 and 7), 20 nM (lanes 3 and 8), 200 nM (lanes 4 and 9), and 200 nM plus excess competitor DNA (250 μM) (lanes 5 and 10) and resolved by nondenaturing gel electrophoresis. ss DNA, single-stranded 24-base complementary oligonucleotide DNAs; ds DNA, annealed double-stranded 28-bp substrate DNA.

vitro assays showed that ApoEsaR can bind to the heterologous *lux* box, which differs from the *esaR* box at 5 of 20 positions. However, EsaR binds less efficiently to the *lux* box DNA than to the native *esaR* box, which may explain the weaker activation exhibited by EsaR in the in vivo assays.

$ExpR_{Ecc}$ fails to repress the 35LB10 promoter fusion in vivo, which may reflect an even lower binding affinity of $ExpR_{Ecc}$ for the *lux* box DNA. $ExpR_{Ecc}$ is able to activate expression of the *luxI-lacZ* fusion at rates just barely above background. RNAP may be required to help $ExpR_{Ecc}$ stabilize its contacts with the DNA in this artificial context. The fact that the low level of activation produced by $ExpR_{Ecc}$ is abolished by addition of 3-oxo-C6-HL suggests that it responds to the signal in a manner similar to EsaR.

It was demonstrated previously that the LuxR homologue, LasR, to a degree, can recognize the *lux* box and activate transcription in the presence of its cognate AHL and, conversely, LuxR can recognize the LasR binding site and activate transcription in the presence of its cognate AHL (12). This suggested that there was functional conservation in the DNA binding domain of the LuxR family of proteins and the targets that they recognize. The data reported are consistent with this study showing the ability of two other LuxR homologues to bind to the *lux* box and activate the *luxI* promoter.

However, what is clear from this and previous studies is that EsaR and ExpR have an ability to fold into a stable DNA binding protein in the absence of AHL. There is good evidence that AHL serves as a scaffold for folding and stabilizes the DNA binding conformation of the activator TraR and that the lack of AHL promotes the proteolytic degradation of the nascent TraR protein (30). Conversely, AHL binding to EsaR promotes structural changes that result in reduced DNA binding potential (18). Whether or not these conformational changes also render AHL-EsaR sensitive to proteolysis remains to be established. Ultimately, more detailed comparative structural analyses of representative members of the LuxR family that bind to the DNA in the presence of AHL versus those that bind to the DNA in the absence of AHL will be necessary to fully understand the functional differences between the two groups.

Future studies that make use of reporter constructs incorporating the native binding sites for EsaR and $ExpR_{Ecc}$ should allow for a thorough analysis of the protein-protein interactions that occur between these proteins and RNAP in the transcription initiation complex. These studies would yield additional insights into the degree of functional conservation that exists among the LuxR family of proteins involved in the quorum sensing response of proteobacteria and contribute to a broader understanding of mechanisms of transcription initiation and protein evolution in bacteria.

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