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Interchangeability and Specificity of Components from the Quorum-Sensing Regulatory Systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*

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Autoinduction is a conserved mechanism of cell density-dependent gene regulation that occurs in a variety of gram-negative bacteria. Autoinducible luminescence in *Vibrio fischeri* requires a transcriptional activator, LuxR, while a LuxR homolog, LasR, activates elastase expression in *Pseudomonas aeruginosa*. Both LuxR and LasR require specific signal molecules, called autoinducers, for activity. We show here the activation in *Escherichia coli* of the *V. fischeri* luminescence (*lux*) operon by LasR and of the *P. aeruginosa* elastase gene (*lasB*) by LuxR when each is in the presence of its cognate autoinducer. Neither LuxR nor LasR showed appreciable activity with the heterologous *V. fischeri* or *P. aeruginosa* autoinducer. This supports the view that there is a direct interaction of each transcriptional activator with its proper autoinducer and suggests that there are conserved, autoinduction-related elements within the promoter regions of these genes.

The autoinduction of gene expression, originally described for luminous bacteria (8, 22), has recently been shown to be a conserved system of gene regulation common to a variety of gram-negative bacteria (2, 13, 23, 25, 26). Autoinduction involves the interaction of a transcriptional activator protein with a signal molecule (the autoinducer) that is released by bacteria into the surrounding environment (8–10, 21, 22). Because the autoinducer must accumulate above an extracellular threshold concentration, gene expression is induced only in situations of relatively high population density, when the bacteria sense a quorum (for a recent review, see reference 13).

Despite the diversity of physiological functions now known to be regulated by autoinduction (see below), the regulatory components involved are conserved at both the physiological level and the genetic level (13). All of the autoinducer molecules currently known are *N*-acyl homoserine lactones, varying primarily in the length and hydrophobicity of their acyl side chains (4, 9, 24, 32). In *Vibrio fischeri*, the autoinducer synthase (LuxI) and transcriptional activator (LuxR) required for activation of the luminescence genes are encoded by a single pair of genes, *luxI* and *luxR*, respectively (10). Genes homologous to these signal generator and response activator regulatory components have recently been identified in several other bacteria. These include the *lasI* and *lasR* genes that regulate virulence functions in *Pseudomonas aeruginosa* (14, 23), the *traI* and *traR* genes regulating conjugal transfer in *Agrobacterium tumefaciens* (12, 13, 17, 25), and the *expI* and *expR* genes from *Erwinia carotovora*, which regulate exoprotease production, cellulase production, and also probably other extracellular products (18, 26).

According to current models of autoinduction, the autoinducer binds directly to its transcriptional activator protein, thus allowing DNA binding and gene activation to occur (1, 13, 29, 30). There is little evidence for the direct binding of the autoinducer by the transcriptional activator (1, 29, 30), however, and some investigators believe that the autoinducer may bind to an undefined accessory protein that in turn signals the transcriptional activator.

Using *Escherichia coli* MG4 (27) containing plasmid-borne *lux* and *las* gene constructs (Fig. 1), we show here activation of the *V. fischeri* luminescence (*lux*) genes by LasR and the synthetic *Pseudomonas* autoinducer (PAI), *N*-(3-oxododecanoyl)homoserine lactone (24), and of the *P. aeruginosa* elastase (*lasB*) gene by LuxR and the synthetic *V. fischeri* autoinducer (VAI), *N*-(3-oxohexanoyl)homoserine lactone (9). The autoinducers from *V. fischeri* and *P. aeruginosa* were not active with the heterologous activator protein, lending support to the model whereby each transcriptional activator binds directly to its cognate autoinducer. In view of these results, the identification of a conserved *lux* box-like promoter sequence upstream of the *P. aeruginosa lasB* gene (13) might be expected. Similar sequences are also found upstream of autoinducible genes from *A. tumefaciens* (13).

Elastase (*lasB*) gene activation by LuxR and the *V. fischeri* autoinducer. The *P. aeruginosa lasB* gene is activated by the LasR protein and PAI (14, 23, 24). As shown with the *lasB*::*lacZ* reporter fusion contained in pKDT17 (Fig. 1), *lasB* promoter activity increased more than 60-fold in the presence of LasR and PAI, compared with the basal levels observed with cultures lacking PAI (Table 1). Substitution of VAI for PAI in these experiments resulted in no autoinduction response. Deletion of LasR from the system also resulted in no autoinduction response (Table 1). When the *V. fischeri* transcriptional activator, LuxR, was substituted for LasR, there was a fourfold stimulation of *lasB* expression in the presence of VAI but no induction in the presence of PAI (Table 1). This low level of activation by LuxR and VAI represented a maximal response to the autoinducer, as increased dosage of VAI had no further effect (data not shown). When LasR and LuxR were both present, the results obtained with PAI and VAI closely matched those seen when each autoinducer was paired with its respective transcriptional activator alone, suggesting that there were no antagonistic or inhibitory effects arising from the presence of both activator proteins (Table 1).

These results agree with previous evidence that VAI and PAI are not cross-reactive inducing signals (24) and argue

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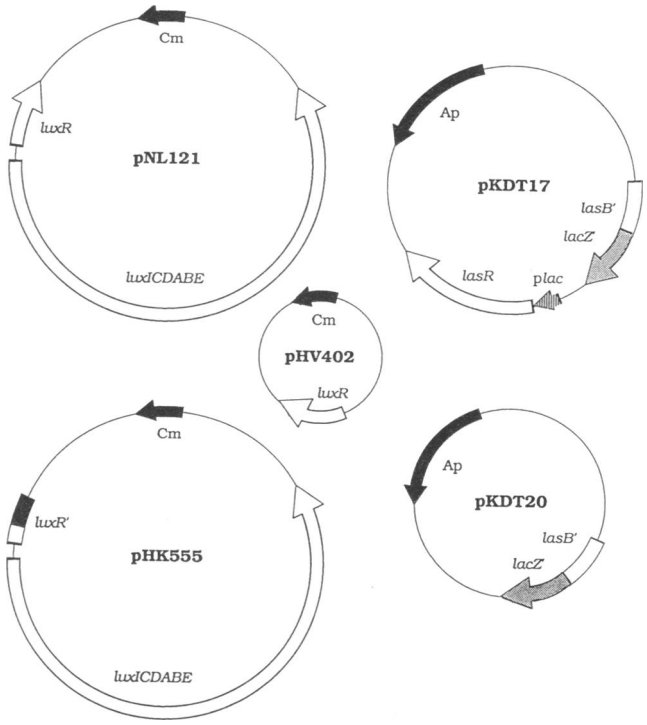

FIG. 1. Plasmids used in this study. pNL121 (7) contains the entire *V. fischeri lux* regulon, while pHK555 (19) contains the same genes with an insertionally inactivated *luxR*. pHV402 contains the 1.1-kb *Hin*cII *luxR* fragment from pHV202 (15) cloned in pACYC184, with *luxR* under the control of its natural promoter. Each of these *lux* plasmids is a P15A replicon. pKDT17 (24) carries a *lasB*::*lacZ* translational fusion under the control of the *lasB* promoter and the *lasR* gene under the control of an *E. coli lac* promoter. pKDT20 was formed by digesting pKDT17 with *Eco*RI and *Hin*dIII to excise the *lasR* gene, filling in the overhanging 5′ termini with DNA polymerase, and ligating the resulting blunt ends. Both of the *las* plasmids, pKDT17 and pKDT20, are ColE1 replicons.

against a report that VAI is the inducer signal produced by *P. aeruginosa* that is required for the activation of *lasB* by LasR (18).

Luminescence (*lux*) gene activation by LasR and the *P. aeruginosa* autoinducer. In the absence of either LuxR or LasR, the background level of luminescence achieved in *E. coli* containing the *lux* genes from *V. fischeri* remained unaffected by the addition of either VAI or PAI (Table 2). In the presence of LuxR, luminescence increased 10-fold in the absence of added autoinducer. Similar low levels of autoinducer-independent *lux* gene activation have been reported previously (for example, see reference 5). There was a further eightfold activation of luminescence in the presence of PAI (Fig. 2 and Table 2). This represented a maximal response to PAI, as

TABLE 1. Activation of the *lasB* promoter in *E. coli* by LasR and LuxR[a]

Plasmid	Activator phenotype	Culture addition[b]	β-galactosidase activity (Miller units)[c]	Relative expression
pKDT20	LasR$^-$ LuxR$^-$	No addition	25 ± 2	1
		VAI	31 ± 3	1
		PAI	27 ± 2	1
pKDT17	LasR$^+$ LuxR$^-$	No addition	28 ± 3	1
		VAI	36 ± 4	1
		PAI	1,569 ± 123	63
pKDT20-pHV402	LasR$^-$ LuxR$^+$	No addition	33 ± 2	1
		VAI	99 ± 6	4
		PAI	34 ± 2	1
pKDT17-pHV402	LasR$^+$ LuxR$^+$	No addition	35 ± 2	1
		VAI	102 ± 2	4
		PAI	1,877 ± 116	75

[a] Quantitative assays for the activation of *lasB* expression were performed as previously described (24) with the *lasB*::*lacZ* reporter fusion contained on pKDT17 and pKDT20.
[b] Synthetic PAI and VAI were tested at final concentrations of 100 ng/ml, which is fully saturating for the autoinduction response of either system (24).
[c] Data are the averages of four assays ± the ranges for each.

TABLE 2. Activation of luminescence (*lux*) gene expression in *E. coli* by LasR and LuxR[a]

Plasmid	Activator phenotype	Culture addition[b]	Culture luminescence (quanta/s/ml)[c]	Relative expression
pKDT20-pHK555	LasR$^-$ LuxR$^-$	No addition	$2.8 \times 10^5 \pm 0.4 \times 10^5$	1
		VAI	$2.8 \times 10^5 \pm 0.5 \times 10^5$	1
		PAI	$2.8 \times 10^5 \pm 0.4 \times 10^5$	1
pKDT17-pHK555	LasR$^+$ LuxR$^-$	No addition	$2.4 \times 10^5 \pm 0.4 \times 10^5$	1
		VAI	$2.7 \times 10^5 \pm 0.8 \times 10^5$	1
		PAI	$1.5 \times 10^{11} \pm 0.1 \times 10^{11}$	5.3×10^5
pKDT20-pNL121	LasR$^-$ LuxR$^+$	No addition	$3.0 \times 10^6 \pm 0.8 \times 10^6$	11
		VAI	$1.5 \times 10^{11} \pm 0.5 \times 10^{11}$	5.4×10^5
		PAI	$2.3 \times 10^7 \pm 0.9 \times 10^7$	82
pKDT17-pNL121	LasR$^+$ LuxR$^+$	No addition	$2.8 \times 10^6 \pm 1.0 \times 10^6$	10
		VAI	$1.1 \times 10^{11} \pm 0.6 \times 10^{11}$	3.9×10^5
		PAI	$1.9 \times 10^{11} \pm 0.5 \times 10^{11}$	6.8×10^5

[a] *E. coli* MG4 containing the plasmids indicated was grown in supplemented A medium (24) overnight at 30°C with shaking, diluted to an optical density at 660 nm of 0.05 in fresh medium, and shaken at 30°C for 3 h. These cultures were then inoculated to an optical density at 660 nm of 0.01 in supplemented A medium ± PAI or VAI and shaken at 30°C for 5.5 h.

[b] Synthetic PAI and VAI were tested at final concentrations of 100 ng/ml.

[c] Culture luminescence was measured as previously described (15). Data are the averages of three assays ± the ranges for each.

increased dosage of PAI had no further effect (data not shown). We consider this eightfold response to PAI minor in comparison with the greater than 50,000-fold increase observed when VAI was added to the system (Fig. 2 and Table 2).

When LuxR was replaced with LasR, the level of luminescence without added autoinducer was similar to that seen in the absence of either transcriptional activator. Addition of PAI to the culture resulted in full induction of luminescence (Fig. 2 and Table 2). VAI had no effect on the LasR$^+$ LuxR$^-$ cells, again supporting the view that the two autoinducers are not cross-reactive.

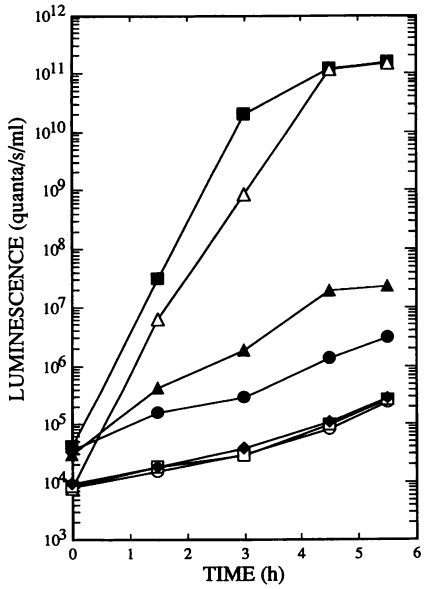

FIG. 2. Inducible luminescence of *E. coli* MG4 containing *lux* gene plasmids. *E. coli* cultures containing either *lasR* (open symbols) or *luxR* (closed symbols) were grown as described in Table 2, footnote *a*, in the presence of no added autoinducer (circles), 100 ng of PAI per ml (triangles), and 100 ng of VAI per ml (squares). Luminescence of *E. coli* lacking both *luxR* and *lasR* (diamonds) remained unaffected by the addition of either autoinducer.

Identification of *lux* box-like sequences. Because our data demonstrated that the *lux* promoter from *V. fischeri* and the *lasB* promoter from *P. aeruginosa* can be activated by either LuxR or LasR, we searched upstream of these genes for conserved sequences that might be involved in the autoinduction response.

A 20-bp palindrome centered around −40 bp from the transcriptional start of the *V. fischeri lux* operon has been implicated as the binding site for LuxR (6, 31). As shown in Fig. 3, representative *V. fischeri* strains from each of four different subgroups identified by restriction analysis (20) show differences at 3 of the 20 positions within this proposed binding site.

As previously reported (13), we examined the sequence upstream of *lasB* for similar 20-bp repeats and found such a sequence in the appropriate position relative to the start of transcription. In the case of *lasB*, which our data show contains a promoter weakly activated by LuxR (Table 1), the palindrome shows sequence identity at 10 of the 17 conserved sites of the *V. fischeri* sequences (Fig. 3).

A prediction implicit in this analysis is that the promoter regions of autoinducible genes from other bacteria might be expected to contain similar repeats at the appropriate positions. In the case of the autoinducible *traA* and *traI* genes carried on the octopine-type Ti plasmid of *A. tumefaciens*, such repeats have been identified (12, 13) (Fig. 3). We show in our alignment (Fig. 3) a similar sequence from the 5′-flanking region of the *A. tumefaciens traI* carried on the nopaline-type Ti plasmid (17).

Conclusions. Despite their structural similarities (24), the autoinducers of *V. fischeri* and *P. aeruginosa* do not show appreciable activity with heterologous transcriptional activator proteins in *E. coli*. It should be mentioned that the levels of autoinducer used in this study were far in excess of the levels required for full activity of their cognate R protein (approximately 50-fold excess). If each autoinducer is provided with its respective activator protein, however, the signal-activator pair is capable of activating expression of autoinducible genes from either system. These results support the view that the autoin-

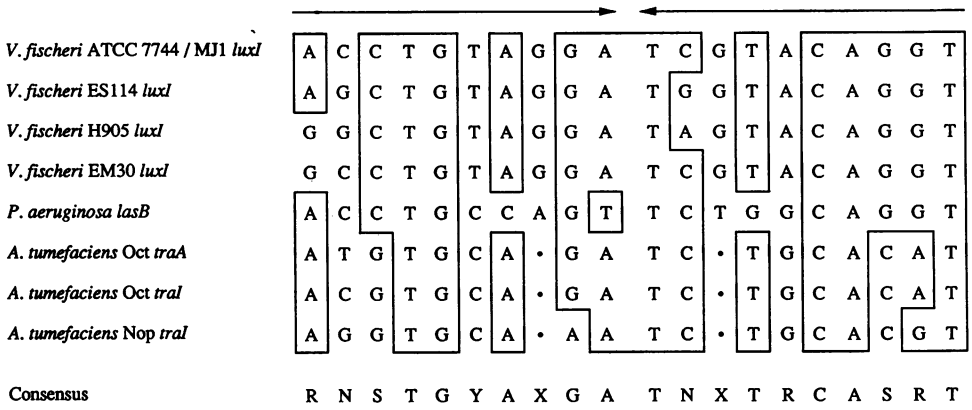

FIG. 3. Analysis of *lux* box-like elements. The palindromic *lux* box identified in *V. fischeri* ATCC 7744 (6) was compared with the published sequences from strains MJ1 (11) and ES114 (16). Sequencing of PCR-amplified *luxI* promoter regions from *V. fischeri* H905 and EM30 (20) was performed as previously described for strain ES114 (16). Strains H905 and EM30 are from Lee et al. (20). We also show alignments of similar palindromes within the published sequences upstream of the *P. aeruginosa lasB* gene (3), the *traA* and *traI* genes from the octopine-type Ti plasmid of *A. tumefaciens* (13), and the *traI* from the nopaline-type Ti plasmid (17). Boxes indicate sites at which five or more nucleotides are identical. Consensus sequence abbreviations: N = A, T, C, or G; R = A or G; S = C or G; Y = T or C; X = N or a gap in the sequence.

ducer must interact directly with its corresponding transcriptional activator protein, rather than with an accessory element encoded by *E. coli*. Absolute proof of this hypothesis awaits the development of in vitro activity assays for LuxR and LasR.

LuxR and VAI only weakly activate the *lasB* promoter (Table 1), while LasR and PAI fully activate the *lux* operon promoter (Fig. 2 and Table 2). One interpretation of these data is that LasR is more flexible in its recognition of the alternative promoter sequence than is LuxR; however, it would be premature to reach such a conclusion. In our experiments, we have made no attempt to control cellular levels of LuxR or LasR. This is of particular concern because *lasR* was under the control of the *lac* promoter on a high-copy-number ColE1 replicon, whereas *luxR* was under the control of its own promoter and on a lower-copy-number P15A replicon. Thus, it is conceivable that higher levels of LuxR than those attained in our experiments might fully activate the *lasB* promoter.

Regardless, the ability of LuxR with VAI and of LasR with PAI to activate expression of heterologous genes suggests that the two systems share similar promoter features related to the autoinduction response. The *P. aeruginosa lasB* promoter region contains a *lux* box-like 20-bp palindrome (Fig. 3) at about −40 (28). Similar sequences in the regions upstream of autoinducible genes from *A. tumefaciens* were also identified (Fig. 3). Thus, the *lux* box sequence may represent a conserved regulatory element centered around −40 bp from the transcriptional start sites of autoinducible genes. From the data presented in Fig. 3, we have deduced a consensus sequence for such *cis*-acting regulatory elements. Whether the interchangeability of *lux* box elements observed with the *V. fischeri* and *P. aeruginosa* systems will extend to other systems, such as that of *A. tumefaciens*, remains to be determined. Regardless, we propose that the existence of an appropriately positioned *lux* box-like element upstream of any bacterial gene suggests the possibility of cell density-dependent regulation by autoinduction.

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