JOURNAL OF BACTERIOLOGY, June 1992, p. 4064–4069
0021-9193/92/124064-06$02.00/0

Vol. 174, No. 12

Genetic Dissection of DNA Binding and Luminescence Gene Activation by the *Vibrio fischeri* LuxR Protein

S. H. CHOI AND E. P. GREENBERG*

Department of Microbiology, The University of Iowa, Iowa City, Iowa 52242

Received 3 February 1992/Accepted 15 April 1992

The *Vibrio fischeri* luminescence (*lux*) genes are regulated by the 250-amino-acid-residue LuxR protein and a *V. fischeri* metabolite termed autoinducer. The *V. fischeri lux* regulon consists of two divergently transcribed units. Autoinducer and LuxR activate transcription of the *luxICDABE* operon and autoregulate the *luxR* transcriptional unit. LuxR proteins with C-terminal truncations of up to 40 amino acid residues coded by plasmids with *luxR* 3′-deletion mutations are functional in negative autoregulation as demonstrated by using a *luxR::lacZ* transcriptional fusion as a *luxR* promoter probe in *Escherichia coli*. The truncated LuxR proteins showed little or no ability to activate transcription of *luxICDABE*, as indicated by using luminescence as a sensitive indicator of promoter strength in *E. coli*. Besides having no detectable activity as positive regulators of *luxICDABE*, LuxR proteins with C-terminal truncations of more than 40 amino acid residues had reduced or no detectable activity as negative autoregulators. The results suggest that amino acid residues in LuxR prior to no. 211 are sufficient for *lux* DNA binding. Residues in the region of 211 to 250 constitute a C-terminal tail that appears to be involved in activation of *luxICDABE* transcription either by interacting physically with the transcription initiation complex or by affecting *lux* DNA in the vicinity of the promoter.

The LuxR protein is required for activation of the luminescence genes of *Vibrio fischeri* (12, 13). A 9-kb fragment of *V. fischeri* DNA that encodes LuxR and the other functions required for luminescence in *Escherichia coli* has been isolated and characterized (12, 13). This fragment contains two transcriptional units (Fig. 1). One unit contains *luxR*, which codes for the LuxR protein. The other unit, which is activated by the LuxR protein together with a substance termed autoinducer [*N*-(3-oxohexanoyl)-homoserine lactone (11)], contains *luxA* and *luxB*, genes coding for the α and β subunits of luciferase; *luxC*, *luxD*, and *luxE*, genes encoding polypeptides involved in synthesis of the aldehyde substrate for luciferase; and *luxI*, the only gene required for synthesis of autoinducer by *E. coli* (13). Additionally, *luxR* is autoregulated (8, 10, 14, 39), and this gene is activated by cyclic AMP (cAMP) and the cAMP receptor protein (6–8).

Because cells are freely permeable to autoinducer, this compound accumulates in cells and in the surrounding medium at equal concentrations (23). When autoinducer reaches a sufficient concentration, it triggers synthesis of the luminescence enzymes (23, 29, 30). Thus, autoinducer and the LuxR protein constitute a chemical communication system that allows *V. fischeri* cells to sense their own population density. *V. fischeri* occurs in specific light organ symbioses with certain marine animals (1, 9, 36). At the high population densities in the light organ symbiosis (10^{10} to 10^{11}/ml), autoinducer can accumulate, and induction of luminescence will occur. However, in seawater, where *V. fischeri* exists at low population densities (<10^2/ml), autoinduction of luminescence would not be expected (1, 9, 30, 35–37).

LuxR is a member of a family of transcriptional activators defined by sequence similarities in a C-terminal helix-turn-helix (H-T-H)-containing region (21). This LuxR family can be subgrouped on the basis of N-terminal sequence similarities. LasR (17) and the UvrC 28-k protein (21) appear to be LuxR homologs. Several members of the family possess related N-terminal sequences that classify them as members of the two-component regulatory system transcriptional activators (21, 43, 44). GerE consists in its entirety of the C-terminal region defining the LuxR family (21), and MalT is so far unique in that it possesses an N-terminal arm that is five times as long as that of LuxR and that it requires two inducers, maltotriose and ATP, for activity (21, 33). Also, MalT is the only member of the LuxR family that has been shown to specifically bind DNA in vitro (32, 45). More recently, a conserved region within the C terminus of LuxR family members that encompasses the H-T-H has been shown to have significant sequence similarity to the conserved region 4 of bacterial RNA polymerase σ factors (22, 44). Region 4 is an H-T-H-containing region considered to recognize the −35 sequences of promoters (20).

Although LuxR activity in vitro has not yet been demonstrated, a general view of the mechanism of LuxR control of luminescence genes has been developed by using a molecular genetic approach in which LuxR activity in *E. coli* is monitored (5, 7, 8, 12, 14, 24, 39, 40, 42). There are 155 bp between the *luxR* and *luxI* transcriptional start sites (15). There is a 20-bp inverted repeat centered at −40 from the *luxI* transcriptional start site that appears from a mutational analysis to be a LuxR binding sequence required for induction of luminescence (5) and for *luxR* autoregulation (39). Also, the cAMP receptor protein binding sequence is centered at position −59 from the *luxR* transcriptional start site (4, 15).

The protein predicted from the *luxR* sequence consists of 250 amino acid residues (4, 5, 15, 24). Studies with *luxR* point mutations indicate that LuxR residues 79 to 127 are an autoinducer binding region (40, 42) and that residues 184 to 230 contain the DNA binding region (42). Residues 196 to 210 constitute a predicted H-T-H thought to be required for DNA binding (21, 22). Studies with LuxR deletion mutants show that residues 163 to 250 constitute an independently folded domain that possesses DNA binding activity and

* Corresponding author. Electronic mail address: cmdepg@uiamvs.bitnet or cmdepg@uiowa.edu.

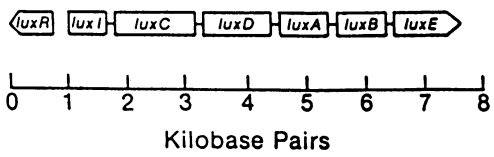

FIG. 1. Organization of the *V. fischeri lux* genes required for autoinducible luminescence in *E. coli*. The *luxICDABE* operon and *luxR* are divergently transcribed. pJR551, which was used in this study, contains the *lux* gene cluster with *luxR* insertionally inactivated with a transcriptional fusion of *lacZ*. There is also a nonpolar mutation in the *luxI* gene on pJR551 (10).

serves as an activator of *luxICDABE*; LuxR proteins without residues 2 to 162 are active and autoinducer independent. Apparently, a region between LuxR residues 20 and 162 blocks binding of the protein to the appropriate *lux* DNA unless autoinducer is provided. Finally, residues between positions 10 and 20 are required for autoregulation of *luxR* (3).

Obviously, the luminescence gene-activating domain constituted by residues 163 to 250 binds DNA and activates *luxICDABE* transcription. Because residues 196 to 210 define the predicted H-T-H DNA binding region, it is possible that amino acid residues following the predicted H-T-H are required specifically for activation of *luxICDABE* by DNA-bound LuxR. In an effort to separate DNA binding activity from activation of *luxICDABE*, we constructed *luxR* 3′-deletion mutations and compared the abilities of their products as activators of *luxICDABE* and as autorepressors of *luxR*. Our results indicate that the C terminus of LuxR is critical for activation of *luxICDABE* but that LuxR proteins without residues 211 to 250 retain the abilities to bind *lux* DNA and to repress *luxR*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The *E. coli* strains used were DH5α (18) and JM109 (46). The following plasmids were used: pKK223-3 and pPD723, which have been described previously (8); the pSC series plasmids, which were constructed as described below; and pJR551, which contains the *lux* gene cluster shown in Fig. 1, with *luxR* insertionally inactivated by Mu dI1681 and with a nonpolar mutation in *luxI* (10). The selectable marker on pJR551 is chloramphenicol resistance, and this plasmid is a P15A replicon. All of the other plasmids are ColE1 replicons with ampicillin resistance markers.

Cultures were grown at 30°C in L broth or on L agar (41) containing the appropriate antibiotic for plasmid screening or maintenance (100 μg of ampicillin or 30 μg of chloramphenicol per ml). Where indicated, isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 1 mM and autoinducer at a final concentration of 200 nM were added to the culture medium (7).

Plasmid purification, transformation, and isolation of *luxR* mutant plasmids. Plasmids were purified by the procedure described by Kraft et al. (25). Manipulations of plasmids were performed according to the methods of Sambrook et al. (38). The transformation procedure used was that described by Hanahan (18).

Specific *luxR* mutations were constructed by using polymerase chain reaction technology (38) to synthesize genes with 3′ deletions. The *luxR* gene in pPD723 served as a template. The primers used to construct these genes are shown in Table 1. Primer N300 has an *Eco*RI site followed by 24 bases corresponding to the 5′ end of the *luxR* open reading frame. N300 was used in conjunction with one of the other primers, each of which contained bases complementary to the indicated region of the 3′ end of the *luxR* open reading frame followed by 6 bases complementary to two consecutive translational stop codons and a *Sma*I site (Table 1). The polymerase chain reaction products were digested with *Eco*RI and *Sma*I and isolated by agarose gel electrophoresis (38). The synthetic genes were then cloned into pKK223-3, which had been digested with *Eco*RI and *Sma*I, and they were treated with calf intestinal phosphatase by standard procedures. The recombinant plasmids constructed in this manner should contain the *tac* promoter from pKK223-3, a Shine-Dalgarno sequence from pKK223-3 followed by a 6-bp *Eco*RI site, and a *luxR* open reading frame with a 3′ deletion. The extent of the 3′ deletion is dependent on which particular primer complementary to the 3′ end of *luxR* was used in the polymerase chain reaction.

Plasmids constructed as described above were used to transform *E. coli* DH5α. Plasmids from transformants were

TABLE 1. Primers used to synthesize *luxR* genes with 3′ deletions

Primer	Primer sequence[a]	Location[b]
N300	5′-CCGGAATTC–ATGAAAAACATAAATGCCGACGAC-3′	1–24
C300	5′-TCCCCCGGGCTA–TTAATTTTTAAAGTATGGGCA-3′	753–733
C310	5′-TCCCCCGGGCTATTA–TGTTAAAATTGCTTTAGAAATACT-3′	720–697
C315	5′-TCCCCCGGGCTATTA–AGAAATACTTTGGCAGCGGTTTGT-3′	705–682
C320	5′-TCCCCCGGGCTATTA–GCGGTTTGTTGTATTGAGTTTCAT-3′	690–667
C325	5′-TCCCCCGGGCTATTA–GAGTTTCATTTGCGCATTGGTTAA-3′	675–652
C330	5′-TCCCCCGGGCTATTA–AATGGTTAAATGGAAAGTGACAGT-3′	660–637
C335	5′-TCCCCCGGGCTATTA–AGTGACAGTACGCTCACTGCAGCC-3′	645–622
C340	5′-TCCCCCGGGCTATTA–ACTGCAGCCTAATATTTTTGAAAT-3′	630–607
C345	5′-TCCCCCGGGCTATTA–TTTTGAAATATCCCAAGAGCTTTT-3′	615–592
C350	5′-TCCCCCGGGCTATTA–AGAGCTTTTTCCTTCGCATGCCCA-3′	600–577
C357	5′-TCCCCCGGGCTATTA–CCACGCTAAACATTCTTTTTCTCT-3′	579–556
C389	5′-TCCCCCGGGCTATTA–AATTAATGGTATGTTCATACACGC-3′	483–460
C434	5′-TCCCCCGGGCTATTA–TGATGTTTTCGCTTCTTTAATTAC-3′	348–325
C488	5′-TCCCCCGGGCTATTA–GTAATTATCTAGAATTGAAATATC-3′	186–163

[a] Boldface characters represent the *Eco*RI site in N300 and the *Sma*I site and two translational stop codons in C300 to C488. Nucleotide sequences to the right of the space are complementary to the template DNA.

[b] Oligonucleotide position, where 1 is the first base of the translation initiation codon.

screened for appropriately sized *Eco*RI-*Sma*I restriction fragments. In selected plasmids, the sequence of the *luxR* gene, through the upstream *tac* promoter and downstream *rrnB* transcription terminator, was confirmed by DNA sequencing as described elsewhere (3, 4, 42). These plasmids, the pSC series plasmids, were used to transform *E. coli* JM109 containing pJR551. The transformants were used in our subsequent experiments.

Gel electrophoresis and Western immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out by the procedure established by Laemmli (27) as described elsewhere (26); the resolving gel contained 15% acrylamide. Western immunoblotting involved the procedure described by Brahamsha and Greenberg (2) with a 1:200 dilution of preabsorbed LuxR antiserum to probe the nitrocellulose blot (42).

Determination of cellular luminescence and β-galactosidase activity. The light-measuring equipment and standard to calibrate the equipment have been described previously (19), as have the procedures for measuring luminescence of broth cultures (7, 16). The $CHCl_3$-sodium dodecyl sulfate method described by Miller (28) was used to measure β-galactosidase activity. Unless otherwise indicated, luminescence and β-galactosidase activity were measured when cultures reached an optical density at 660 nm of 0.5 (3).

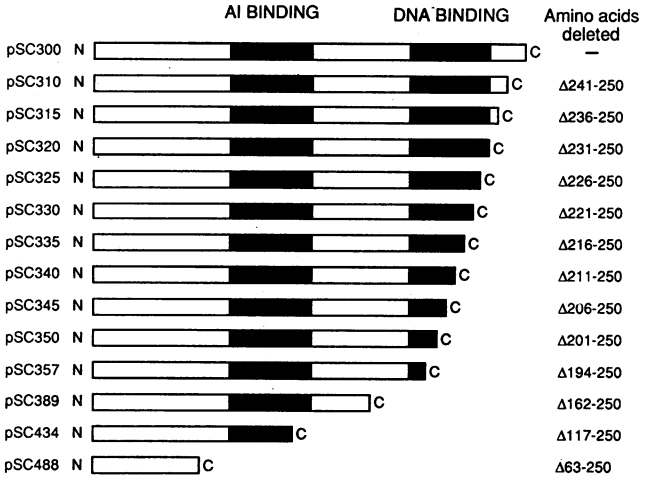

FIG. 2. Diagram of the pSC-encoded LuxR proteins described in this report. The pSC300-encoded wild-type LuxR is shown on top with the proposed autoinducer (AI)-binding region and DNA binding and *luxICDABE* activator region indicated. These regions were proposed on the basis of analysis of point mutations (40, 42). The truncated polypeptides encoded by the other pSC plasmids are shown below the pSC300 LuxR, and the amino acid residues deleted from these polypeptides are shown on the right.

RESULTS AND DISCUSSION

Polypeptides encoded by *luxR* 3′-deletion genes. Thirteen different *luxR* 3′-deletion plasmids were studied together with a plasmid that encoded the wild-type LuxR. The wild-type *luxR* plasmid was constructed so that the DNA sequences flanking the gene were identical to those flanking the mutant genes. The 13 *luxR* mutant plasmids were predicted to code for polypeptides containing as few as the N-terminal 62 amino acid residues and as many as the N-terminal 240 residues of LuxR (Fig. 2).

To confirm that cells containing a *luxR* 3′-deletion plasmid and pJR551 contained truncated LuxR proteins of the predicted molecular mass and to estimate the level of each truncated LuxR protein in these cells, we performed a Western immunoblot analysis (Fig. 3). When LuxR synthesis was induced with IPTG, the truncated product encoded by each of the *luxR* 3′-deletion mutant plasmids except pSC488 could be detected, and the molecular masses of these truncated polypeptides were consistent with the values predicted from the DNA sequences. As judged from band intensities, the cellular level of each of the 12 mutant proteins detected on the Western immunoblot was roughly comparable to the level of the wild-type LuxR (Fig. 3). The truncated product encoded by pSC488 was detected on immunoblots of overloaded gels. The molecular mass of this *luxR* product was in agreement with the predicted value. Thus, it seems likely that this truncated LuxR protein is less stable than the others or that its reactivity with the antiserum is relatively low.

Activities of *luxR* 3′-deletion products. The LuxR protein together with autoinducer has been shown to affect both *luxR* and *luxICDABE* promoter activity. Because LuxR activity has not yet been demonstrated in vitro, we assessed the ability of the truncated *luxR* 3′-deletion products to regulate the *luxICDABE* and *luxR* promoters on pJR551 in *E. coli*. Culture luminescence was used as a measure of the ability of a mutant LuxR protein to activate transcription of *luxICDABE*, and β-galactosidase activity was used to assess transcription of the *lacZ* gene fused to the *luxR* promoter in pJR551. Because pJR551 contains a *luxI* mutation and directs *E. coli* to make little or no autoinducer at 30°C (10), autoinducer was added to the culture medium.

As expected from previous studies (3, 7), the wild-type LuxR encoded by pSC300 in *E. coli* cells containing pJR551 increased the level of luminescence 10^5-fold over the level in cells without pSC300. This corresponds roughly to a 100-fold increase in cellular levels of luciferase (7). The LuxR encoded by pSC310, which has a truncation of the C-terminal 10 amino acid residues, showed a low but detectable activation of *luxICDABE*. LuxR proteins with truncations of 15 or more amino acid residues from the C terminus showed no detectable activation (Table 2). Apparently, the C-terminal tail of LuxR is critical for DNA binding or for activation of *luxICDABE* transcription by DNA-bound LuxR.

The *luxR* gene is autoregulated at the level of transcription and at a posttranscriptional level (8, 10, 14, 39). The transcriptional autoregulation can be positive or negative, depending on the experimental conditions (39). It appears that LuxR binding to a palindromic sequence centered at −40 from the start of *luxICDABE* transcription is required for either positive or negative autoregulation of the *luxR* pro-

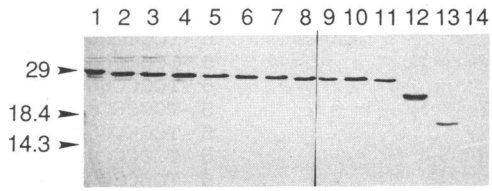

FIG. 3. Western immunoblot analysis of *E. coli* cells containing pJR551 and a pSC plasmid. Cultures were grown in the presence of IPTG and autoinducer. Lanes: 1, pSC300; 2, pSC310; 3, pSC315; 4, pSC320; 5, pSC325; 6, pSC330; 7, pSC335; 8, pSC340; 9, pSC345; 10, pSC350; 11, pSC357; 12, pSC389; 13, pSC434; 14, pSC488. The migrations of protein molecular size standards are indicated on the left, in kilodaltons.

TABLE 2. Luminescence and β-galactosidase activity in *E. coli* JM109 containing pJR551 and a pSC series *luxR* plasmid[a]

luxR plasmid	Luminescence[b]	β-Galactosidase activity[c]
pSC300	130 ± 30	24.5 ± 2.0
pSC310	0.02 ± 0.005	20.8 ± 1.7
pSC315	<0.0007	22.5 ± 1.3
pSC320	<0.0007	20.9 ± 1.6
pSC325	<0.0007	23.3 ± 1.5
pSC330	<0.0007	24.5 ± 1.9
pSC335	<0.0007	21.1 ± 1.9
pSC340	<0.0007	22.2 ± 1.7
pSC345	<0.0007	44.5 ± 2.0
pSC350	<0.0007	48.8 ± 4.8
pSC357	<0.0007	47.1 ± 4.8
pSC389	<0.0007	63.0 ± 2.3
pSC434	<0.0007	63.0 ± 2.6
pSC488	<0.0007	62.7 ± 2.2
—[d]	<0.0007	62.2 ± 3.0

[a] Cells were grown in the presence of IPTG and 0.2 μM autoinducer. Numbers are the averages of two separate experiments, each done in quadruplicate, ± the range around the average.

[b] In quanta · second^{-1} · milliliter^{-1} · optical density unit^{-1} · 10^{-9}.

[c] In Miller units (28).

[d] —, *E. coli* containing only pJR551.

moter. This sequence is also required for activation of the *luxICDABE* promoter by LuxR (5, 39). It is not yet known whether LuxR might bind to any other regions of the *lux* regulon.

Under the conditions of our experiments, where there is the complete *lux* gene cluster with a reporter gene fused to *luxR*, the concentration of autoinducer is relatively high, and cellular levels of LuxR are relatively high, negative autoregulation of *luxR* is expected. The *luxR* promoter strength should be reduced by LuxR and autoinducer to about one-third that in the absence of LuxR (3, 8, 10, 39). Our results were consistent with this expectation: *E. coli* containing pJR551 alone had 62.2 U of β-galactosidase, and *E. coli* containing pJR551 and pSC300, which codes for wild-type LuxR, had 24.5 U of β-galactosidase (Table 2).

Although truncated LuxR proteins missing the C-terminal 10 amino acid residues or more retained little or no ability to activate transcription of *luxICDABE*, LuxR proteins with C-terminal truncations of up to 40 amino acids showed *luxR* autorepression comparable to that of wild-type LuxR (Table 2). This indicates that these truncated LuxR proteins retained DNA binding activity and that the C-terminal tail of the wild-type LuxR is required for activation of *luxICDABE* but not for DNA binding. A similar conclusion was reached by Perego et al. (31) when they were working with missense mutations in the *Bacillus subtilis spo0A* gene. The Spo0A protein activates transcription of *spoIIA* and represses *abrB*. Spo0A proteins with an amino acid substitution at a location 11 residues from the C terminus negatively regulate *abrB* in a normal fashion but are unable to activate transcription of the *spoIIA* operon.

Residues 196 to 210 of LuxR constitute an H-T-H motif thought to be involved in DNA binding (21, 22). The LuxR protein encoded by pSC340 contained the N-terminal 210 amino acid residues only and thus contained the complete H-T-H. This truncated LuxR retained the ability to repress transcription from the *luxR* promoter threefold. However, there was only a 1.3- to 1.4-fold reduction in transcription from the *luxR* promoter by the N-terminal 205-amino-acid-residue, truncated LuxR protein encoded by pSC345 (Table 2). The lack of repression by the pSC345-encoded LuxR, which is missing part of the proposed H-T-H, is consistent with the proposal that this region is involved in DNA binding.

It is interesting that a small (1.3- to 1.4-fold) but significant reduction in transcription from the *luxR* promoter was observed in the presence of truncated LuxR proteins containing the N-terminal 205, 200, and 193 amino acid residues (Table 2). These truncated LuxR proteins are missing all or part of the proposed H-T-H. We do not know whether these mutant proteins retained some weak DNA binding activity or whether the small reduction in β-galactosidase activity in cells containing these proteins was due to other, unknown factors. On the basis of the β-galactosidase activity in cells containing pSC389, pSC434, and pSC488, which code for truncated LuxR proteins containing the N-terminal 161, 116, and 62 amino acids, respectively, it appears that these proteins did not repress transcription of *luxR* to any extent (Table 2).

With the exception of a very much reduced level of luminescence in cells containing the pSC300-encoded, wild-type LuxR, levels of luminescence and β-galactosidase in the absence of autoinducer were similar to levels in the presence of autoinducer (Table 2). Although luminescence in cells containing the wild-type LuxR was stimulated 300-fold by the addition of autoinducer, even in the absence of autoinducer luminescence in the presence of wild-type LuxR was more than 250-fold higher than in the absence of LuxR (0.2 U compared with <0.0007 U). These results with the wild-type LuxR are similar to our previously reported results obtained by using a different plasmid to direct the synthesis of high levels of LuxR in *E. coli* (3). Our findings that autoregulation of *luxR* is autoinducer independent and that partial activation of *luxICDABE* occurs in the absence of autoinducer are not consistent with those of previous studies (10, 12, 39). The major experimental difference between our current report of LuxR activity in the absence of autoinducer and the previous reports of strict autoinducer dependence is that in our experiments the cellular levels of LuxR were much higher (well out of the normal physiological range) than in the experiments reported by others (10, 12, 39). We suggest that autoinducer dependence of relatively low levels of LuxR can be compensated for by high levels of LuxR. This sort of compensation at high concentrations has been shown for other transcription regulatory factors (34). It is interesting that we did not detect any effect of autoinducer on *luxR* autorepression in *E. coli* containing pSC300 and pJR551, whereas full activation of *luxICDABE* required autoinducer.

Although activity of LuxR in vitro has not yet been demonstrated, genetic analyses of LuxR activity in *E. coli* have led to a general view of LuxR structure and function, and this study adds to the general understanding of LuxR. Like other members of the LuxR family (21, 22), LuxR appears to be modular (Fig. 4). According to our current model of the LuxR polypeptide, there is an N-terminal regulator module required to block binding of a C-terminal *luxICDABE* activator module to the appropriate *lux* DNA (3). Within the regulator module, there is an autoinducer binding region (3, 40, 42). Autoinducer binding to the regulatory region allows the C-terminal module to bind DNA and influence *lux* gene transcription (3, 40, 42). A region at the N terminus of LuxR (between residues 10 and 20) is required for *luxR* autoregulation (3).

There is an H-T-H around residues 196 to 210 in the C-terminal activator module, and this H-T-H is thought to be

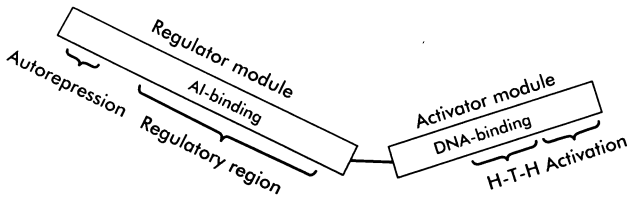

FIG. 4. Model depicting general features of the 250-amino-acid-residue, modular LuxR polypeptide. The regulator module constitutes approximately two-thirds of LuxR. A region within residues 20 to 157 is involved in blocking DNA binding by LuxR. Autoinducer (AI) binds within this region. DNA binding is not blocked by the regulator module when LuxR is in the AI-bound state. A region near the N terminus (between residues 10 and 20) is required for *luxR* autorepression. The C-terminal one-third of LuxR constitutes the *luxICDABE* activator module. Residues within the first 50 to 60% of this module are required for DNA binding, which is a prerequisite for *luxR* autoregulation and *luxICDABE* activation. Residues in the region of no. 210 to 250 are required for *luxICDABE* activation but not for DNA binding or for autoregulation of *luxR*.

involved in DNA binding (3, 22). This is supported by studies of *luxR* point mutations, which led to the hypothesis that LuxR residues between 184 and 230 are involved in *lux* DNA recognition and binding (42). However, in these studies no effort was made to discriminate between DNA binding and subsequent transcriptional activation by LuxR. We have demonstrated that mutant LuxR proteins with C-terminal truncations of as many as 40 amino acid residues negatively autoregulate *luxR* in a normal fashion but do not activate *luxICDABE* as does the wild-type LuxR (Table 2). Because DNA binding is required for both activities of LuxR, each of the truncated proteins that retain normal *luxR* autoregulation must retain DNA binding activity. The facts that proteins with C-terminal truncations up to the border of the predicted H-T-H negatively autoregulate *luxR* in a normal fashion and that truncated proteins missing the C-terminal 5 amino acid residues or more of the H-T-H do not show normal autoregulation (Table 2) support the contention that the predicted H-T-H region is involved in *lux* DNA binding. Thus, we have indicated in our model of LuxR (Fig. 4) that there is a C-terminal tail required for activation of the *luxICDABE* promoter but not for *lux* DNA binding or autorepression. It is of interest to determine the molecular mechanism by which the C-terminal tail of the C-terminal module of LuxR activates transcription of *luxICDABE*.

ACKNOWLEDGMENTS

We thank Dana Kolibachuk for providing LuxR antiserum and P. V. Dunlap for providing pJR551.

S.H.C. was supported in part by a fellowship from The University of Iowa Biocatalysis and Bioprocessing Center, and this research was supported by the Office of Naval Research (N00014-80-K-0570).

REFERENCES

1. **Boettcher, K. J., and E. G. Ruby.** 1990. Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*. J. Bacteriol. **172:**3701–3706.
2. **Brahamsha, B., and E. P. Greenberg.** 1988. A biochemical and cytological analysis of the complex periplasmic flagella from *Spirochaeta aurantia*. J. Bacteriol. **170:**4023–4032.
3. **Choi, S. H., and E. P. Greenberg.** 1991. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer independent *lux* gene activating domain. Proc. Natl. Acad. Sci. USA **88:**11115–11119.
4. **Devine, J. H., C. Countryman, and T. O. Baldwin.** 1988. Nucleotide sequence of the *luxR* and *luxI* genes and the structure of the primary regulatory region of the *lux* regulon of *Vibrio fischeri* ATCC 7744. Biochemistry **27:**837–842.
5. **Devine, J. H., G. S. Shadel, and T. O. Baldwin.** 1989. Identification of the operator of the *lux* regulon from *Vibrio fischeri* strain ATCC 7744. Proc. Natl. Acad. Sci. USA **86:**5688–5692.
6. **Dunlap, P. V.** 1989. Regulation of luminescence by cyclic AMP in *cya*-like and *crp*-like mutants of *Vibrio fischeri*. J. Bacteriol. **171:**1199–1202.
7. **Dunlap, P. V., and E. P. Greenberg.** 1985. Control of *Vibrio fischeri* luminescence gene expression in *Escherichia coli* by cyclic AMP and cyclic AMP receptor protein. J. Bacteriol. **164:**45–50.
8. **Dunlap, P. V., and E. P. Greenberg.** 1988. Control of *Vibrio fischeri lux* gene transcription by a cyclic AMP receptor protein-LuxR protein regulatory circuit. J. Bacteriol. **170:**4040–4046.
9. **Dunlap, P. V., and E. P. Greenberg.** 1991. Role of intercellular chemical communication in the *Vibrio fischeri*-monocentrid fish symbiosis, p. 219–253. *In* M. Dworkin (ed.), Microbial cell-cell interactions. American Society for Microbiology, Washington, D.C.
10. **Dunlap, P. V., and J. M. Ray.** 1989. Requirement for autoinducer in transcriptional negative autoregulation of the *Vibrio fischeri luxR* gene in *Escherichia coli*. J. Bacteriol. **171:**3549–3552.
11. **Eberhard, A., A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, and N. J. Oppenheimer.** 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry **20:**2444–2449.
12. **Engebrecht, J., K. Nealson, and M. Silverman.** 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell **32:**773–781.
13. **Engebrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA **81:**4154–4158.
14. **Engebrecht, J., and M. Silverman.** 1986. Regulation of expression of bacterial genes for bioluminescence, p. 31–44. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering 8. Plenum Publishing Corp., New York.
15. **Engebrecht, J., and M. Silverman.** 1988. Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. Nucleic Acids Res. **15:**10455–10467.
16. **Friedrich, W. F., and E. P. Greenberg.** 1983. Glucose repression of luminescence and luciferase in *Vibrio fischeri*. Arch. Microbiol. **134:**87–91.
17. **Gambello, M. J., and B. H. Iglewski.** 1991. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. J. Bacteriol. **173:**3000–3009.
18. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
19. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotopic sources. J. Opt. Soc. Am. **53:**1410–1415.
20. **Helman, J. O., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57:**839–872.
21. **Henikoff, S., J. C. Wallace, and J. P. Brown.** 1990. Finding protein similarities with nucleotide sequence data bases. Methods Enzymol. **183:**111–132.
22. **Kahn, D., and G. Ditta.** 1991. Modular structure of FixJ: homology of the transcriptional activator domain with the −35 binding domain of sigma factors. Mol. Microbiol. **5:**987–997.
23. **Kaplan, H. B., and E. P. Greenberg.** 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J. Bacteriol. **163:**1210–1214.
24. **Kaplan, H. B., and E. P. Greenberg.** 1987. Overproduction and purification of the *luxR* gene product: the transcriptional activator of the *Vibrio fischeri* luminescence system. Proc. Natl. Acad. Sci. USA **84:**6639–6643.
25. **Kraft, R., J. Tardiff, K. S. Krauter, and L. A. Leinwand.** 1988. Using mini-prep plasmid DNA for sequencing double stranded templates with Sequenase. BioTechniques **6:**544–547.
26. **Kropinski, A. M., T. R. Parr, Jr., B. L. Angus, R. E. W. Hancock, W. C. Ghiorse, and E. P. Greenberg.** 1987. Isolation of

the outer membrane and characterization of the major outer membrane protein from *Spirochaeta aurantia*. J. Bacteriol. **169:**172–179.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase: occurrence, mechanism and significance. Arch. Microbiol. **112:** 73–79.
30. **Nealson, K. H., and J. W. Hastings.** 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. **43:**496–518.
31. **Perego, M., J.-J. Wu, G. B. Spigelman, and J. A. Hoch.** 1991. Mutational dissociation of the positive and negative regulatory properties of the Spo0A sporulation transcription factor of *Bacillus subtilis*. Gene **100:**207–212.
32. **Raibaud, O.** 1989. Nucleoprotein structures at positively regulated bacterial promoters: homology with replication origins and some hypotheses on the quantinary structure of the activator proteins in these complexes. Mol. Microbiol. **3:**455–458.
33. **Raibaud, O., and E. Richet.** 1987. Maltotriose is the inducer of the maltose regulon of *Escherichia coli*. J. Bacteriol. **169:**3059–3061.
34. **Raibaud, O., and M. Schwartz.** 1984. Positive control of transcription initiation in bacteria. Annu. Rev. Genet. **18:**173–206.
35. **Ruby, E. G., E. P. Greenberg, and J. W. Hastings.** 1980. Planktonic marine luminous bacteria: species distribution in the water column. Appl. Environ. Microbiol. **39:**302–306.
36. **Ruby, E. G., and K. H. Nealson.** 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*: a model of symbiosis based on bacterial studies. Biol. Bull. **151:**574–586.
37. **Ruby, E. G., and K. H. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore waters. Limnol. Oceanogr. **23:**530–533.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Shadel, G. S., and T. O. Baldwin.** 1991. The *Vibrio fischeri* LuxR protein is capable of bidirectional stimulation of transcription and both positive and negative regulation of the *luxR* gene. J. Bacteriol. **173:**568–574.
40. **Shadel, G. S., R. Young, and T. O. Baldwin.** 1990. Use of regulated cell lysis in a lethal genetic selection in *Escherichia coli*: identification of the autoinducer-binding region of the LuxR protein from *Vibrio fischeri* ATCC 7744. J. Bacteriol. **172:**3980–3987.
41. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 217. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Slock, J., D. VanRiet, D. Kolibachuk, and E. P. Greenberg.** 1990. Critical regions of the *Vibrio fischeri* LuxR protein defined by mutational analysis. J. Bacteriol. **172:**3974–3979.
43. **Stewart, V., J. Parales, Jr., and S. M. Merkel.** 1989. Structure of genes *narL* and *narX* of the *nar* (nitrate reductase) locus in *Escherichia coli* K-12. J. Bacteriol. **171:**2229–2234.
44. **Stout, V., A. Torres-Cabassa, M. R. Maurizi, D. Gutnick, and S. Gottesman.** 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. J. Bacteriol. **173:**1738–1747.
45. **Vidal-Ingigliardi, D., E. Richet, and O. Raibaud.** 1991. Two MalT binding sites in direct repeat: a structural motif involved in the activation of all promoters of the maltose regulon in *Escherichia coli* and *Klebsiella pneumoniae*. J. Mol. Biol. **218:**323–334.
46. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.