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AinS and a New Family of Autoinducer Synthesis Proteins†

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In *Vibrio fischeri*, the autoinducer *N*-3-oxohexanoyl-L-homoserine lactone (AI-1) governs the cell density-dependent induction of the luminescence operon via the LuxR transcriptional activator. The synthesis of AI-1 from bacterial metabolic intermediates is dependent on *luxI*. Recently, we found a second *V. fischeri* autoinducer molecule, *N*-octanoyl-L-homoserine lactone (AI-2), that in *E. coli* also activates the luminescence operon via LuxR. A locus independent of *luxI* was identified as being required for AI-2 synthesis. This 2.7-kb *ain* (autoinducer) locus was characterized by transposon insertion mutagenesis, deletion and complementation analysis, and DNA sequencing. A single 1,185-bp gene, *ainS*, was found to be the sole exogenous gene necessary for the synthesis of AI-2 in *Escherichia coli*. In addition, a *V. fischeri ainS* mutant produced AI-1 but not AI-2, confirming that in its native species *ainS* is specific for the synthesis of AI-2. *ainS* is predicted to encode a 45,580-Da protein which exhibits no similarity to LuxI or to any of the LuxI homologs responsible for the synthesis of *N*-acyl-L-homoserine lactones in a variety of other bacteria. The existence of two different and unrelated autoinducer synthesis genes suggests the occurrence of convergent evolution in the synthesis of homoserine lactone signaling molecules. The C-terminal half of AinS shows homology to a putative protein in *Vibrio harveyi*, LuxM, which is required for the synthesis of a *V. harveyi* bioluminescence autoinducer. Together, AinS and LuxM define a new family of autoinducer synthesis proteins. Furthermore, the predicted product of another gene, *ainR*, encoded immediately downstream of *ainS*, shows homology to LuxN, which is similarly encoded downstream of *luxM* in *V. harveyi* and proposed to have sensor/regulator functions in the bioluminescence response to the *V. harveyi* autoinducer. This similarity presents the possibility that AI-2, besides interacting with LuxR, also interacts with AinR under presently unknown conditions.

Changing environmental conditions can cause bacteria to experience wide fluctuations in population density. As a mechanism for sensing and adapting to cell density, bacterially produced autoinducer molecules induce the expression of specific genes in a dose-dependent manner (for a review, see reference 16). Since autoinducers diffuse freely across bacterial membranes following a concentration gradient (20), their cellular concentrations remain low when autoinducer-producing cells are living at low cell density; however, at high cell densities, intracellular concentrations of autoinducers increase, allowing the autoinducers to activate the transcriptional regulator molecules for the specified genes.

Autoinducers have been found in a variety of proteobacteria and are homoserine lactone (HSL) derivatives with various acyl groups (16). In the extensively studied system of autoinduction of bioluminescence in the marine bacterium *Vibrio fischeri*, the *luxI* gene is responsible for the synthesis of the first identified autoinducer, *N*-3-oxohexanoyl-L-HSL (AI-1) (11, 14). At threshold concentrations, AI-1 is thought to interact with the regulatory protein, LuxR, to induce transcription of the luminescence operon, *luxICDABEG*, which contains the genes for synthesis of luciferase, its aldehyde substrate, and AI-1 (14). In a homologous regulatory system in *Pseudomonas aeruginosa*, the *lasI* gene is necessary for the production of *N*-3-oxododecanoyl-L-HSL (PAI), which mediates the induction of virulence determinants (26). Similarly, synthesis of *N*-3-oxooctanoyl-L-HSL, which mediates induction of the conjugal transfer genes in *Agrobacterium tumefaciens*, is dependent on *traI* (15, 31). In *Erwinia carotovora*, *N*-3-oxohexanoyl-L-HSL is suspected to be the regulator of carbapenem and exoenzyme production; two genes, *carI* and *expI*, have been suggested to induce, separately, *N*-3-oxohexanoyl-L-HSL synthesis (3, 28).

In the systems described above, both the autoinducer and a gene necessary for its synthesis have been identified; in each system, the autoinducer is an *N*-acyl-L-HSL and the gene is a *luxI* homolog. Although the pathway for autoinducer synthesis is poorly understood, the cloned *luxI* and *lasI* genes have been shown to be necessary and sufficient to produce AI-1 and PAI, respectively, in *Escherichia coli* (14, 26) as well as their native bacteria. Apparently, only a single dedicated protein is required for the synthesis of these autoinducers from precursors in the cell (12, 14), and thus LuxI and the LuxI homologs are thought of as synthases. However, the specific biochemical activity of this family of proteins remains to be confirmed.

Additional situations exist in which only a putative synthase gene or only an autoinducer is known. For example, a *luxI* homolog has been cloned from *Enterobacter agglomerans*, but the autoinducer which is synthesized remains unknown (30). In *Vibrio harveyi*, expression of the luminescence genes is controlled by *N*-3-hydroxybutanoyl-L-HSL (HAI); however, no *luxI* homolog has been found. Two polypeptides, LuxL and LuxM, are reported to be involved in HAI synthesis; possible roles remain difficult to elucidate, however, since *luxLM*-induced production of HAI has not been readily detected in *E. coli* (4).

Recently, multiple autoinducer molecules have been isolated from *V. fischeri*. As with AI-1, the second autoinducer, AI-2, was identified through its ability to induce bioluminescence. AI-2 is also an HSL derivative, *N*-octanoyl-L-HSL; however, the locus responsible for its synthesis, *ain*, is distinct from *luxI*, demonstrating that the production of the two autoinduc-

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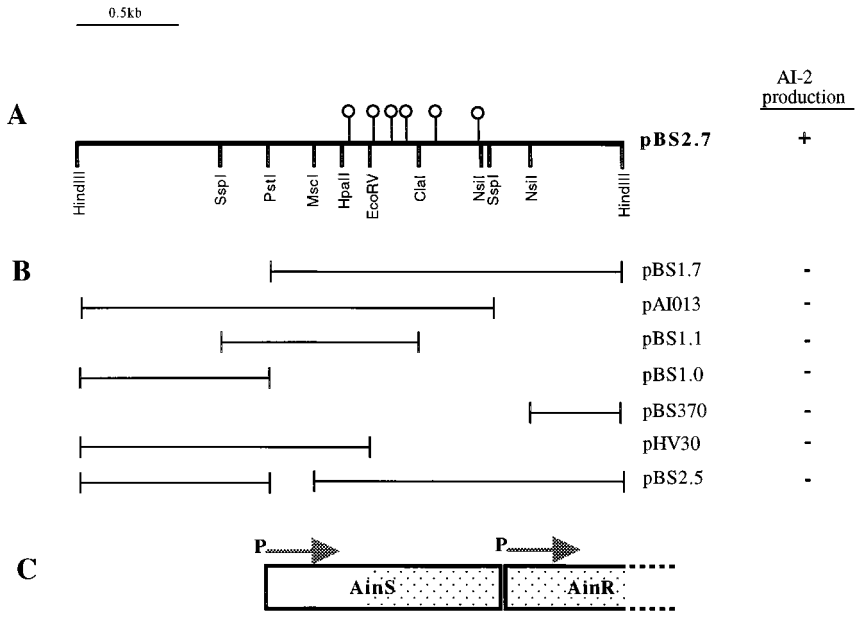

FIG. 1. Genetic structure of the *ain* locus. (A) Restriction map of the 2.7-kb fragment cloned into pBluescript to form pBS2.7. Lollipops indicate sites of representative mγδ-1 insertions in pBS2.7 that prevented the production of AI-2 in *E. coli*. These insertions were used as primer annealing sites for DNA sequencing. (B) The location of *ainS* was further defined by subcloning the depicted fragments into either pBluescript (pBS1.7, pBS1.1, pBS1.0, pBS370, and pBS2.5), pBR322 (pAI013), or pSUP102 (pHV30); none showed AI-2 activity. Some of these subclones were also used as part of the sequencing strategy. (C) Locations of the predicted ORFs encoding AinS and AinR. Stippled areas indicate the regions of similarity with LuxM and LuxN from *V. harveyi*. Arrows show directions of transcription. P, promoter.

ers is under the control of different genes (21). The genetic analysis of *ain* presented here shows that the single gene product responsible for the critical step in catalyzing AI-2 synthesis in both *V. fischeri* and *E. coli* is not a member of the LuxI autoinducer synthesis family of proteins. Thus, the production of AI-2 via a new pathway presents a clear divergence from the paradigm that the synthesis of autoinducers depends on LuxI homologs.

MATERIALS AND METHODS

Plasmids and strains. pAK211 is a pSUP102 (Cm^r) (29) derivative constructed by Kuo et al. (21) such that it is carrying the *luxR* and *luxICDABEG* genes with a ~250-bp nonpolar deletion in *luxI*. pAI002 and pAI009 are pSUP102 derivatives carrying *ain*-bearing 10.5- and 2.7-kb fragments, respectively (21). The 2.7-kb *Hin*dIII fragment of pAI002 was moved into pBluescript KS+ (Stratagene, La Jolla, Calif.) to form pBS2.7. Transformations, performed as described by Chung et al. (8), were generally into ZK4, a *recA56* derivative of *E. coli* MC4100 (17). Other routine DNA manipulations were performed by using standard procedures (2). *V. fischeri* MJ-100 is a spontaneous Nx^r derivative of MJ-1 (10).

Media and culture conditions. *E. coli* strains were grown on LB medium as described by Miller (25). Antibiotics were used at the following concentrations: ampicillin, 150 μg/ml; chloramphenicol, 38 μg/ml; and kanamycin, 10 or 50 μg/ml, depending on whether it was necessary to select for a low- or a high-copy-number plasmid. *V. fischeri* strains were grown at 28°C in LBS medium (10) with nalidixic acid (20 μg/ml) and, where appropriate, neomycin (200 μg/ml) or chloramphenicol (34 μg/ml).

Transposon mutagenesis. A set of mini-γδ transposon insertions which carry *kan* (mγδ-1) was generated in pBS2.7, using the strains and method developed by Berg et al. (6). To enhance the number of independent insertions, the procedure was repeated five times. Each procedure gave thousands of colonies with mγδ-1 insertions in pBS2.7. Pooled plasmid preparations were used to transform ZK4 carrying pAK211, after which Ap^r Cm^r Km^r transformants were selected. Approximately 42% of these grew as colonies which did not produce light, indicating that in those clones, the mγδ-1 element had inserted into *ain*. Thirty-five insertions were mapped with *Bam*HI digests, using the precisely known *Bam*HI restriction sites in pBluescript and the mγδ-1 element. During DNA sequencing, six relatively evenly spaced insertions, including the two outermost ones, were mapped precisely (Fig. 1A).

Complementation analysis. pBS2.5 was paired with pHV30 (Fig. 1B) and used to cotransform ZK4. Ap^r Cm^r transformants were analyzed on LB plates for AI-2 synthesis. A zone of diffused autoinducer which caused a cross-streak of ZK4(pAK211) to produce light was considered positive complementation.

DNA sequencing. Double-stranded nucleotide sequencing was performed according to the protocol for the Sequenase kit (U.S. Biochemical, Cleveland, Ohio). Sequencing from the mγδ-1 insertion sites required the use of *kan* and *res* primers (gifts from R. Kolter), which anneal to opposite ends of the mγδ-1 inserts (6). The SK 17-mer primer from Stratagene (La Jolla, Calif.) was also used to sequence the ends of fragments subcloned into pBluescript.

Construction of a *V. fischeri ainS* mutant. pAI009 cut once with *Cla*I was blunt ended, purified by agarose gel electrophoresis, and ligated in the presence of the similarly blunt-ended and purified 1.8-kb *Bam*HI-*Hin*dIII fragment containing *neo* from Mu dI1734 (7). *E. coli* transformants were selected on chloramphenicol and kanamycin and screened for lack of autoinducer activity. An *ainS*::*neo* plasmid, pAI014, so isolated was transferred to *V. fischeri* MJ-100 by using the transconjugation procedure described previously (10). Of 1,899 Nm^r transconjugants screened, 13 were Cm^s and presumably lacked plasmid. Double homologous recombination in the regions of DNA flanking the *neo* insert prior to the loss of the unstable plasmid was confirmed in one of the clones, MJ-216, by Southern hybridization using the 2.7-kb *ain* fragment to probe a *Hin*dIII-*Cla*I double digest of MJ-216 chromosomal DNA. Chemical detection of autoinducers by reverse-phase high-pressure liquid chromatography (RP HPLC) and autoinducer activity assays were performed as described previously (21).

Nucleotide sequence accession number. The nucleotide sequence determined in this study has been deposited in the GenBank database under accession number L37404.

RESULTS and DISCUSSION

Insertion mutagenesis and deletion mapping of the cloned *ain* locus. Isolation of the *ain* locus (21) was based on the ability of *E. coli* to express the cloned *lux* genes and emit light in a LuxI/LuxR-dependent manner. Thus, plasmid pAI002 was isolated from a *V. fischeri* plasmid library by virtue of its ability to complement in *E. coli* a plasmid carrying the *lux* system with a mutation in *luxI*. The 10.5-kb insertion fragment from pAI002 contained a 2.7-kb *Hin*dIII fragment which maintained the same Lux-inducing phenotype and directed the synthesis of AI-2. This locus was shown to be distinct from *luxI* and designated *ain*, for autoinducer (21).

To localize the gene(s) necessary for AI-2 synthesis, we

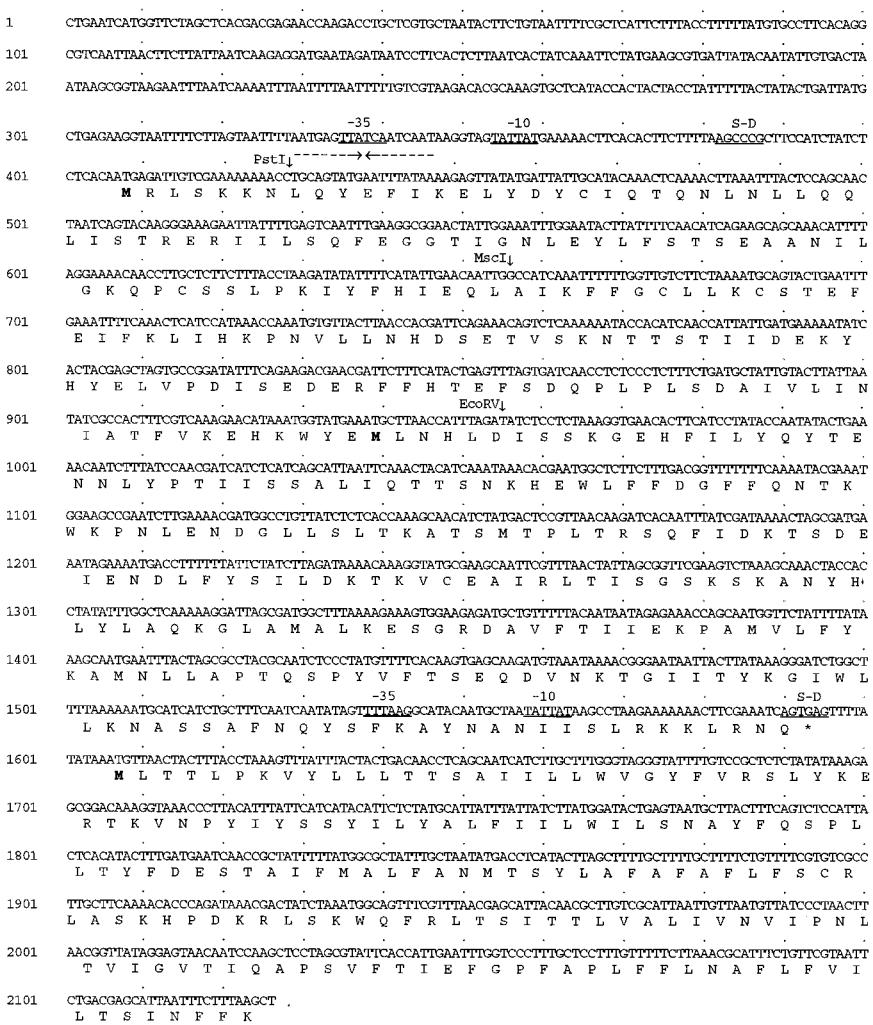

FIG. 2. DNA sequence of the *ainS* region, including the 5′ terminus of *ainR*. The predicted amino acid sequences for the ORFs are given, with the initial methionines in boldface. An internal methionine in AinS, after which the amino acid sequence shows homology to LuxM in *V. harveyi*, is also in boldface. The *lux* box-like palindrome is underlined with dashed arrows. Potential −10, −35, and Shine-Dalgarno (S-D) sequences are underlined, and pertinent restriction sites are indicated.

mutagenized the *ain*-carrying plasmid pBS2.7 with a mini-γδ transposon, mγδ-1 (6) (Materials and Methods), and then employed the same functional complementation screen used in isolating *ain* to identify insertion mutations in *ain. E. coli* was cotransformed with the mutagenized plasmids and pAK211 (which carries the *luxR* and *luxICDABEG* operons with a nonpolar deletion in *luxI*), and Lux$^-$ colonies were isolated. The mγδ-1 insertion sites for 35 such mutants were determined by restriction site analysis; the locations of six evenly spaced representative *ain*::γδ insertions are depicted in Fig. 1A. All insertions mapped within a 660-bp region, indicating that the area responsible for the Lux$^+$ phenotype of *ain* was greater than or equal to this region.

Deletion mapping by generating the subclones shown in Fig. 1B and cotransforming *E. coli* with pAK211 further defined the *ain* locus. Neither pBS1.7 nor pAI013 induced luminescence, demonstrating that the region necessary for AI-2 expression was greater than 1 kb, extending both to the left of the *Pst*I site and to the right of the second *Ssp*I site. Accordingly, all of the mγδ-1 insertions which blocked AI-2 synthesis mapped within this region.

Identification of the novel autoinducer synthesis gene, *ainS*. DNA sequencing of the *ain* locus (Materials and Methods) used a combined strategy of sequencing from mγδ-1 transposon insertions and from subcloned fragments (Fig. 1A and B). The DNA sequence (Fig. 2) revealed a 1,185-bp open reading frame (ORF), designated *ainS*, beginning just upstream of the *Pst*I site and extending to between the two *Nsi*I sites. The location and size of *ainS* concurred with the earlier transposon mutagenesis and deletion mapping. The 395-amino-acid sequence predicts a 45,580-Da AinS protein and was used in a BLAST (basic local alignment search tool) (1) search of nonredundant protein data banks supplied by the National Center for Biotechnology Information. It exhibited no obvious similarity to LuxI or to any of the LuxI homologs, which was surprising since all previously identified autoinducer synthesis proteins with similar phenotypes are members of the LuxI family of proteins. The C-terminal 218 amino acids, however, showed homology to the putative 216-amino-acid LuxM protein of *V. harveyi*. The region of similarity extends from an internal methionine in AinS to near the end of the protein. When aligned (19), this region is 34% identical to the full-length LuxM protein (Fig. 3A). Mutations in *luxM* result in diminished induction of luminescence and presumably in di-

FIG. 3. Amino acid sequence comparison between (A) *V. fischeri* AinS and *V. harveyi* LuxM and (B) *V. fischeri* AinR and *V. harveyi* LuxN. Dashes in the sequences represent gaps. Identical amino acids are represented by vertical bars, whereas colons denote conservative substitutions. The conservative residue substitutions used are Gly and Ala, Phe and Tyr, Glu and Asp, Ser and Thr, Arg and Lys, Gln and Asn, and Ile, Leu, and Val.

minished production of a bioluminescence autoinducer, HAI (4). Immediately upstream of *luxM* is a phenotypically indistinguishable gene, *luxL*. Since *luxL* encodes a predicted polypeptide of 168 amino acids (4), we compared the N-terminal 177 residues of AinS with the LuxL sequence. No significant similarity was found.

Demonstration that a possible internal translational initiation site does not produce a protein capable of autoinducer synthesis. Both the similarities between AinS and LuxM and the observation that none of the mγδ-1 insertion mutations mapped in the 5′ end of *ainS* suggested that perhaps the internal ATG in the *ainS* transcript can be employed to produce an active, truncated form of AinS. Indeed, the internal ATG is preceded by a plausible ribosome binding site. To explore the possibility that only the carboxy-terminal domain of AinS is required for function, pBS2.5 (Fig. 1B) was constructed such that the natural transcriptional promoter region was left intact and the 230-bp *Pst*I-*Msc*I in-frame deletion would not interfere with the potential internal translational start site. This plasmid gave a Lux$^-$ phenotype when used with pAK211 in a cotransformation of *E. coli*, indicating that a carboxyl AinS capable of AI-2 synthesis is not made.

To test the idea that the 5′ and 3′ halves of the *ainS* transcript were both necessary but could be translated separately (analogous to the *luxL* and *luxM* genes), with the resulting polypeptides functioning as a complex in AI-2 synthesis, we performed a complementation analysis by using pBS2.5 and pHV30, a plasmid carrying only the 5′ portion of *ainS*. pHV30 (Fig. 1B) was constructed by cloning the 1.5-kb *Hin*dIII-*Eco*RV fragment of *ainS* into *Hin*dIII and *Hin*cII sites in pSUP102, generating a stop codon 15 bp downstream of the *Eco*RV-*Hin*cII fusion site. The *E. coli* double transformants were screened for AI-2 activity on the basis of light production in a cross-streak of an *E. coli* strain carrying pAK211. No autoinducer was detected from the double transformants, demonstrating that the N-terminal and C-terminal regions of AinS cannot be translated separately and that full-length *ainS* is necessary for AI-2 production.

Although the gene products of *luxLM* apparently are required for synthesis of HAI, the specific roles of LuxL and LuxM and the requirement for additional dedicated genes in HAI production have not yet been defined (4). The possibility exists that LuxL and/or LuxM are involved in the synthesis of substrates for subsequent catalysis by a LuxI homolog or that they function at some later step in HAI production. The possibility that *luxL* mutations are polar mutations of *luxM* and that LuxL is not directly involved in HAI production also has not been eliminated. The *ainS*-catalyzed production of AI-2 in *E. coli* and the similarities between AinS and LuxM, however, suggest that LuxM, as part of a complex, is an HAI synthesis protein.

Since half of AinS is homologous to LuxM whereas the other half shows no resemblance to LuxL or any other protein in the data bank, it appears that AinS is made up of at least two distinct domains. The 5′ domain of *ainS* is similar in size to *luxL*; therefore, we speculate that LuxL may function analogously to the N terminus of AinS by physically interacting with LuxM to form a protein complex. Since there is no similarity between the N-terminal amino acid sequence of AinS and LuxL, the N-terminal portion of AinS would recognize a substrate or specify a function in *V. fischeri* different from that of LuxL in *V. harveyi*.

The existence of two different and unrelated genes, *luxI* and *ainS*, directing the synthesis of *N*-acyl HSL signaling molecules points to an interesting convergence of evolution in autoinducer production. The two genes may have arisen independently to perform essentially similar reactions, or alternatively, members of the AinS and LuxI families may use different precursors which demand enzymes with completely different structures and functions to synthesize HSL derivatives.

Analysis of a second ORF, *ainR*. A second ORF in the same orientation as AinS but in a different reading frame begins 12 bp downstream of *ainS* and apparently continues beyond the *Hin*dIII cloning site. From the sequenced 521 bp, we found that the predicted 173 N-terminal residues are 38% identical to the amino terminus of the putative *V. harveyi luxN* gene product (Fig. 3B). *luxN* is contiguously encoded with *luxM* in an arrangement analogous to that of *ainS* and the second ORF. Hydropathy profiles of the second ORF and LuxN N-terminal regions (23) are also similar; both fragments appear to be integral inner membrane domains (data not shown). Since LuxN$^-$ cells do not luminesce upon the addition of HAI, LuxN has been proposed to function as a sensor/receptor for HAI (4). This hypothesis is supported by both the *V. harveyi* LuxN$^-$ phenotype and the presence of conserved amino acid sequence motifs found in sensor kinases and response regulators of two-component signal transduction systems (4). Thus, it has been postulated that HAI interacts with LuxN, causing autophosphorylation and intramolecular phosphate transfer within LuxN; this modified protein then activates, either directly or indirectly, the transcription of *luxCDABEGH* (4). Because of the sequence similarity and similar genetic arrangements of the second *ain* ORF and *luxN*, we predict that the gene product from this second ORF will function in a receptor role, and we have named the gene *ainR*. However, unlike the case for the HAI/LuxN system of *V. harveyi*, in *V. fischeri*, induction of *luxICDABEG* by AI-2 is dependent on LuxR, the transcriptional regulator which interacts with AI-1 (21). Therefore, it appears that if AI-2 does regulate genes via AinR, the object(s) of this regulation is an operon(s) other than *luxICDABEG* and should be identifiable by examining the transcripts and proteins induced by AI-2 and AinR.

Multiple induction systems for bioluminescence have been hypothesized to exist in *V. harveyi*. Although neither a second

autoinducer molecule nor a second synthase gene has been identified, phenotypic evidence suggests that there are two independent systems which each pair a single autoinducer with its particular regulatory or sensor protein (4). Two autoinducer synthesis genes are also suspected to exist in *P. aeruginosa*; in this case too it appears that the second autoinducer cannot interact with LasR, a LuxR homolog and the putative regulator for the first autoinducer (27). In contrast, the multiple autoinduction systems shown here in *V. fischeri* exhibit cross talk between the autoinducer of one system, AI-2, and the transcriptional activator of the other system, LuxR. This intriguing complexity of signaling might permit *V. fischeri* to fine-tune its response to diverse habitats. *V. fischeri* has been isolated from environments as different as coastal seawater, open ocean, surfaces of fish and squid, and the light organs of monocentrid fish and sepiolid squid (5). Many factors besides population density are likely to vary in these habitats; consequently, it may be reasonable for the luminescence system to have input from multiple signaling molecules. Fine-tuning by AI-2 may take the role of enhancing or inhibiting *luxICDABEG* induction (13, 22), depending on the particular environmental conditions. It is also possible that environmental factors that affect *lux* regulation, such as iron, catabolites, and oxygen (24), are involved in determining the positive or negative action of AI-2.

Identification of a *lux* box-like sequence in *ainS*. A 20-bp palindrome in the regulatory region of the *luxICDABEG* operon in *V. fischeri* has been shown to be an important operator element for regulation by LuxR (9). Upon examination of the *ainS* and *ainR* promoter regions, we find a similar 20-bp region of dyad symmetry, TAATGAGTTA|TCAATCAATA, centered at the predicted −35 site of *ainS*. Gray et al. (18) recently aligned eight similar palindromic sequences from promoter regions of genes thought to be autoinducible: *luxI* genes from various strains of *V. fischeri*, *lasB* from *P. aeruginosa*, and *traA* and *traI* from *A. tumefaciens*. When our sequence is added to this compilation, the palindromic consensus sequence can be represented as $X_3TGX_4(A/T)X_4CAX_3$.

Gray et al. (18) proposed that a *lux* box-like sequence, present approximately 40 bp upstream of a transcriptional start site, may represent a conserved regulatory element for cell density-dependent regulation by autoinduction. The presence of a *lux* box-like element in the proposed *ainS* promoter, which is indicative of an autoinducible promoter (18), suggests yet another level of interaction between *ain* and *lux* via LuxR. Expression of *ainS*, like that of *luxICDABEG*, may be controlled by LuxR and an autoinducer. Consistent with this possibility, production of AI-2 in *V. fischeri* exhibits a population density dependence, at least in the absence of *luxI* (21). Presumably, the autoinducer controlling *ainS* autoinduction is AI-2. If so, this would indicate an interesting parallel between *lux* and *ain*, with both AI-1 and AI-2 autocatalytically controlling their own production. Expression of *ainS* and synthesis of AI-2 in *E. coli*, in which LuxR is not present, presumably depends on the −35 region of the proposed *ainS* promoter acting as a housekeeping promoter recognizable to RNA polymerase. Furthermore, we cannot exclude the possibility that AinR plays a role in *ainS* expression by recognizing the *ainS lux* box-like element.

***ainS* is required for synthesis of AI-2 in *V. fischeri*.** To confirm that *ainS* is necessary for the synthesis of AI-2 in its native background, we constructed a *V. fischeri ainS* chromosomal mutant and demonstrated that the mutant did not synthesize AI-2. A 1.8-kb *neo* cassette was inserted into the *Cla*I site of pAI009, and the resultant plasmid was introduced into MJ-100 by conjugation; double homologous recombinants were selected as described in Materials and Methods. Southern hybridization with one of the recombinants, MJ-216, showed the *ainS*-containing chromosomal fragment increased in size by approximately 1.8 kb and that the *Cla*I site was destroyed. Autoinducer syntheses by the mutant and parent strain were compared by fractionating culture supernatants via RP HPLC and assaying the fractions for autoinducer activity. Culture supernatants of MJ-100 contained three distinct autoinducer activities: AI-1 and AI-3 (*N*-hexanoyl-L-HSL), both of which are *luxI* dependent (21), and AI-2. Similarly treated supernatants of MJ-216 yielded active fractions that coeluted with AI-1 and AI-3 but not with AI-2.

Since chemical analysis confirmed that synthesis of AI-2, but not AI-1, was abolished in both *E. coli* and *V. fischeri* by mutation of *ainS*, we have shown, by the same critieria used to define *luxI* as the AI-1 autoinducer synthesis gene, that *ainS* is necessary and sufficient for the critical step in the synthesis of AI-2 from metabolic precursors and enzymes present within these bacterial cells. Recently, much attention has been focused on autoinducers as signaling molecules involved in sensing bacterial population density. One approach to identifying autoinducers in different organisms is to search for *luxI* homologs. The presence of a new family of autoinducer synthesis proteins encoded by genes distinct from *luxI* opens up a new avenue for investigation, one that is likely to provide substantial insight into the evolution and functional biology of autoinducer-mediated cell signaling.

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