JOURNAL OF BACTERIOLOGY, Oct. 1985, p. 45–50
0021-9193/85/100045-06$02.00/0

Vol. 164, No. 1

Control of *Vibrio fischeri* Luminescence Gene Expression in *Escherichia coli* by Cyclic AMP and Cyclic AMP Receptor Protein

PAUL V. DUNLAP AND E. P. GREENBERG*

Department of Microbiology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853

Received 20 May 1985/Accepted 12 July 1985

Under certain conditions glucose represses the autoinducible synthesis of luminescence enzymes in *Vibrio fischeri*. To examine the genetic regulation of luminescence more closely, *Escherichia coli* catabolite repression mutants were transformed with a plasmid (pJE202) that contains *V. fischeri* genes specifying the luminescence enzymes and encoding regulatory functions for luminescence (the *lux* genes) or with plasmids (pJE413 and pJE455) containing transcriptional fusions between the *lacZ* gene on transposon mini-Mu and specific genes in each of the two *lux* operons. Unless cyclic AMP (cAMP) was added to the growth medium, an adenylate cyclase deletion mutant containing pJE202 produced very little light and low levels of the light-emitting enzyme luciferase. When grown in the presence or absence of cAMP, a cAMP receptor protein (CRP) deletion mutant produced low levels of light and luciferase. A mutant that does not make cAMP but does make an altered CRP which does not require cAMP for activity produced induced levels of luminescence after transformation with pJE202. To test the effects of cAMP and CRP on each of the two *lux* operons separately rather than on both together, the *E. coli* catabolite repression mutants were transformed with pJE413 and pJE455. From measurements of β-galactosidase and luciferase activities it appeared that cAMP and CRP affected transcription of both *lux* operons. In the presence of autoinducer and its receptor, transcription of the operon encoding all of the luminescence genes except the receptor gene appeared to be activated by cAMP and CRP, whereas in the absence of the receptor, cAMP and CRP appeared to decrease transcription of this operon. Transcription of the operon encoding the autoinducer receptor appeared to be stimulated by cAMP and CRP in the absence of the receptor itself. These results demonstrate that cAMP and CRP are required for proper control of the *V. fischeri* luminescence system and suggest that *lux* gene transcription is regulated by a complex mechanism.

Luminescence of *Vibrio fischeri*, a cardinal feature of its light organ symbiosis with pinecone fish, requires autoinduction. *V. fischeri* produces a diffusable substance termed autoinducer [*N*-(3-oxo-hexanoyl) homoserine lactone] that accumulates in the medium during growth, and when this autoinducer reaches a concentration corresponding to a few molecules per cell it triggers synthesis of luciferase and other enzymes involved in luminescence (8, 14, 19, 28). Autoinduction can be considered as an environmental sensing mechanism which provides communication between cells of *V. fischeri*; the autoinducer serves as the chemical signal or pheromone. At high population densities, autoinducer can accumulate, and induction of the *V. fischeri* luminescence enzymes will occur. Presumably, this is the case in the light organ symbiosis where *V. fischeri* occurs at densities of 10^{10} cells per ml of organ fluid; however in seawater, where *V. fischeri* exists at much lower densities ($<10^3$ cells per ml), autoinduction of luminescence would not be expected (21, 27, 29).

The recent isolation of a DNA fragment from *V. fischeri* that encodes all of the functions necessary for luminescence and that contains regulatory elements sufficient for their expression in *Escherichia coli* (9) has made possible various studies of the regulation of luminescence at a molecular level. The luminescence genes (*lux* genes) are organized on the cloned DNA fragment in two operons (the left and right) which are transcribed divergently. The *luxR* gene in the left operon encodes a function required for cells to respond to autoinducer (the receptor function). Together with the *luxR* function, autoinducer stimulates transcription of the right operon. There are six genes in this operon. The first, *luxI*, specifies a function required for autoinducer synthesis. The next two, *luxC* and *luxD*, encode activities required for production of the aldehyde substrate for luciferase. These are followed by *luxA* and *luxB*, the genes encoding the two subunit polypeptides of luciferase, and by the last gene, *luxE*, which encodes a third, aldehyde-related function (9, 10). Of particular interest, the gene for autoinducer synthesis is controlled by the autoinducer itself, thus creating what appears to be a positive feedback circuit (9).

Besides autoinduction, catabolite repression of luminescence in *V. fischeri* has been reported (11). The effect of glucose on the luminescence of batch cultures of *V. fischeri* is difficult to interpret in that it is transient, eliminated by prior culture growth in the presence of glucose, and not reversible by cyclic AMP (cAMP) (11, 28). Glucose can permanently repress luminescence in phosphate-limited chemostat cultures, and this repression can be overcome by the addition of cAMP. Of note, a transient addition of cAMP (or of excess autoinducer) permanently relieves luminescence from glucose repression in the chemostat (11). It has been suggested that this is related to the positive-feedback circuit mentioned above (11).

Compared with those for *E. coli*, the techniques for genetic manipulation of *V. fischeri* are primitive, and regulatory mutants of *V. fischeri* are not available. Thus, even though it is apparent that some sort of catabolite repression is involved in regulation of *V. fischeri lux* gene transcription, there is no information on the molecular mechanism of this control. If cAMP and a cAMP receptor protein (CRP) are required to activate autoinduction of *V. fischeri* lumines-

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source (reference)
E. coli K-12		
LS340	*cya*$^+$ *crp*$^+$	CGSC[a] (33)
LS853	Δ*cya-2*[b] *crp*$^+$	CGSC (3)
LS854	*cya*$^+$ Δ*crp-3*	CGSC (30)
CA8306	Δ*cya-854 crp*$^+$	C. Manoil (3)
CA8404	Δ*cya-854 crp-1**[c]	C. Manoil (30)
SG404	P1::Tn*9 clr-100*	N. Kleckner (32)
SH205	*zah-735*::Tn*10* Δ(*argF-lac*)*U169*	M. Weiner (31)
PD50	SH205, P1::Tn*9 clr-100*	This study
PD100	LS340, *zah-735*::Tn*10* Δ(*argF-lac*)*U169*	This study
PD200	LS853, *zah-735*::Tn*10* Δ(*argF-lac*)*U169*	This study
PD300	LS854, *zah-735*::Tn*10* Δ(*argF-lac*)*U169*	This study
ED8654	Mu *cts*	J. Engebrecht (9)
PD101	PD100, Mu *cts*	This study
PD201	PD200, Mu *cts*	This study
PD301	PD300, Mu *cts*	This study
Plasmids		
pJE202	pBR322 with 9-kilobase *Sal*I fragment of *V. fischeri lux* DNA (*luxR*$^+$ *luxICDABE*$^+$)	J. Engebrecht (9, 10)
pJE413	pJE202, *luxC*::mini-Mu(Kan^r *lacZ*$^+$)	J. Engebrecht (9)
pJE455	pJE202, *luxR*::mini-Mu(Kan^r *lacZ*$^+$)	J. Engebrecht

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[b] *cyaA* mutation (2,26).
[c] *crp-1**, Produces CRP which activates transcription of catabolite-repressible operons without cAMP (30).

cence, then it is logical to expect that the *E. coli* CRP mediates autoinduction of luminescence (because *lux* gene activation in *V. fischeri* and *E. coli* appears to be analogous). To test this hypothesis and to study the molecular details of *lux* gene regulation, we monitored *lux* gene expression in *E. coli* adenylate cyclase and CRP mutants that were transformed with a plasmid containing all of the *V. fischeri lux* genes or with plasmids containing transcriptional fusions between the *lacZ* gene on transposon mini-Mu and specific genes in each of the two *lux* operons.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used in this study are listed in Table 1. PD100, PD200, and PD300 were constructed by P1::Tn*9 clr-100*-mediated transduction (32) of *zah-735*::Tn*10*Δ(*argF-lac*)*U169* (31). Transductants were selected on L agar containing tetracycline (15 μg/ml). These transductants were then screened for growth as white colonies on L agar containing 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (32) and for sensitivity to chloramphenicol (30 μg/ml). PD100, PD200, and PD300 were transduced with Mu *cts* (isolated from *E. coli* ED8654 [9]) to construct PD101, PD201, and PD301. PD101, PD201, and PD301 were immune to Mu, and cultures of these strains lysed after incubation at 42°C (4, 5).

Plasmids and transformations. The plasmids used in this study are listed in Table 1. For transformation of LS340, LS853, and LS854 with pJE202, the $CaCl_2$ method (16) was employed, and transformants were selected on L agar containing ampicillin (80 μg/ml). PD101, PD201, and PD301 were transformed with pJE413 or pJE455 by the previously described method for transformation of *E. coli* χ1776 (17) except that the heat shock was for 4 min at 30°C to minimize Mu *cts* induction (5). PD101, PD201, and PD301 transformed with pJE413 or pJE455 were selected on L agar containing ampicillin (80 μg/ml) and kanamycin (100 μg/ml).

Media and culture conditions. For selections and screening, cells were plated on L agar (18) supplemented as indicated and incubated for 1 to 2 days at 30°C. Cultures for all experiments were grown in modified L broth containing 5 g of tryptone (Difco Laboratories, Detroit, Mich.), 2.5 g of yeast extract (Difco), 43 mM NaCl, 20 mM glycerol, and 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (pH 7.5) plus ampicillin (80 μg/ml) to assure plasmid maintenance. This medium was supplemented with D-glucose (10 mM), cAMP (4 mM), and autoinducer (0.2 μM) as indicated.

For experiments with strains harboring pJE202, cultures were grown in 50 ml of medium contained in 300-ml sidearm flasks with shaking (100 cycles per min). For experiments with strains harboring pJE413 or pJE455, cultures were grown in 3 ml of medium contained in culture tubes (13 by 100 mm) with shaking (200 rpm). Cultures were inoculated to an initial cell density (optical density at 660 nm [OD_{660}]; Bausch & Lomb Spectronic 21 spectrophotometer) of 0.002 to 0.008. Inocula were from cultures that had been grown in modified L broth (without additions) to a density of 0.5 (OD_{660}) that were themselves inoculated from overnight cultures.

All broth cultures and plates were incubated at 30°C.

Determination of cellular luminescence and luciferase activity. The light-measuring equipment and standard to calibrate this equipment have been described previously (11, 12), except that levels of light below 10^6 quanta per s were measured with the single-photon-counting accessory on a Beckman model LS 1800 scintillation counter. The techniques for measuring luminescence in a culture sample and the procedure for measuring luciferase in cell extracts by its reaction with excess reduced flavin mononucleotide, decanal, and oxygen have been described previously by Rosson and Nealson (25). Cell extracts were prepared as follows. Cells from 1 ml of culture were harvested by microcentrifugation (13,000 × *g* for 2 min at 4°C), frozen, and then stored for up to 1 week at −70°C. Cells were then thawed in 1 ml of lysis buffer (25), refrozen, and finally thawed on ice. These cell extracts were used for luciferase activity measurements. As demonstrated by direct counting by phase-contrast microscopy, >94% of the cells lysed as a result of the freeze-thaw procedure employed. The values given are means of at least two assays, and the range between assays for a given sample never exceeded 17%. Luciferase activity is related to cell extract from 1 ml of culture. Luciferase activity units have been normalized to a cell density of 1.0 (OD_{660}), and actually, in experiments for which units of luciferase activity are reported, cells were harvested from cultures at a density of 1.0 (OD_{660}).

Determination of β-galactosidase activity. For experiments on strains containing plasmids with transcriptional fusions between the *lacZ* on transposon mini-Mu and the *lux* genes, β-galactosidase activity was measured by the $CHCl_3$-sodium dodecyl sulfate method described by Miller (18). Activity units have been defined by Miller (18), and cells for β-galactosidase activity measurements were harvested from

cultures at a density of 1.0 (OD_{660}). The values reported are means of quadruplicate assays (range, <10%).

Chemicals. Antibiotics, cAMP, decanal, reduced flavin mononucleotide, HEPES, and 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside were purchased from Sigma Chemical Co., St. Louis, Mo., and autoinducer [*N*-(3-oxo-hexanoyl) homoserine lactone] was provided by H. B. Kaplan.

RESULTS

Luciferase activity and cellular luminescence in *E. coli* and *E. coli* catabolite repression mutants containing pJE202. For comparison with results from studies of *V. fischeri* (11, 28) and as a control for experiments with catabolite repression mutants, we studied the effects of glucose, cAMP, and autoinducer on the parent strain (LS340) containing pJE202. In this and in other experiments both luciferase activity and cellular luminescence were measured. Luminescence is the phenotypic consequence of *lux* gene transcriptional activation, but luciferase activity in cell extracts is a more direct measure of transcriptional activation of the right operon (the operon encoding the enzymes for the luminescence reaction). Luciferase activity exhibited a characteristic autoinduction response (19, 25): activity remained at a constant uninduced level for the first 4 to 5 h of growth and was then induced to a level about 1,000-fold greater than the uninduced level (Fig. 1A). Glucose effected a slight delay of at most 1 to 2 h in the onset of induction that was not reversed by cAMP (Fig. 1A). When high concentrations of autoinducer were added to the growth medium together with glucose, there was no lag before the increase in luciferase activity (Fig. 1A). Measurements of cellular luminescence were consistent with measurements of luciferase activity except that luminescence exhibited the characteristic drop before induction (Fig. 1B). These results are similar to those obtained with batch cultures of *V. fischeri* (11, 28). As mentioned above, studies with chemostat cultures were required to demonstrate that the *V. fischeri* luminescence system was sensitive to catabolite repression (11). The glucose effect on luminescence of batch cultures may or may not be related to catabolite repression.

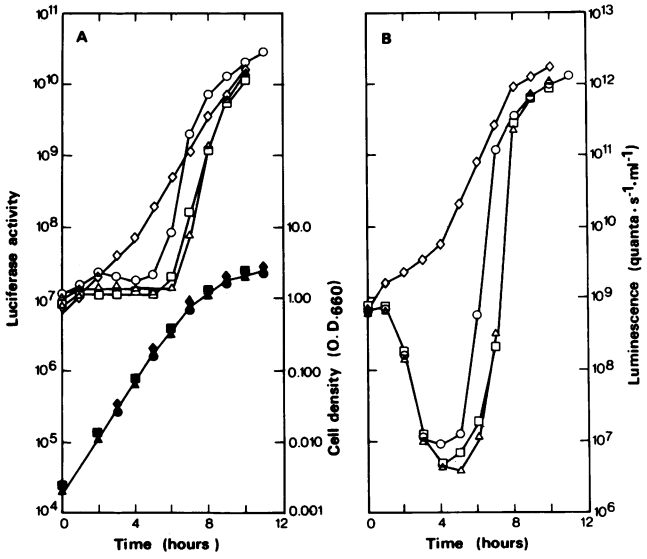

FIG. 1. Effects of D-glucose, cAMP, and autoinducer on growth, luciferase activity, and in vivo luminescence of *E. coli* LS340 containing pJE202. (A) Cell density (solid symbols) and luciferase activity (open symbols). (B) Luminescence. Symbols: △, ▲, glucose added to the medium; □, ■, glucose and cAMP added; ◇, ◆, glucose and autoinducer added; ○, ●, no addition.

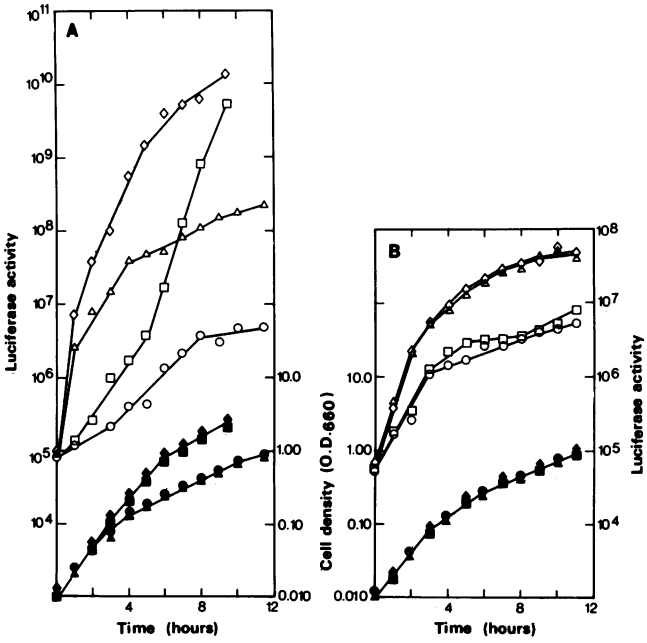

FIG. 2. Effects of cAMP and autoinducer on luciferase activity and growth in cultures of *E. coli* catabolite repression mutants. Open symbols, luciferase activity; solid symbols, cell density. (A) *E. coli* LS853 (Δ*cyaA*) containing pJE202. (B) *E. coli* LS854 (Δ*crp*) containing pJE202. Symbols: △, ▲, autoinducer added to the medium; □, ■, cAMP added; ◇, ◆, autoinducer and cAMP added; ○, ●, no addition.

In the absence of cAMP the Δ*cyaA* mutant (LS853) containing pJE202 produced a low level of luciferase (lower than uninduced levels in the parent) that increased in concert with growth (Fig. 2A). That is, luciferase activity per cell remained constant. When cAMP was added with or without autoinducer, luciferase activity reached levels similar to the induced level in the parent (Fig. 2A). This fully induced level was reached sooner when autoinducer was added (Fig. 2A), presumably because added autoinducer eliminated the delay that occurs while autoinducer produced by the cells builds to the critical concentration necessary for induction (5 to 10 nM; 14). Addition of excess autoinducer without cAMP provided some stimulation of luciferase synthesis, but the maximum level of luciferase activity was about 100-fold less than the fully induced level in the presence of cAMP (Fig. 2A).

For the Δ*crp* mutant (LS854) containing pJE202, addition of cAMP did not stimulate luciferase synthesis. Instead, in the presence or absence of added cAMP, luciferase synthesis in this strain was remarkably similar to luciferase synthesis in the Δ*cyaA* mutant without added cAMP (Fig. 2). Another CRP mutant was studied also. This mutant (CA8404) produces a CRP which activates transcription of catabolite-repressible operons without cAMP (30). When CA8404, which also has a deletion mutation in *cyaA*, was transformed with pJE202, it produced induced levels of luminescence, whereas the isogenic strain CA8306, which carries crp^+, produced very little light unless cAMP was added to the growth medium (data not shown).

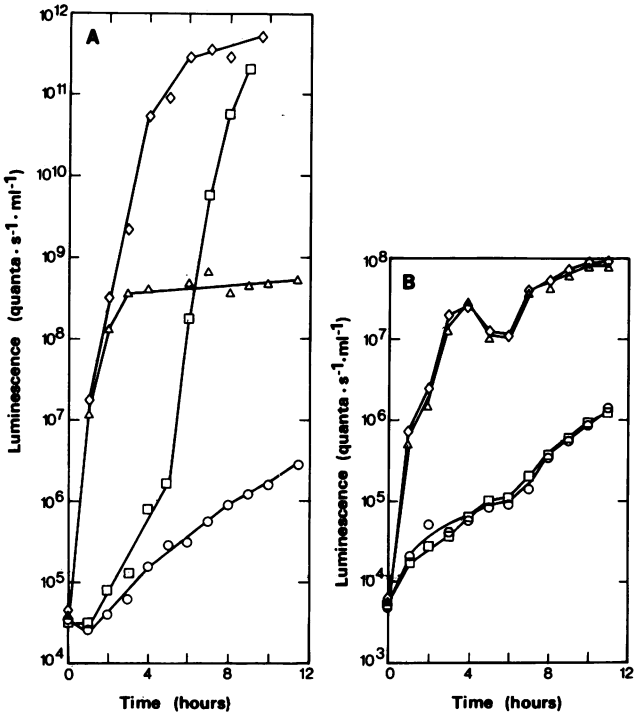

FIG. 3. Effects of cAMP and autoinducer on luminescence in cultures of *E. coli* catabolite repression mutants containing pJE202. (A) *E. coli* LS853 (Δ*cyaA*). (B) *E. coli* LS854 (Δ*crp*). Symbols: △, autoinducer added to the medium; □, cAMP added; ◇, autoinducer and cAMP added; ○, no addition. These luminescence measurements were made with the same cultures as those described in the legend to Fig. 2.

Luminescence of LS853 and LS854 containing pJE202 was measured, and the results were consistent with those for luciferase activity. However, the difference between basal and induced levels of cellular luminescence was even greater than that for luciferase activity (Fig. 3). Presumably, this reflects the fact that several enzymes are required for luciferase activity and are coinduced with luciferase (9, 19, 22). These results indicate that in the future, effects of cAMP and autoinducer on *lux* gene activation in *E. coli* mutants containing pJE202 may be conveniently and continuously monitored in vivo by measuring cellular luminescence.

Addition of cAMP stimulated growth of the Δ*cyaA* mutant (LS853) containing pJE202 (Fig. 2A). Thus, it is important to mention that when glucose was present in the culture medium, growth rate was not affected by cAMP, yet glucose did not alter *lux* gene regulation with respect to regulation in the absence of glucose (Fig. 2A and 3A).

β-Galactosidase activity in *E. coli* and *E. coli* catabolite repression mutants containing pJE413 or pJE455. From the experiments described above it is apparent that cAMP and CRP are required for full induction of luciferase synthesis (and cellular luminescence) in *E. coli* containing pJE202. However, these experiments do not define the mechanism of this involvement. The mechanism could involve direct stimulation of the right operon which encodes the functions required for luminescence and a function required for autoinducer synthesis in *E. coli*. Another possible mechanism could involve cAMP and CRP stimulation of left operon transcription. This should increase levels of the autoinducer receptor and might thereby serve to fully activate transcription of the right operon. There are other possibilities as well. For example, this mechanism could involve an indirect stimulation of *lux* transcription. To begin studies of the details of cAMP and CRP involvement in the regulation of luminescence, we examined β-galactosidase synthesis directed by *lux* plasmids containing transcriptional fusions between *lacZ* in transposon mini-Mu and *luxC* in the right operon (pJE413) or *luxR* in the left operon (pJE455). For these experiments, β-galactosidase activity is reported in units as defined by Miller (18). This is an indication of activity per cell.

Experiments with strains containing pJE413 were consistent with the notion that cAMP and CRP are involved in transcriptional activation of the right *lux* operon (Table 2). In a *cya*$^+$ *crp*$^+$ strain (PD101), autoinduction resulted in high levels of β-galactosidase activity, and these levels were little affected by addition of cAMP or autoinducer to the growth medium. In a Δ*cyaA* mutant (PD201), cAMP was required for induction of β-galactosidase synthesis, and β-galactosidase activity in cells grown with added autoinducer or autoinducer and cAMP mirrored luciferase activity measurements in a Δ*cyaA* mutant containing pJE202 (Fig. 2A). In a Δ*crp* mutant (PD301) containing pJE413, levels of β-galactosidase activity were low with or without cAMP (Table 2). Since this approach with *lacZ* operon fusions has been established as a method for monitoring transcription in vivo (6), these experiments lend credence to the idea that cAMP and CRP regulate luminescence at the transcriptional level.

These studies with pJE413 (Table 2) do not provide any information regarding the mechanism of *lux* transcriptional activation. Is transcription of the right operon affected directly, is transcription of the left operon affected, are both operons affected, or is neither operon affected directly? To begin to answer these question, strains containing pJE455 were studied. pJE455 possesses an intact right operon but has transposon mini-Mu inserted into *luxR* of the left operon (10). Thus, the receptor function is inactivated, and levels of β-galactosidase activity should reflect levels of transcription

TABLE 2. β-Galactosidase activity in *E. coli* catabolite repression mutants containing pJE413

Addition(s)	β-Galactosidase activity units[a]		
	PD101 (*cya*$^+$ *crp*$^+$)	PD201 (Δ*cyaA* *crp*$^+$)	PD301 (*cya*$^+$ Δ*crp*)
None	7,700	20	20
Autoinducer (0.2 μM)	8,900	80	30
cAMP (4 mM)	12,300	2,000	20
Autoinducer and cAMP	7,200	15,300	25

[a] Units of β-galactosidase are defined in Materials and Methods.

TABLE 3. β-Galactosidase activity of *E. coli* catabolite repression mutants containing pJE455

Addition(s)	β-Galactosidase activity units[a]		
	PD101 (*cya*$^+$ *crp*$^+$)	PD201 (Δ*cyaA* *crp*$^+$)	PD301 (*cya*$^+$ Δ*crp*)
None	2,100	110	190
Autoinducer (0.2 μM)	2,000	110	190
cAMP (4 mM)	2,900	590	180
Autoinducer and cAMP	2,900	480	200

[a] Units of β-galactosidase are defined in Materials and Methods.

TABLE 4. Luciferase activity of *E. coli* catabolite repression mutants containing pJE455

Addition(s)	Luciferase activity units (10^5)[a]		
	PD101 (*cya*$^+$ *crp*$^+$)	PD201 (Δ*cyaA crp*$^+$)	PD301 (*cya*$^+$ Δ*crp*)
None	23	47	58
Autoinducer (0.2 μM)	22	37	59
cAMP (4 mM)	23	9	63
Autoinducer and cAMP	16	9	47

[a] Luciferase activity units are defined in Materials and Methods.

of the left operon. Addition of cAMP to a *cya*$^+$ *crp*$^+$ strain (PD101) containing pJE455 resulted in a slight elevation of β-galactosidase synthesis, and cAMP stimulation of β-galactosidase activity in the Δ*cyaA* mutant (PD201)) was more obvious (Table 3). Addition of cAMP to the Δ*crp* mutant (PD301) containing pJE455 did not increase β-galactosidase activity, and addition of autoinducer to any of these organisms did not affect β-galactosidase activity (Table 3). Apparently, cAMP and CRP can stimulate transcription of *luxR* (left operon). Not surprisingly, autoinducer, which together with the *luxR* function stimulates transcription of the right operon, did not affect β-galactosidase activity in these strains that do not produce a functional *luxR* gene product.

Luciferase activity in *E. coli* and *E. coli* catabolite repression mutants containing pJE455. Together with monitoring transcription of the left operon (β-galactosidase activity; Table 3), it was possible to monitor levels of right operon transcription in cells containing pJE455 by measuring luciferase activity (Table 4). In the parent (PD101), luciferase activity was extremely low compared with luciferase activity in cells with a functional *luxR* (Fig. 1 and 2; Table 4). This is in agreement with previous reports (9, 10). Furthermore, it is evident that in the absence of the *luxR* function, cAMP together with CRP decreased transcription of the right operon. In the Δ*cyaA* strain (PD201) containing pJE455, luciferase activity was about fivefold lower in the presence of cAMP than in the absence of cAMP. In the Δ*crp* strain (PD301), luciferase activity was similar when cells were grown with or without cAMP (Table 4).

DISCUSSION

By using *E. coli* catabolite repression mutants containing a plasmid with the *V. fischeri lux* genes (pJE202), it was demonstrated that induction of luminescence requires cAMP and a CRP (Fig. 3). In vivo experiments indicate that cAMP and CRP are involved in transcriptional activation of the luciferase genes and other genes in the right *lux* operon (Fig. 2; Table 2). Presumably, the cAMP- (or autoinducer-) reversible glucose repression of luminescence in chemostat cultures of *V. fischeri* (11) involves an analogous mechanism.

That *E. coli* CRP functions as a regulator of the *V. fischeri lux* genes is both fortuitous and interesting. It is fortuitous in that this was necessary for the isolation of a DNA fragment from *V. fischeri* sufficient for expression of induced levels of luminescence in *E. coli* (9). In this regard, it is of interest that the CRP from another luminous marine *Vibrio* sp., *V. harveyi*, cross-reacts immunologically with *E. coli* CRP (1) and that purified *V. harveyi* CRP stimulates transcription of catabolite-repressible genes from *E. coli* in vitro (P.-F. Chen, S.-C. Tu, N. Hagag, F. Y.-H. Wu, and C. W. Wu, Arch. Biochem. Biophys. in press).

The effects of glucose and cAMP on batch cultures of *V. fischeri* (11, 28) and batch cultures of *E. coli* (pJE202) without catabolite repression mutations (Fig. 1) are similar and are difficult to interpret. It is possible that the observed glucose effect in batch cultures of cells with functional CRPs and adenylate cyclase may in fact not be related to catabolite repression. Nevertheless, studies with the catabolite repression mutants demonstrate that cAMP and CRP are required for full expression of the *lux* genes in *E. coli*. For *V. harveyi*, catabolite repression of luminescence has been demonstrated by simple batch-culture experiments. Luciferase synthesis in batch cultures of *V. harveyi* is permanently repressed by D-glucose, and this repression can be overcome by addition of cAMP to the growth medium (20). Furthermore, pleiotropic mutants have been isolated which make light only when cAMP is added to the growth medium (34). For other luminous bacterial species the picture is not clear, but it is possible that transcription of *lux* genes in these species may involve activation by cAMP and CRP (7, 13, 15).

Apparently, expression of the *V. fischeri lux* genes in *E. coli* is under dual positive control: both autoinducer and its receptor and cAMP and its receptor are required for induction of luminescence. In a general sense this is similar to transcriptional regulation of the *E. coli* arabinose operon (23, 24); however, details of *lux* gene regulation are lacking. We do know from experiments with *E. coli* containing pJE455 (Tables 3 and 4) that at least in the absence of autoinducer receptor, transcription of both *lux* operons is influenced by CRP together with cAMP. Transcription of the left operon in a Δ*cyaA* mutant was stimulated (Table 3) and transcription of the right operon was deactivated (Table 4) by cAMP. Based on this study and previous publications on expression of *V. fischeri lux* genes in *E. coli* (9, 10), it appears that cAMP and CRP can serve to activate transcription of one *lux* operon while deactivating transcription of the other. In the absence of the receptor for autoinducer, the left operon is activated and the right deactivated (Tables 3 and 4). It is known that in the presence of sufficient autoinducer and receptor the right operon is activated, and it has been suggested that the left operon is deactivated (9, 10). This would result in a dramatic increase in luminescence and a decrease in the concentration of the receptor for autoinducer. To understand the molecular details of the cAMP and CRP involvement in the regulation of luminescence and to develop a model for transcriptional regulation of luminescence, further studies, both in vivo and in vitro, are necessary.

ACKNOWLEDGMENTS

We thank J. Engebrecht who generously provided the recombinant plasmids, B. Bachmann, M. Weiner, and C. Manoil for bacterial strains, and H. B. Kaplan for pure autoinducer and helpful advice.

This work was supported by a grant from the Cornell Biotechnology Program which is sponsored by the New York State Science and Technology Foundation and a consortium of industries.

LITERATURE CITED

1. **Anderson, W. B., and I. Pastan.** 1973. The cyclic AMP receptor of *Escherichia coli*: immunological studies in extracts of *Escherichia coli* and other organisms. Biochim. Biophys. Acta **320**:577–587.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12,

edition 7. Microbiol. Rev. **47**:180–230.
3. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which affect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenyl cyclase. J. Bacteriol. **116**:582–587.
4. **Bukhari, A. I., and E. Ljungquist.** 1977. Bacteriophage Mu: methods for cultivation and use, p. 749–756. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Casadaban, M. J.** 1975. Fusion of the *Escherichia coli lac* genes to the *ara* promoter: a general technique using bacteriophage Mu-1 insertions. Proc. Natl. Acad. Sci. USA **72**:809–813.
6. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
7. **Dunlap, P. V.** 1985. Osmotic control of luminescence and growth in *Photobacterium leiognathi* from ponyfish light organs. Arch. Microbiol. **141**:44–50.
8. **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.
9. **Engebrecht, J., K. Nealson, and M. Silverman.** 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell **32**:773–781.
10. **Engbrecht, J., and M. Silverman.** 1984. Identification of genes and gene products necessary for bacterial bioluminescence. Proc. Natl. Acad. Sci. USA **81**:4154–4158.
11. **Friedrich, W. F., and E. P. Greenberg.** 1983. Glucose repression of luminescence and luciferase in *Vibrio fischeri*. Arch. Microbiol. **134**:87–91.
12. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotopic sources. J. Opt. Soc. Am. **53**:1410–1415.
13. **Henry, J.-P., and A. M. Michelson.** 1970. Etudes de bioluminescence. Regulation de la bioluminescence bacterienne. C. R. Acad. Sci. **270**:1947–1949.
14. **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.
15. **Makemson, J. C.** 1973. Control of *in vivo* luminescence in psychrophilic marine photobacterium. Arch. Mikrobiol. **93**:347–358.
16. **Mandel, M., and A. Higa.** 1970. Calcium dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual, p. 254–255. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase. Occurrence, mechanism and significance. Arch. Microbiol. **112**:73–79.
20. **Nealson, K. H., A. Eberhard, and J. W. Hastings.** 1972. Catabolite repression of bacterial bioluminescence: functional implications. Proc. Natl. Acad. Sci. USA **69**:1073–1076.
21. **Nealson, K. H., and J. W. Hastings.** 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. **43**:496–518.
22. **Nealson, K. H., T. Platt, and J. W. Hastings.** 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**:313–322.
23. **Ogden, S., D. Haggerty, C. M. Stoner, D. Kolodrubetz, and R. Schleif.** 1980. The *Escherichia coli* L-arabinose operon: binding sites of the regulatory proteins and a mechanism of positive and negative regulation. Proc. Natl. Acad. Sci. USA **77**:3346–3350.
24. **Raibaud, O., and M. Schwartz.** 1984. Positive control of transcription initiation in bacteria. Annu. Rev. Genet. **18**:173–206.
25. **Rosson, R. A., and K. H. Nealson.** 1981. Autoinduction of bacterial bioluminescence in a carbon limited chemostat. Arch. Microbiol. **129**:299–304.
26. **Roy, A., and A. Danchin.** 1981. Restriction map of the *cya* region of the *Escherichia coli* K12 chromosome. Biochimie **63**:719–722.
27. **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.
28. **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.
29. **Ruby, E. G., and K. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore waters. Limnol. Oceanogr. **23**:530–533.
30. **Sabourin, D., and J. Beckwith.** 1975. Deletion of the *Escherichia coli crp* gene. J. Bacteriol. **122**:338–340.
31. **Schweizer, H., and W. Boos.** 1983. Transfer of the Δ(*argF-lac*) *U169* mutation between *Escherichia coli* strains by selection for a closely linked Tn*10* insertion. Mol. Gen. Genet. **192**:293–294.
32. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 107–112. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Soll, L., and P. Berg.** 1969. Recessive lethals: a new class of nonsense suppressors in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **63**:392–399.
34. **Ulitzur, S., and J. Yashphe.** 1975. An adenosine 3′,5′-monophosphate-requiring mutant of the luminous bacteria *Beneckea harveyi*. Biochim. Biophys. Acta **404**:321–328.