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Control of *Vibrio fischeri lux* Gene Transcription by a Cyclic AMP Receptor Protein-LuxR Protein Regulatory Circuit

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Expression of the *Vibrio fischeri* luminescence genes (*lux* genes) requires two transcriptional activators: the *V. fischeri luxR* gene product with autoinducer and the cyclic AMP (cAMP) receptor protein (CRP) with cAMP. It has been established that autoinducer and the *luxR* gene product are required for transcriptional activation of the *luxICDABE* operon, which contains a gene required for autoinducer synthesis and genes required for light emission. However, the role of cAMP-CRP in the induction of luminescence is not clear. We examined transcriptional control of the *lux* genes in *Escherichia coli*, using catabolite repression mutants carrying *lux* DNA-containing plasmids. Transcriptional fusions between the *lacZ* gene on Mu dI and *luxR* were used to assess *luxR* promoter activity, and the *luxAB* genes (which encode the two luciferase subunits) were used as a natural reporter of *luxICDABE* promoter activity. A plasmid containing *luxR* under control of the cAMP-CRP-independent *tac* promoter was constructed to direct the synthesis of the *luxR* gene product in cells containing compatible *luxR*::Mu dI insertion mutant plasmids. In *E. coli*, cAMP-CRP activated transcription of *luxR* and concurrently decreased *luxICDABE* transcription. In the presence of relatively high levels of the *luxR* gene product, cAMP and CRP were not required for induction of the *luxICDABE* operon. The *luxR* gene product in the presence of autoinducer activated transcription of the *luxICDABE* operon, as has been shown previously, and we demonstrate that it also decreased *luxR* transcription. Apparently, control of the *V. fischeri* luminescence genes involves a regulatory circuit in which cAMP and CRP activate *luxR* transcription and in turn the *luxR* gene product activates transcription of the operon responsible for light emission (*luxICDABE*). Furthermore, in *lux* gene regulation cAMP-CRP and autoinducer-LuxR protein appear to function as transcriptional antagonists.

Luminescence of *Vibrio fischeri*, the light organ symbiont of pinecone fish, requires autoinduction. The autoinducer [*N*-(3-oxo-hexanoyl)homoserine lactone] is a specific *V. fischeri* metabolite that activates transcription of the luminescence genes (*lux* genes) when it reaches a critical concentration of a few molecules per cell. Cells are permeable to autoinducer, so this molecule accumulates within *V. fischeri* and in the external medium at equal concentrations (7, 15, 18, 22). At high cell densities, autoinducer can accumulate and cause induction of the luminescence enzymes. This is thought to be the case in the light organ symbiosis in which *V. fischeri* occurs at densities of 10^9 to 10^{10} cells per ml of organ fluid. In environments in which *V. fischeri* density is low, such as seawater ($<10^3$ cells per ml), autoinducer does not accumulate and *V. fischeri* luminescence would not be expected (19, 21–23).

A 9-kilobase (kb) fragment of *V. fischeri* DNA that encodes all the functions for luminescence and contains regulatory elements sufficient for their expression in *Escherichia coli* has been isolated (8). The cloned DNA contains seven *lux* genes that constitute the *lux* regulon, organized as two divergently transcribed units (Fig. 1). One transcriptional unit contains *luxR*, which encodes a protein required for cells to respond to autoinducer (the LuxR protein). The other transcriptional unit contains *luxA* and *luxB*, which encode the α and β subunits of luciferase; *luxC*, *luxD*, and *luxE*, which encode proteins involved in synthesis of the aldehyde substrate for luciferase; and *luxI*, which is the only *V. fischeri* gene required for synthesis of autoinducer in *E. coli* (2, 8, 9). As a consequence of the arrangement of *lux* genes, autoinducer controls expression of *luxI*, creating a positive autoregulation of autoinducer synthesis (8). Furthermore, a recent publication indicates that expression of *luxR* is negatively autoregulated at a posttranscriptional level (10).

Besides control by autoinduction, *V. fischeri* luminescence is thought to be regulated by the cyclic AMP (cAMP) receptor protein (CRP) and cAMP (6, 12). Since cAMP and CRP mutants of *V. fischeri* have not been isolated, much of the existing information on cAMP-CRP control of *lux* transcription has been derived from studies with *E. coli* mutants containing the cloned *lux* genes (6). It is not clear from the existing evidence how cAMP and CRP activate luminescence in *E. coli*. Using a plasmid containing a Mu dI(*lacZ*) fusion in *luxR*, we previously demonstrated that in the absence of a functional *luxR* gene, cAMP and CRP stimulate transcription from the *luxR* promoter (6). Thus, it seems possible that cAMP and CRP activate *luxICDABE* indirectly by effecting an increase in the *luxR* gene product to a level above the threshold required for autoinduction. In this article, we present evidence in support of this hypothesis. A strategy was used that involved subcloning of *luxR* on a *tac* promoter vector and use of this construct to direct synthesis of relatively high levels of the *luxR* gene product in *E. coli* cAMP and CRP mutants containing the *luxICDABE* operon and *luxR*::Mu dI(*lacZ*). This also allowed us to examine the affect of the *luxR* gene product on transcription from the *luxR* promoter. Transcription of the *luxICDABE* operon in vivo was monitored by measuring luciferase content, and

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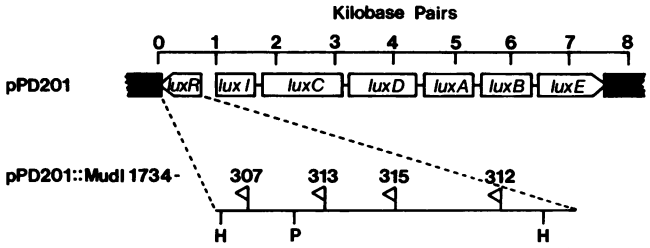

FIG. 1. Organization of *V. fischeri lux* genes and map locations of the *luxR*::Mu dI1734(*lacZ*) insertions used to study *luxR* promoter activity. (Top) Physical map of *lux* DNA in pPD201 showing the two divergently transcribed operons; the *luxR* operon and the *luxICDABE* operon. (Bottom) Restriction map of *luxR* in pPD201. H, *Hin*dIII; P, *Pst*I. The flags indicate the positions of four Mu dI1734 insertions and point in the direction of *lacZ* transcription.

transcription from the *luxR* promoter was monitored by measuring β-galactosidase content (6).

MATERIALS AND METHODS

Bacterial strains and culture conditions. All the strains used in this study are derivatives of *E. coli* K-12 and are listed in Table 1. For selection and screening of strains containing various plasmids, cells were transformed by the procedure of Hanahan (13) and plated on LB agar (24) supplemented as indicated and incubated for 1 to 2 days at 30°C. To monitor in vivo *luxR* and *luxICDABE* promoter activity, cultures were grown in LB broth or LB agar to which glucose (final concentration, 10 mM), Tris buffer (final concentration, 50 mM) (pH 7.5), and the appropriate antibiotics for plasmid maintenance (chloramphenicol [30 μg/ml] or chloramphenicol and ampicillin [80 μg/ml]) were added after sterilization. Cultures were grown in 3-ml volumes as described previously (6).

Construction of *luxR* insertion mutant plasmids. To construct plasmids containing the *lux* regulon with Mu dI1734 insertions in *luxR*, a 9-kb *Sal*I fragment of DNA containing the *lux* regulon was first removed from pJE202 and inserted into the *Sal*I site of pACYC184. Recombinant plasmids were selected by transformation of *E. coli* PD100 with the ligation mixture followed by plating of the transformed cells on LB agar containing chloramphenicol. An appropriate plasmid, pPD201, was obtained by screening transformants for luminescence (indicating the presence of the *lux* regulon) and ampicillin sensitivity (indicating the absence of pJE202). Mu dI1734 insertions in the *luxR* gene of pPD201 were then generated by the method of Castilho et al. (3). *E. coli* PD1734 was transformed with pPD201, Mu transposition was heat induced, and the resulting lysate was used to transduce *E. coli* PD100, with selection on LB agar containing chloramphenicol and kanamycin (20 μg/ml). Transductants which were not luminescent were isolated and screened for high-level expression of β-galactosidase on LB agar containing 5-bromo-4-chloro-3-indoyl-β-galactoside (X-gal) (suggestive of alignment of the *lacZ* gene of Mu dI with an active promoter). To identify *luxR*::Mu dI1734 insertions specifically, we used plasmid DNA from each of 85 strains containing pPD201 *lux*::Mu dI insertions to transform *E. coli* DH1 containing a *tac* promoter-*luxR* complementing plasmid, pPD749 (see below for construction), with selection on LB agar plus chloramphenicol and ampicillin followed by screening for luminescence in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG, 1 mM). Of the 85 plasmids examined by this method, 16 appeared to contain Mu dI insertions in *luxR*. To determine the orientation of the insertions and their positions in *luxR*, each of the 16 *luxR*::Mu dI insertion plasmids was digested with *Sal*I, *Eco*RI and *Bgl*II, and *Pst*I. Restriction maps of Mu dI1734 (provided by N. Kent) and the *lux* regulon (8) were used to estimate fragment sizes. Examination of the digestion fragments by agarose gel electrophoresis allowed mapping of the insertions with an accuracy of ±80 base pairs. Of the 16 *luxR*::Mu dI insertions examined, 8 were found to have *lacZ* in alignment with the *luxR* promoter, and 4 that had insertions

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics[a]	Source or reference
E. coli K-12		
JM109	F′ *lacI*^{q}	M. Weiner (25)
DH1	*recA1 hsdR17*	CGSC[b] (13)
PD1734	POI1734 [Mu *cts*, Mu dI1734 (Km^r *lacZYA*)] *recA56*	3, P. Dunlap, unpublished data
PD100	*cya*$^+$ *crp*$^+$ Δ(*argF-lac*)*U169*	6
PD200	Δ*cyaA-2 crp*$^+$ Δ(*argF-lac*)*U169*	6
PD300	*cya*$^+$ Δ*crp-3* Δ(*argF-lac*)*U169*	6
Plasmid		
pJE202	pBR322 with 9-kb *Sal*I fragment of *V. fischeri* DNA (*luxR luxICDABE*) Ap^r	J. Engebrecht (8)
pACYC184	Cm^r Tc^r	M. Weiner (4)
pHK555	pACYC184 with *luxICDABE luxR*::Mu Δ(*c*, *nerAB*) dI (Km^s *lacZYA*) Cm^r	16
pPD201	pACYC184 with *Sal*I *lux* fragment from pJE202, Cm^r	This study
pPD307	pPD201, *luxR*::Mu dI1734	This study
pPD312	pPD201, *luxR*::Mu dI1734	This study
pPD313	pPD201, *luxR*::Mu dI1734	This study
pPD315	pPD201, *luxR*::Mu dI1734	This study
pHK705	pUC18 with 1-kb *luxR* fragment, Ap^r	16
pKK223-3	*tac* promoter vector, Ap^r	1
pPD723	pKK223-3 with *luxR* fragment from pHK705, Ap^r	This study
p*lacI*^{q}	pACYC184 with 1.2-kb *lacI*^{q} fragment, Tc^r	L. Zumstein (1)
pPD749	pPD723 with *lacI*^{q} fragment from p*lacI*^{q}, Ap^r	This study

[a] Ap^r, Ampicillin resistant; Cm^r, chloramphenicol resistant; Tc^r, tetracycline resistant.
[b] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

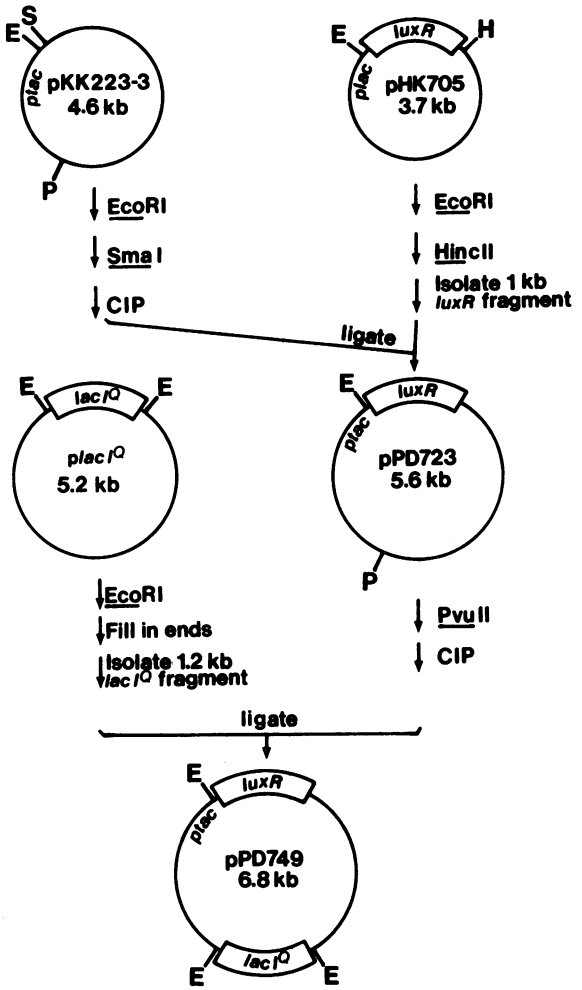

FIG. 2. Construction of the *luxR* complementing plasmid, pPD749. Restriction enzyme cleavage sites: E, *Eco*RI; H, *Hin*dIII; S, *Sma*I; and P, *Pvu*II. P_{lac} and P_{tac} indicate the *lac* promoter and *tac* promoter, respectively. CIP indicates treatment with calf intestinal phosphatase. The 1-kb *luxR* fragment and the 1.2-kb *lacI*^{q} fragment were isolated after separation by electrophoresis through low-melting-point agarose.

widely separated from each other were chosen for further studies (Fig. 1).

Construction of plasmid containing *tac* promoter-controlled *luxR* gene. The construction of pPD749, the *luxR* complementing plasmid used in this study, is depicted in Fig. 2. The *luxR* gene was excised from pHK705, removing the *lac* ribosome-binding site and translation initiation sequence, and this gene was cloned downstream of the pKK223-3 *tac* promoter. An appropriate recombinant plasmid was obtained by transformation of *E. coli* JM109 containing pHK555 (*luxICDABE*$^+$ *luxR*), using ampicillin resistance (Ap^r) as a selectable marker and screening for transformants which were luminescent when grown in the presence of IPTG (1 mM), indicating complementation of the pHK555 *luxR* mutation by a *tac* promoter-controlled *luxR* gene. A plasmid from one of the transformants, designated pPD723, was chosen for further studies. A DNA sequence analysis (C. Countryman and T. Baldwin, personal communication) indicated that pPD723 contained only 15 base pairs of *lux* DNA 5′ to the *luxR* translational start site (5, 11). This plasmid did not contain the native *luxR* transcriptional start nor did it contain the *lux* DNA matching the consensus sequence for cAMP-CRP recognition (5, 11).

To permit IPTG-regulated expression of the *tac-luxR* construct in *E. coli* PD100, PD200, and PD300, which do not contain a *lacI* gene, we subcloned the *lacI*^{q} gene into pPD723 to form pPD749 (Fig. 2). To obtain pPD749, we transformed *E. coli* PD410 (*lacI*; P. V. Dunlap, unpublished data) containing pHK555 with the pPD723-*lacI*^{q} ligation mixture and plated it on LB agar plus chloramphenicol and ampicillin. Colonies that did not appear luminous upon visual examination were transferred to IPTG agar plates to identify transformants that were luminous upon induction of the *tac* promoter. One such transformant harbored pPD749, which was purified and used in our subsequent investigations. Restriction mapping was used to confirm the plasmid constructions.

Determination of cellular luminescence, luciferase activity, and β-galactosidase activity. The light-measuring equipment and standard to calibrate the equipment have been described previously (6, 12, 14), as have the procedures for measuring luminescence of broth cultures (6). Permanent records of colony luminescence on agar plates were obtained by exposing X-ray film to the agar plates. A neutral-density filter (25% transmittance) was placed between the film and the plates, and exposure time was 20 s. The procedure for measuring luciferase activity in cell extracts involved a reaction with excess flavin mononucleotide, decanal, and oxygen (20) as described previously (6). β-Galactosidase activity was measured by the $CHCl_3$-sodium dodecyl sulfate method described by Miller (17). As described previously, cells for β-galactosidase activity measurements and for luciferase activity measurements were harvested from cultures at an optical density at 660 nm of 1.0 (6).

Chemicals. Antibiotics, cAMP, decanal, flavin mononucleotide, IPTG, Tris, and X-gal were purchased from Sigma Chemical Co. (St. Louis, Mo.). *N*-(3-Oxo-hexanoyl)homoserine lactone (autoinducer) was synthesized and supplied by H. B. Kaplan and A. Eberhard.

RESULTS

CRP and cAMP are not required for induction of luciferase synthesis in the presence of sufficient *luxR* gene product. *E. coli* Δ*cyaA* mutants carrying pJE202 (*luxICDABE luxR*) required the addition of cAMP to the growth medium for luminescence, and Δ*crp* mutants were not brightly luminous even in the presence of added cAMP (6) (Fig. 3). This demonstrated that cAMP and CRP are required for activation of the *luxICDABE* operon on pJE202. This cAMP-CRP requirement may be indirect in that these effectors may act by inducing the synthesis of the *luxR* gene product which in turn is needed for *luxICDABE* activation (6) (see Introduction). When the *luxR* gene was supplied under control of the cAMP-independent *tac* promoter (on pPD749) to *E. coli* Δ*cyaA* or Δ*crp* mutants containing the *luxICDABE* operon on pHK555 [*luxICDABE luxR*::Mu dI1681(*lacZ*)], luminescence was not dependent on cAMP or CRP; luminescence was dependent only on IPTG, the inducer of the *tac* promoter (Fig. 3).

To quantitate the effects of cAMP and CRP on transcription of the *luxICDABE* operon in the presence of *luxR*, we measured luciferase activities in extracts of *E. coli* strains carrying pHK555 and pPD749 (Table 2). In the presence of IPTG, induced levels of luciferase were achieved in the Δ*crp* mutant and in the Δ*cyaA* mutant with or without added

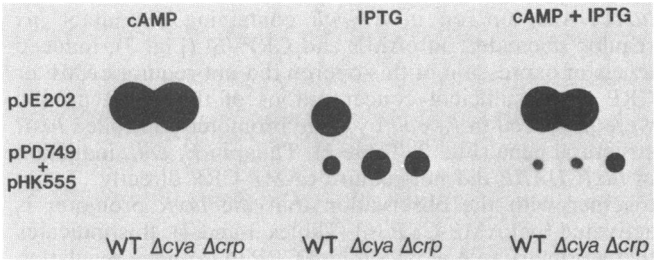

FIG. 3. Relationship between colony luminescence, cAMP, CRP, and *luxR* protein. Three agar plates, one containing cAMP (5 mM), one containing IPTG (1 mM), and one containing cAMP and IPTG, were spotted with small drops of six different bacterial cultures. Each plate was spotted with *E. coli* PD100 (*cya*$^+$ *crp*$^+$), PD200 (Δ*cyaA*), and PD300 (Δ*crp*) containing either pJE202 or pPD749 and pHK555. After 24 h at 30°C, colony luminescence was recorded on X-ray film as described in Materials and Methods. WT, Wild type.

cAMP. In the absence of IPTG, LuxR protein concentrations were apparently not sufficient for activation of luciferase synthesis even in the *cya*$^+$ *crp*$^+$ strain. Since pHK555 contains a functional *luxI* gene, the *E. coli* strains in these experiments were capable of synthesizing autoinducer, and exogenously added autoinducer had little effect on the synthesis of luciferase (Table 2). It should be noted here that in the presence of IPTG these strains exhibited the characteristic autoinducer response; luciferase activity remained at a constant uninduced level for the first 4 to 5 h of growth and was then induced to a level about 100-fold greater than the uninduced level. The induction lag could be overcome by the addition of autoinducer (0.2 μM) to the growth medium (data not shown). These in vivo experiments demonstrate that in the presence of sufficient *luxR* gene product, *luxICDABE* transcription did not require cAMP and CRP.

Effects of cAMP and CRP on *lux* promoter activity. Based on experiments in which β-galactosidase levels in *E. coli* Δ*cyaA* and Δ*crp* mutants containing pJE455 [*luxICDABE luxR*::Mu dI1681(*lacZ*)] were monitored, it was concluded that transcription from the *luxR* promoter is stimulated by cAMP-CRP (6). This, taken together with the results presented in Table 2 and Fig. 3, suggests that for the intact *lux* regulon, cAMP and CRP are required for the establishment of levels of the LuxR protein sufficient for induction of luminescence. This hypothesis was examined further by addressing two remaining questions. (i) Is the transcriptional stimulation from the *luxR* promoter evident regardless of the position of the *lacZ* insertion in *luxR* (only one transcriptional fusion was examined previously)? (ii) Does transcriptional activation of *luxR* by cAMP and CRP occur in the presence of a functional LuxR protein (cells in the previous study were *luxR*)?

TABLE 2. Luciferase activity in *E. coli* catabolite repression mutants containing pHK555 and pPD749

Addition(s)	Luciferase activity units (10^5)		
	PD100 (*cya*$^+$ *crp*$^+$)	PD200 (Δ*cyaA* *crp*$^+$)	PD300 (*cya*$^+$ Δ*crp*)
None	10	45	15
cAMP (5 mM)	10	25	15
IPTG (1 mM)	2,800	5,600	3,300
cAMP and IPTG	3,300	3,400	3,500
cAMP, IPTG, and autoinducer (0.2 μM)	3,600	3,800	5,600

TABLE 3. Influence of cAMP on β-galactosidase synthesis and luciferase synthesis in *E. coli* PD200 (Δ*cyaA*) containing *lux* plasmids with Mu dI(*lacZ*) insertions in *luxR*

Insertion plasmid[a]	β-Galactosidase activity units		Luciferase activity units (10^5)	
	−cAMP	5 mM cAMP	−cAMP	5 mM cAMP
pPD312	80	960	11.8	4.8
pPD315	30	320	5.7	3.6
pPD313	40	500	8.1	3.9
pHK555	30	360	7.2	4.0
pPD307	20	130	5.1	2.1

[a] For positions of Mu dI insertions in *luxR*, see Fig. 1 and text.

To examine the relationship between cAMP stimulation of *luxR-lacZ* transcription and the position of the *lacZ* fusion, we measured β-galactosidase activity in *E. coli* PD200 (Δ*cyaA*) with each of four *luxR*::Mu dI1734 insertion plasmids or with one *luxR*::Mu dI1681 insertion plasmid (Table 3). The map positions of each of the Mu dI1734 insertions are shown in Fig. 1. There appears to be some correlation between distance of the *lacZ* insertion from the *luxR* promoter and the level of transcription. More importantly, in every case cAMP stimulated transcription from the *luxR* promoter about 6- to 12-fold (Table 3). In these experiments, the presence of cAMP in the growth medium did not stimulate growth (data not shown). These data confirm our previous conclusion that cAMP stimulates transcription from the intact *luxR* promoter (6). Also, the cAMP stimulation is independent of the *lux* DNA orientation in the plasmid—compare the results with pPD313 to those with pHK555, the *luxR*::Mu dI1681 insertion plasmid; the insertions map at approximately the same position in *luxR* but the *lux* DNA in pHK555 is oriented in the vector DNA opposite to its orientation in pPD313.

In the experiment described above, transcription from the *luxICDABE* promoter was monitored concurrently with transcription from the *luxR* promoter by measuring luciferase activity (Table 3). Consistent with our previous study (6), cAMP decreased transcription from this promoter by about 50% in these *luxR* cells.

To examine the influence of cAMP and CRP on the *luxR* promoter in the presence of the LuxR protein, we used the *tac* promoter-controlled *luxR* on pPD749 to complement the *luxR*::Mu dI(*lacZ*) mutation on pHK555. As in the experiments described above, the level of β-galactosidase was measured as an indication of *luxR* promoter activity. Without induction of the *tac* promoter-controlled *luxR* gene by IPTG, cAMP stimulated transcription of *lacZ* from the *luxR* promoter in the *E. coli* Δ*cyaA* mutant and the *cya*$^+$ *crp*$^+$ strain containing pHK555 and pPD749. The basal level of *luxR* promoter activity was higher in the parent, presumably because of the ability of this strain to synthesize cAMP. The *luxR* promoter activity was not affected by the addition of cAMP to the *E. coli* Δ*crp* mutant (Table 4). This is in agreement with our conclusion that cAMP and CRP can stimulate transcription from the *luxR* promoter when the LuxR protein is not abundant (6). When IPTG was added to induce synthesis of the LuxR protein, the level of *luxR* promoter activity was affected (see below). Nevertheless, for the parent strain and particularly for the Δ*cyaA* mutant (PD200), it is evident that cAMP stimulated *luxR* promoter activity in the presence of relatively high levels of the LuxR protein, as it did in the absence of this protein. For the

TABLE 4. β-Galactosidase activity in *E. coli* catabolite repression mutants containing pHK555 and pPD749

Addition(s)	β-Galactosidase activity units		
	PD100 (*cya*$^+$ *crp*$^+$)	PD200 (Δ*cyaA crp*$^+$)	PD300 (*cya*$^+$ Δ*crp*)
None	170	35	45
cAMP (5 mM)	690	470	40
IPTG (1 mM)	95	15	25
cAMP and IPTG	160	150	25
cAMP, IPTG, and autoinducer (0.2 μM)	100	70	25

Δ*cyaA* mutant, the cAMP stimulation was 10-fold (Table 3), comparable to the magnitude of stimulation in the absence of LuxR protein (Table 3). In PD300, the Δ*crp* strain, addition of cAMP did not influence the level of transcription from the *luxR* promoter in IPTG-grown cells (Table 4). Apparently, transcriptional activation of the *luxR* promoter by cAMP did occur in the presence of a functional LuxR protein and this stimulation depended on CRP. Similar results for cAMP control of *luxR* promoter activity were obtained with PD200 (Δ*cyaA*) containing pPD749 plus each of the other four *luxR*::Mu dI(*lacZ*) insertion plasmids: pPD312, pPD315, pPD313, and pPD307 (data not shown).

Influence of *luxR* gene product on *luxR* promoter activity. The ability to control synthesis of the LuxR protein with the cAMP-CRP-independent *tac* promoter on pPD749 and to monitor transcription of the *luxR-lacZ* fusion on pHK555 in Δ*cyaA* and Δ*crp* backgrounds enabled us to study the influence of the LuxR protein on *luxR* promoter activity in vivo. We sought to examine this question because it has been reported that *luxR* is negatively autoregulated, apparently at a posttranscriptional level rather than at the level of transcription (10). The evidence presented here indicates the *luxR* gene product was involved in a negative autoregulation of transcription from the *luxR* promoter (Table 4). When cultures of the *cya*$^+$ *crp*$^+$ strain or either of the mutants were grown in the presence of IPTG to induce synthesis of the LuxR protein, the level of transcription from the *luxR* promoter was about 50% of that when cells were grown without IPTG. The *cya*$^+$ *crp*$^+$ strain and the Δ*cyaA* mutant grown with cAMP and IPTG exhibited only about 25% of the *luxR* promoter activity exhibited by cAMP-grown cells. When saturating concentrations of autoinducer (15) were added with IPTG and cAMP, levels of transcription from the *luxR* promoter were about 15% of the levels in cAMP-grown cells of either the parent or the Δ*cyaA* mutant. The effect of added autoinducer suggests that for maximum negative regulation of the *luxR* promoter by the LuxR protein, autoinducer is required. However, because the cells in these experiments possessed an intact *luxI* gene on pHK555 and thus could synthesize autoinducer, the role of autoinducer in *luxR* regulation awaits clarification. Similar results for *luxR* negative autoregulation were obtained with PD200 (Δ*cyaA*) containing pPD749 plus each of the other four *luxR*::Mu dI(*lacZ*) insertion plasmids: pPD312, pPD315, pPD313, and pPD307 (data not shown).

DISCUSSION

The results of this study extend our understanding of the basis for cAMP-CRP control of the *V. fischeri* luminescence regulon and provide evidence for negative autoregulation of *luxR* at the level of transcription. Although expression of the *luxICDABE* operon in *E. coli* containing the intact *lux* regulon depended on cAMP and CRP (6) (Fig. 3), induced levels of expression of this operon did not require cAMP or CRP when sufficient concentrations of the LuxR protein were produced in *E. coli* by a *tac* promoter-controlled *luxR* structural gene (Fig. 2; Table 2). Thus, in *E. coli*, induction of *luxICDABE* did not require cAMP-CRP directly. Taken together with the observation that the *luxR* promoter is activated by cAMP-CRP (6) (Tables 3 and 4), this indicates that a primary role of cAMP and CRP in *lux* gene regulation is to activate transcription of *luxR*, thereby increasing the concentration of the LuxR protein to a level sufficient for it to serve in conjunction with autoinducer as a *luxICDABE* transcriptional activator.

The results of experiments in which a *luxR-lacZ* transcriptional fusion was used to monitor *luxR* promoter activity in the presence of the *tac-luxR*-containing plasmid indicated that in *E. coli* the *luxR* gene product repressed transcription from the *luxR* promoter and that cAMP-CRP partially relieved that repression (Table 4). Regulation of the *luxR* promoter appears substantial; i.e., the fully repressed levels in IPTG-grown cells of the Δ*cyaA* mutant were only about 3% of the fully induced levels exhibited by cAMP-grown cells (Table 4). Our results appear to be at variance with those of Engebrecht and Silverman (10). Using a *luxR* complementing plasmid with *luxR* under control of its native promoter, these investigators did not observe repression of β-galactosidase in *luxR*::Mu dI (transcriptional) fusions. However, in *luxR*::Mu dII (translational) fusions located distal to the *luxR* promoter, a decrease in β-galactosidase activity in the presence of the *luxR* complementing plasmid was observed (10). It is possible that removal of *luxR* from control by its native promoter and the use of catabolite repression mutants helped reveal the negative autoregulation of *luxR* at the transcriptional level (Table 4). These findings do not preclude the possibility that there is also posttranscriptional negative autoregulation of *luxR* as has been suggested elsewhere (10).

Our investigations of *lux* gene control in *E. coli* lead to the proposal that CRP and the LuxR protein are transcriptional antagonists. Transcription from the *luxR* promoter was activated by cAMP-CRP, whereas transcription from the *luxICDABE* promoter was repressed (Tables 3 and 4). The LuxR protein, apparently together with autoinducer, repressed transcription from the *luxR* promoter (Table 4) and activated *luxICDABE* transcription (Table 2). The evidence for autoinducer involvement in the *luxR* negative autoregulation is not firm, but it has been well established that autoinducer is required for induction of the *luxICDABE* operon (6, 8, 9).

The data presented here led us to develop a working model for transcriptional control of the *lux* regulon (Fig. 4) that is consistent with all other published data of which we are aware. The model indicates that there is a region of approximately 220 base pairs between the *luxR* and *luxI* open reading frames (5, 11) and that together with cAMP, CRP binds to a site about midway between the two open reading frames. Placement of the CRP-binding site is based on identification of a DNA sequence which compares favorably with the consensus sequence for *E. coli* CRP-binding sites (5, 11). According to the model, CRP binding activates transcription of *luxR*, thus driving up the concentration of the LuxR protein, and decreases transcription of *luxICDABE* to an even lower level than in the absence of the LuxR protein. The small available quantities of the *luxI* gene product catalyze the synthesis of autoinducer at a slow rate.

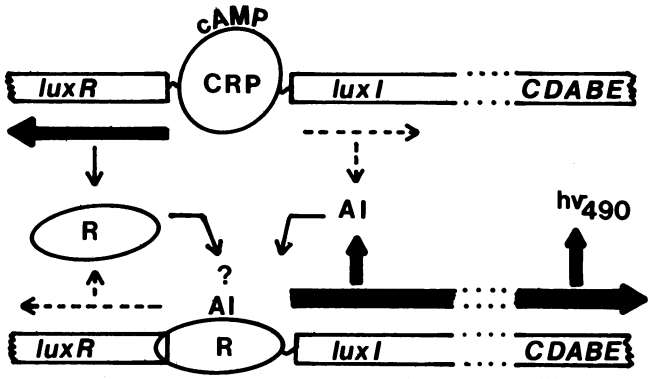

FIG. 4. Model for transcriptional regulation of the *V. fischeri lux* regulon. CRP and cAMP bind to the region between *luxR* and *luxI*, activate transcription of *luxR* (indicated by the heavy arrow) and decrease transcription of *luxICDABE* (indicated by the light arrow). The activation of *luxR* transcription increases the concentration of the LuxR protein (R) to a sufficient level for interaction with the low concentration of autoinducer (AI) that has accumulated owing to basal expression of the *luxI* gene and the resulting low activity of the *luxI* gene product. The LuxR protein and autoinducer then bind to a region upstream of the *luxICDABE* promoter, activating transcription of *luxICDABE*. This leads to increased levels of autoinducer and to induced levels of luminescence (hv_{490}). Since LuxR protein and autoinducer also deactivate transcription of *luxR*, the level of LuxR protein will be fine tuned continually. As this protein becomes limiting at any given autoinducer concentration, the apparent repression of *luxR* will be relieved and the cAMP-CRP complex will bind to the DNA, thus activating transcription of *luxR*.

Nevertheless, autoinducer will accumulate to concentrations of 5 to 10 nM, which are sufficient for an undefined interaction with the LuxR protein and induction of the *luxICDABE* operon (8, 15). We propose that the LuxR protein and autoinducer also deactivate *luxR* transcription (Table 4), thereby counterbalancing the effect of cAMP and CRP. Thus, the LuxR protein will be adjusted continually to levels appropriate for activity at any given concentration of autoinducer. This model is based in large part on experiments with *E. coli* in which *lux* genes were provided in a variety of dosages. Further studies with chromosomal genes in a *V. fischeri* background will serve to test the validity of the model.

DNA sequencing has revealed a 20-base-pair palindrome at −62 with respect to the translational start of *luxI* which is a potential site for binding of a regulatory protein and may represent the LuxR protein-binding site (5). It should be noted, however, that in *E. coli*, overproduction of the LuxR protein directed by a high-copy-number *tac-luxR*-containing plasmid is suppressed by the presence of the *luxICDABE* operon on a low-copy-number plasmid (16). This suggests that there is a *luxR* operator within the *luxR* structural gene or that an additional control element such as the posttranscriptional control postulated by Engebrecht and Silverman (10) is significant (5). The question mark above the autoinducer-LuxR protein-DNA complex in the model (Fig. 4) is meant to indicate several points. Autoinducer binding to the LuxR protein has not been demonstrated, and although it has been demonstrated that the LuxR protein can bind DNA, specific binding to *lux* DNA has not been demonstrated (16). Finally, there is some evidence to suggest that autoinducer and the LuxR protein do not act alone to suppress the synthesis of the LuxR protein (10, 16). The limited data are consistent with the idea that the *luxI* gene product has a dual function: it is required for autoinducer synthesis and it is involved more directly at some level (perhaps a posttranscriptional level) in *luxR* regulation (10, 16). Clearly, this idea remains to be tested.

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LITERATURE CITED

1. **Amann, E., J. Brosius, and M. Ptashne.** 1983. Vectors bearing a hybrid *trp-lac* promoter useful for regulated expression of cloned genes in *Escherichia coli*. Gene **25**:167–178.
2. **Boylan, M., A. F. Graham, and E. A. Meighen.** 1985. Functional identification of the fatty acid reductase components encoded in the luminescence operon of *Vibrio fischeri*. J. Bacteriol. **163**: 1186–1190.
3. **Castilho, B. A., P. Olfson, and M. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
4. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
5. **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.
6. **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.
7. **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.
8. **Engebrecht, J., K. Nealson, and M. Silverman.** 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell **32**:773–781.
9. **Engebrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA **81**:4154–4158.
10. **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.
11. **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.
12. **Friedrich, W. F., and E. P. Greenberg.** 1983. Glucose repression of luminescence and luciferase in *Vibrio fischeri*. Arch. Microbiol. **134**:87–91.
13. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
14. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotopic sources. J. Opt. Soc. Am. **53**:1410–1415.
15. **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.
16. **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.
17. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

18. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase. Occurrence, mechanism and significance. Arch. Microbiol. **112:** 73–79.
19. **Nealson, K. H., and J. W. Hastings.** 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. **43:**469–518.
20. **Rosson, R. A., and K. H. Nealson.** 1981. Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch. Microbiol. **129:**299–304.
21. **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.
22. **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.
23. **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.
24. **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.
25. **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.