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The *Vibrio fischeri* LuxR Protein Is Capable of Bidirectional Stimulation of Transcription and Both Positive and Negative Regulation of the *luxR* Gene

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Regulation of the genes required for bioluminescence in the marine bacterium *Vibrio fischeri* (the *lux* regulon) is a complex process requiring coordination of several systems. The primary level of regulation is mediated by a positive regulatory protein, LuxR, and a small diffusible molecule, *N*-(3-oxo-hexanoyl)-homoserine lactone, termed autoinducer. Transcription of the *luxR* gene, which encodes the regulatory protein, is positively regulated by the cyclic AMP-CAP system. The *lux* regulon of *V. fischeri* consists of two divergently transcribed operons designated operon$_L$ and operon$_R$. Transcription of the rightward operon (operon$_R$; *luxICDABE*), consisting of the genes required for autoinducer synthesis (*luxI*) and light production (*luxCDABE*), is activated by LuxR in an autoinducer-dependent fashion. The leftward operon (operon$_L$) consists of a single known gene, *luxR*. The LuxR protein has also been shown to decrease transcription of operon$_L$ through an autoinducer-dependent mechanism, thereby negatively regulating its own synthesis. In this paper we demonstrate that the autoinducer-dependent repression of operon$_L$ transcription requires not only LuxR but also DNA sequences within operon$_R$ which occur upstream of the promoter for operon$_L$. In the absence of these DNA sequences, the LuxR protein causes an autoinducer-dependent activation of transcription of operon$_L$. The *lux* operator, located in the control region between the two operons, was required for both the positive and negative autoinducer-dependent responses. By titration of high levels of LuxR supplied in *trans* with synthetic autoinducer, we found that low levels of autoinducer could elicit a positive response even in the presence of the negative-acting DNA sequences, while higher levels of autoinducer resulted in a negative response. Without these DNA sequences in operon$_R$, LuxR and autoinducer stimulated transcription regardless of the level of autoinducer. These results suggest that a switch between stimulation and repression of operon$_L$ transcription is mediated by the levels of the LuxR-autoinducer complex, which in these experiments reflects the level of autoinducer in the growth medium.

The expression of bioluminescence in the marine bacterium *Vibrio fischeri* is regulated by a unique cell-density-dependent mechanism, termed autoinduction (20). Autoinduction is mediated through the production of a small molecule termed the autoinducer which in *V. fischeri* was identified as *N*-(3-oxo-hexanoyl)-homoserine lactone (8, 9). Autoinducer is freely diffusible across the cytoplasmic membrane (16), affording *V. fischeri* a mechanism for sensing cell density. At low cell density, the autoinducer diffuses into and is diluted by the growth medium, while at higher cell density the autoinducer can reach a critical intracellular concentration capable of inducing bioluminescence.

The genes required for bioluminescence (*lux*) have been isolated from *V. fischeri* and expressed from a variety of plasmids in *E. coli*; the autoinduction mechanism observed in *V. fischeri* appears to function in *E. coli* (10, 12). The nucleotide sequence of the entire *lux* regulon from *V. fischeri* ATCC 7744 has been determined (1). The *lux* genes are organized into two operons, termed rightward and leftward, which are divergently transcribed from a common regulatory region separating the two operons (3, 10, 13). The rightward operon (operon$_R$) consists of six known *lux* genes (*luxICDABE*) which encode the enzyme required for autoinducer synthesis (*luxI*), the α and β subunits of the enzyme luciferase (*luxAB*) and the enzymes which comprise the fatty acid reductase complex (*luxC*, *luxD*, and *luxE*) which provide luciferase with the aldehyde substrate necessary for bioluminescence (11, 18). The leftward operon (operon$_L$) consists of a single gene, *luxR*, which encodes the regulatory protein LuxR. The LuxR protein and autoinducer comprise the primary *lux*-encoded regulatory molecules responsible for autoinduction. The current model describing the induction of luminescence asserts that a low basal level of operon$_R$ transcription leads to the production of low levels of autoinducer by the LuxI protein. At high cell densities, the autoinducer can accumulate and interact with the LuxR protein to form a complex capable of binding the *lux* operator and stimulating operon$_R$ transcription (4). This autoinducer-dependent transcriptional activation leads to the establishment of positive feedback induction of operon$_R$ transcription. Positive feedback results because *luxI* is located in operon$_R$ so that transcriptional activation by LuxR and autoinducer leads to the production of more autoinducer by increased levels of LuxI protein. The positive feedback regulation of operon$_R$ transcription leads to the observed sharp induction of luminescence characteristic of the autoinduction response.

A fundamental aspect of this model of the autoinduction response (3) involves direct interaction of the LuxR-autoinducer complex with a specific site within the control region of the *lux* regulon (4). We have shown that certain mutations within a 20-bp palindromic sequence, ACCTGTAGGATCG TACAGGT, located near the start point of transcription of

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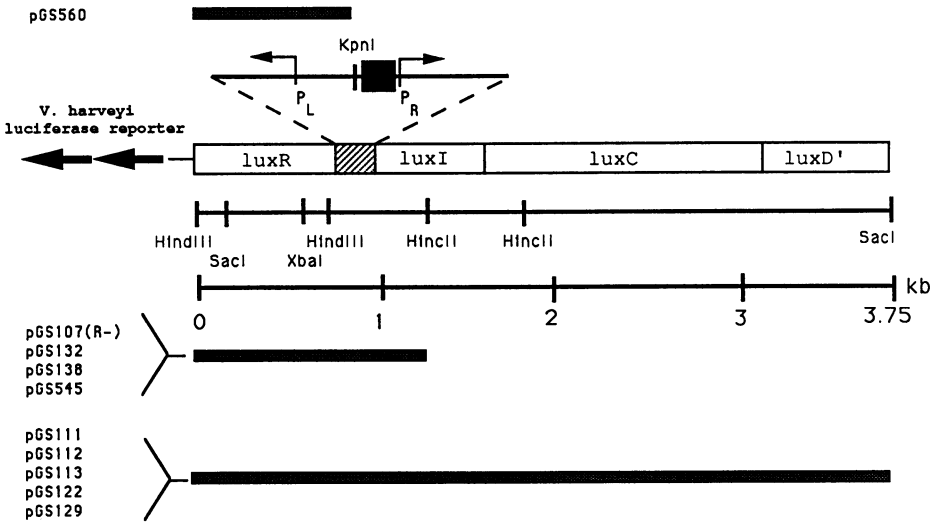

FIG. 1. Summary of operon$_L$ reporter vectors used in this study. The locations of restriction enzyme sites utilized to construct the various plasmid vectors are labeled below the regulon map, and the *V. fischeri lux* DNA fragments contained in each vector are designated by shaded bars. All vectors listed have the *luxA* and *luxB* genes (bold arrows), encoding the luciferase enzyme from *V. harveyi*, located downstream of the *luxR* gene. Important characteristics of each plasmid are as follows: pGS138, pGS545, and pGS111, the *V. fischeri* inserts are wild-type DNA sequence; pGS560 is identical to pGS545 except that sequences between *Kpn*I and *Hin*cII (*luxI*), which include the *lux* operator, are deleted; pGS107(R$^-$) has a frameshift mutation in *luxR*; pGS132 and pGS129 have a mutant *lux* operator (position 3 C to T); pGS122 has a mutant *lux* operator (central 12 bp deleted); pGS112 has a nonsense codon in *luxC*; pGS113 has a nonsense mutation in *luxI*. Within the expanded control region (hatched box), the black box represents the *lux* operator and the arrows indicate the direction of transcription from the two characterized *lux* promoters (13).

operon$_R$, block the ability of the LuxR-autoinducer to stimulate transcription. Furthermore, we have isolated mutations in *luxR* that result in a protein product requiring elevated levels of autoinducer to stimulate transcription (22). However, until recently, the possibility remained that the effect of the LuxR-autoinducer was mediated through some other DNA-binding factor to alter transcription rates. Recent experiments (2) have shown that the negative effects of point mutations within the defined operator sequence can be reversed in an allele-specific fashion by single amino acid changes in the LuxR protein. These mutants demonstrate convincingly that the LuxR protein binds to the palindromic sequence defined as the *lux* operator (4).

The expression of bioluminescence in *V. fischeri* is also controlled by catabolite repression (14, 19). Studies utilizing *Escherichia coli* strains deficient in either cyclic AMP (cAMP) (*cya*) or catabolite gene activator protein (CAP) (*crp*) demonstrated that operon$_L$ was the target for catabolite repression and that transcription of operon$_L$ was activated by the cAMP-CAP regulatory circuit (5, 6). The transcription of operon$_L$, and thus the *luxR* gene, was also shown to be negatively regulated by LuxR through an autoinducer-dependent mechanism providing negative autoregulation of LuxR synthesis (7). Negative autoregulation by LuxR required the production of relatively high levels of LuxR protein produced from a *tac* promoter expression vector (6, 7). In this paper we present new aspects of *lux* regulation which were revealed during experiments aimed at understanding the mechanism of LuxR negative autoregulation.

MATERIALS AND METHODS

Strains and plasmids. All experiments used *E. coli* TB1 [*hsdR* Δ(*lac-proAB*) *ara rspL lacZ*Δ*M15*] or LE392 (*hsdR514 hsdM supE44 supF58* Δ*lacIZY galK2 galT22 metB1 trpR55*).

The *V. fischeri* inserts used to construct the operon$_L$ luciferase reporter plasmids used in this study are summarized in Fig. 1. All plasmids listed in Fig. 1 contain the *luxA* and *luxB* genes from *Vibrio harveyi* located downstream of a full-length *luxR* gene. The *luxA* and *luxB* genes encode the luciferase enzyme and comprise the luminescence reporter which allowed quantitation of the level of transcription of operon$_L$.

The pGS545 and pGS560 plasmids are derivatives of pBR322. The pGS560 plasmid only contains *V. fischeri* DNA extending from the 3′ end of *luxR* to a *Kpn*I restriction site located next to the *lux* operator (Fig. 1). This *Kpn*I site was introduced by site-directed mutagenesis and was described previously (4). All of the remaining reporter vectors are derivatives of pACYC184. To create pGS107(R$^-$), pGS107 was digested with *Xba*I, treated with T4 DNA polymerase, and ligated to create a four-base insertion at the unique *Xba*I site in *luxR* (Fig. 1) resulting in a shift in reading frame of the *luxR* gene. The plasmids pGS129 and pGS132 have a single base change (C to T; described previously [4]) at position 3 of the *lux* operator. The pGS112 and pGS113 plasmids have nonsense codons in *luxC* and *luxI*, respectively. The pGS122 plasmid has a mutant *lux* operator from which the central 12 bp were deleted. The plasmid pPD749 (a gift from Pete Greenberg) was used to supply LuxR in *trans* in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG) and was described previously (6).

Site-directed mutagenesis. Site-directed mutagenesis was done by the method of Kunkel (17) with slight variations and using a derivative of the phagemid vector pTZ18R (Pharmacia) as described previously (22).

Growth of cultures and measurement of operon$_L$ transcription as bioluminescence in vivo. The level of transcription of operon$_L$ was determined by monitoring aldehyde-stimulatable luminescence of samples (1 ml) withdrawn from cultures of *E. coli* transformed with various operon$_L$ reporter

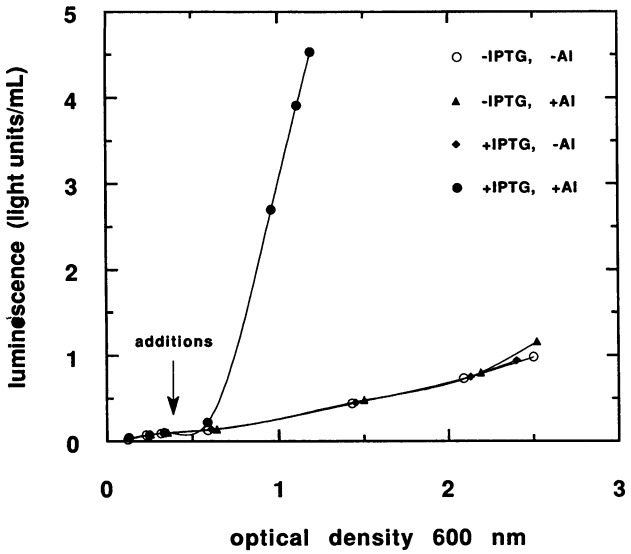

FIG. 2. Expression of luminescence in *E. coli* TB1 transformed with pGS107(R⁻) and pPD749. IPTG and autoinducer (AI) were added where indicated at an OD_{600} of 0.4 to final concentrations of 1 mM and 2.5 μM, respectively. Luminescence in vivo, given as light units per milliliter of culture, was plotted versus the OD_{600} of the culture and was monitored throughout growth.

plasmids. LB (Luria-Bertani) medium containing carbenicillin (100 μg/ml) and/or chloramphenicol (34 μg/ml) was used for growth and selection of plasmid-transformed strains. Culture growth and measurement of cell density and luciferase activity in vivo (22) and autoinducer synthesis (19) were performed as described previously. Complete growth curves were done for all experiments and the values (light units per milliliter) presented in Tables 1, 2, and 3 were determined from graphs of data plotted as light units per milliliter versus optical density at 600 nm (OD_{600}) and estimated to the nearest 0.5 light units per ml at a given OD_{600}.

RESULTS

LuxR stimulates transcription of operon$_L$ in an autoinducer-dependent fashion. The *luxR* gene in operon$_L$ reporter plasmid pGS107(R⁻) was inactivated by creating a frameshift at the unique *Xba*I site (Fig. 1). The pGS107(R⁻) plasmid contains *V. fischeri* DNA spanning the entire *luxR* gene, regulatory region, and approximately half of the *luxI* gene (Fig. 1). Synthesis of autoinducer was not possible in strains carrying this plasmid since the *luxI* gene was inactivated by deletion. Transcription of operon$_L$ was monitored from cultures of *E. coli* carrying pGS107(R⁻) as the reporter vector and pPD749 as the source for LuxR under control of IPTG. By using this system, the effects of autoinducer and LuxR on operon$_L$ transcription were determined through the addition of autoinducer and/or IPTG. The results (Fig. 2) demonstrate that neither LuxR nor autoinducer alone affect transcription from pGS107(R⁻) since the addition of autoinducer or IPTG to a culture resulted in the same level of luminescence as that of a culture to which no additions were made. However, when IPTG and autoinducer were both added to a culture, an increase in luminescence was observed (Fig. 2). The dual requirement for both IPTG and autoinducer to increase luciferase synthesis from pGS107(R⁻) demonstrated that LuxR was able to activate transcription of operon$_L$ in an autoinducer-dependent fashion. The absence of stimulation in the presence of autoinducer alone demonstrated that the levels of LuxR produced from pPD749 in the absence of IPTG must be very low indeed.

TABLE 1. Luciferase activity in vivo from *E. coli* LE392 carrying operon$_L$ reporter plasmids harboring intact *luxR*

Operon$_L$ reporter plasmid	*lux* operator genotype	Light units/ml[a]	
		− Autoinducer	+ Autoinducer
pGS545	Wild type	9	22.5
pGS560	Operator deleted	4.5	5.5
pGS138	Wild type	6	23
pGS132	Position 3 C to T	6	7

[a] Values given represent the peak light emitted from 1 ml of culture at an OD_{600} of 2.0. Autoinducer was added at an OD_{600} of 0.2 to a final concentration of 1.5 μM where indicated.

Autoinducer-dependent stimulation of operon$_L$ transcription by LuxR requires the *lux* operator. The plasmid pPD749 directs the synthesis of relatively high levels of LuxR protein in the presence of IPTG since the *luxR* gene is transcribed from the strong *tac* promoter and it is a high-copy-number plasmid. The plasmids pGS545 and pGS138 were constructed to determine if the observed autoinducer-dependent stimulation of operon$_L$ transcription could be duplicated by levels of LuxR produced from a controlled-copy-number plasmid and from the natural operon$_L$ promoter. Both pGS545 and pGS138 contain the identical *V. fischeri* DNA insert harboring the entire *luxR* gene and regulatory region (Fig. 1). The results (Table 1) demonstrate that the addition of autoinducer to cultures of *E. coli* LE392 carrying pGS545 or pGS138 results in the stimulation of operon$_L$ transcription. These observations were consistent with the ability of LuxR to stimulate operon$_L$ transcription when supplied in *trans* to the pGS107(R⁻) reporter vector (Fig. 2).

The autoinducer-dependent stimulation of operon$_R$ transcription requires participation of the *lux* operator located in the control region (4). One observation that led to this conclusion was that a change of position 3 of the *lux* operator sequence from C to T almost completely removed the stimulatory response. To determine if the *lux* operator was likewise required to observe the stimulatory response of operon$_L$ transcription, two plasmids were constructed which either deleted the *lux* operator altogether (pGS560) or changed the *lux* operator at position 3 from C to T (pGS132). Addition of autoinducer to cultures of *E. coli* carrying pGS560 resulted in no significant increase in luminescence compared with the 2.5-fold increase exhibited by the pGS545 plasmid which contains a wild-type operator (Table 1). Cultures carrying pGS132 exhibited only a very slight increase in luminescence in response to autoinducer instead of the almost fourfold stimulation exhibited by the pGS138 plasmid with an intact operator (Table 1). These results suggest that the ability of the LuxR-autoinducer to stimulate operon$_L$ transcription requires an intact *lux* operator.

Autoinducer-dependent repression of operon$_L$ transcription (LuxR-negative autoregulation) requires DNA sequences in operon$_R$. Early in the investigations of the mechanism of control of the *lux* regulon, we made the decision to employ vectors lacking a functional *luxI* gene so that levels of autoinducer could be controlled through our addition rather than by the *lux* system. By using constructs with a truncated

TABLE 2. Luciferase activity in vivo from *E. coli* LE392 (pPD749) carrying operon$_L$ reporter plasmids harboring *luxI* or *luxC* nonsense mutations

Operon$_L$ reporter plasmid	Relevant *V. fischeri* *lux* genotype	Light units/ml[a]		
		− IPTG, − autoinducer	+ IPTG, − autoinducer	+ IPTG, + autoinducer
pGS111	*luxI*$^+$ *luxC*$^+$	9	4	1
pGS112	*luxI*$^+$ *luxC*	8	6.5	1.5
pGS113	*luxI* *luxC*$^+$	8	8	2.5

[a] Values given represent the peak light emitted from 1 ml of culture at an OD_{600} of 1.2. Autoinducer and IPTG were added at an OD_{600} of 0.3 to final concentrations of 2.5 μM and 1 mM, respectively, where indicated.

and therefore inactive *luxI* gene, we discovered that LuxR, produced either from the *tac* promoter (pPD749) or from the natural operon$_L$ promoter located on the reporter vector itself, stimulated transcription of operon$_L$ in an autoinducer-dependent fashion (Fig. 2 and Table 1). This was contrary to the results reported by other investigators who used a reporter vector consisting of a *luxR*::MudI(*lac*) transcriptional fusion and an intact operon$_R$ (6, 7). These investigators reported an autoinducer-dependent negative regulation of LuxR production. The plasmid pGS111 was constructed to determine if the lack of negative regulation in our system was due to deletion of *V. fischeri* DNA in operon$_R$. This plasmid contains *V. fischeri* DNA spanning all of *luxR*, the control region, *luxI*, *luxC*, and a portion of *luxD* (Fig. 1). This plasmid therefore contains intact *luxR*, *luxI*, and *luxC* genes. The addition of IPTG to cultures of *E. coli* transformed with pPD749 and pGS111 resulted in a decrease in luminescence when compared with a control culture (Table 2). The addition of both IPTG and synthetic autoinducer to an identical culture resulted in an even further reduction in the level of luminescence (Table 2). The decrease in luminescence observed in the presence of IPTG alone was presumably due to production in vivo of autoinducer by the LuxI protein encoded by the intact *luxI* gene on the pGS111 plasmid. The results of the pGS111 experiment presented in Table 2 were similar to the reports of LuxR-negative autoregulation by other investigators using the *luxR*::MudI(*lac*) operon$_L$ reporter vector (6).

To distinguish whether the protein products of the *luxI* or *luxC* genes or DNA sequences in operon$_R$ were responsible for the observed repression, nonsense codons were introduced into *luxI* and *luxC* separately by site-directed mutagenesis and cloned into the pGS111 reporter plasmid. Synthesis of the LuxC polypeptide was interrupted by changing the CAA (glutamine) codon at position 68 of *luxC* to a TAA nonsense codon by site-directed mutagenesis to yield the plasmid pGS112. Cultures of *E. coli* LE392 transformed with pPD749 and pGS112 were indistinguishable from cultures transformed with the wild-type plasmid (pGS111) (Table 2). Addition of IPTG to a culture resulted in a decrease in luminescence produced from pGS112, and addition of both IPTG and autoinducer further decreased the amount of luminescence produced (Table 2). These results showed that the LuxC polypeptide was not required to observe the repression of operon$_L$ transcription by LuxR and autoinducer.

Production of the LuxI polypeptide was prevented by changing the TAT (tyrosine) codon at position 26 to a TAA nonsense codon by site-directed mutagenesis, yielding the plasmid pGS113. This mutation was expected to eliminate production of autoinducer in vivo. Addition of IPTG to a culture of *E. coli* transformed with pGS113 and pPD749 was without effect. However, when both IPTG and synthetic autoinducer were added to a culture, a decrease in luminescence was observed (Table 2). These results demonstrated that the production of autoinducer was not possible from the pGS113 plasmid since addition of IPTG alone failed to elicit a negative response. The fact that the negative effect was not observed in the presence of IPTG alone, but required the addition of both IPTG and autoinducer, demonstrated that transcription of operon$_L$ was repressed by LuxR in an autoinducer-dependent fashion. These results confirm the report of autoinducer dependence of negative regulation of operon$_L$ transcription by Dunlap and Ray (7). These results also demonstrated that the LuxI polypeptide was not required for repression of operon$_L$ transcription.

The demonstration that neither the LuxI nor the LuxC polypeptides were required for repression of operon$_L$ transcription suggested that DNA sequences located in operon$_R$ were required in addition to LuxR and autoinducer. In the absence of these sequences, LuxR and autoinducer stimulate transcription of operon$_L$ through interaction with the *lux* operator (Table 1 and Fig. 2).

Low levels of autoinducer stimulate operon$_L$ transcription in the presence of negative-acting operon$_R$ sequences. The plasmid pGS113 constituted a good operon$_L$ reporter vector with which to perform a titration experiment using synthetic autoinducer, since production of autoinducer in vivo by LuxI was eliminated through introduction of the nonsense codon in *luxI*. Thus, the effect of a variety of autoinducer concentrations on operon$_L$ transcription could be determined in the presence of the negative-acting operon$_R$ sequences. IPTG and various concentrations of synthetic autoinducer were added to cultures of *E. coli* LE392 transformed with pGS113 and pPD749. Luminescence in vivo was monitored and plotted as a function of OD_{600} (Fig. 3A). The addition of IPTG and 250 nM autoinducer to a culture resulted in a decrease in the amount of luminescence produced from pGS113 compared with a culture to which no additions were made. Increasing the concentration of autoinducer to 2.5 μM resulted in a further decrease in luminescence in the presence of IPTG. However, when IPTG and a low concentration of autoinducer (25 nM) were added to a culture, the level of luminescence increased relative to the control culture. In the presence of a high level of LuxR, low autoinducer levels caused an increase in transcription from operon$_L$, while at higher concentrations of autoinducer, repression of operon$_L$ transcription was observed. To determine which of the autoinducer responses would be observed with the negative-acting sequences present and with LuxR protein produced from its native promoter, autoinducer was added to cultures of *E. coli* containing pGS111. Addition of 1.0 μM autoinducer to a culture resulted in an increase in luminescence compared with a culture to which no autoinducer was added (Fig. 3B). This level of autoinducer caused repression of *luxR* transcription when higher levels of LuxR protein were produced from a separate plasmid (Fig. 3A). This result demonstrated that inactivation of the *luxI* gene is not required to observe stimulation of *luxR* transcription and that positive autoregulation of *luxR* transcription is a biologically relevant component of *luxR* regulation even when the negative-acting sequences are present.

The *lux* operator is required to observe the autoinducer-dependent repression of operon$_L$ transcription by LuxR. The *lux* operator located in the control region is required for the autoinducer-dependent stimulation of both operon$_R$ and op-

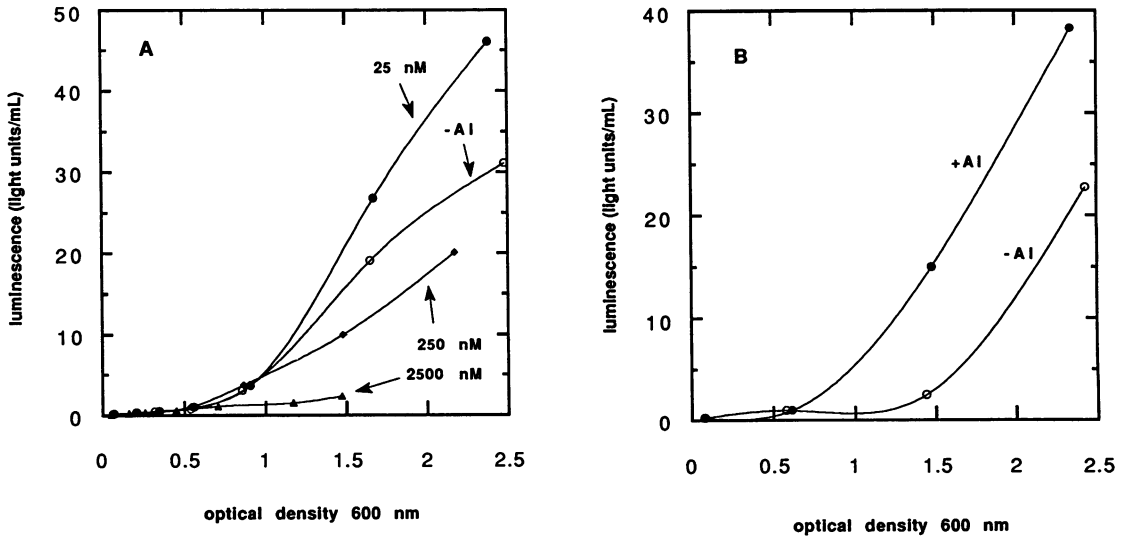

FIG. 3. (A) Effect of concentration of autoinducer on expression of luminescence in *E. coli* LE392 transformed with pGS113 and pPD749. IPTG was added to all cultures to a final concentration of 1 mM. The final concentration of autoinducer in each culture is indicated on the figure. −AI, No autoinducer. All additions were made at an OD_{600} of 0.2. (B) Autoinducer response of *E. coli* TB1 transformed with pGS111. Autoinducer was present at a concentration of 1,000 nM from the beginning of the experiment.

eron$_L$ transcription (4) (Table 1). To determine if it was likewise required for the observed repression of operon$_L$ transcription, the plasmids pGS122 and pGS129 were constructed. Both of these plasmids contain the negative-acting operon$_R$ sequences, and both have altered operators. The central 12 bp had been removed from the operator in the construction of pGS122, and position 3 of the operator had been changed from C to T in the construction of pGS129. Both operator mutations have been described (4). Both the pGS122 and pGS129 reporter plasmids were transformed separately into *E. coli* LE392 along with the plasmid pPD749. The addition of both IPTG and autoinducer to cultures of the above strains was without significant effect (Table 3), demonstrating that the *lux* operator was required for the autoinducer-dependent repression of operon$_L$ transcription.

DISCUSSION

The results of this study have elucidated several new regulatory features involved in the control of the *lux* regulon from *V. fischeri*. At low levels of autoinducer, interaction of the LuxR-autoinducer complex with the operator stimulated transcription of operon$_L$. However, at higher levels of autoinducer, interaction of the LuxR-autoinducer complex with the operator caused repression of operon$_L$ transcription. Thus it was concluded that LuxR is capable of both positive and negative transcriptional autoregulation. The switch from stimulation to repression appeared to be mediated both by the level of LuxR in the cell and by the amount of autoinducer supplied to the system. The autoinducer-dependent repression of operon$_L$ transcription required both the *lux* operator located in the control region and DNA sequences in operon$_R$ residing upstream of the promoter for operon$_L$. In the absence of these negative-acting sequences, interaction of the LuxR-autoinducer complex with the *lux* operator stimulated operon$_L$ transcription regardless of the amount of autoinducer added to the growth medium. These results demonstrate the central role of the *lux* operator in controlling the *lux* regulon since the stimulation of operon$_R$ transcription and both stimulation and repression of operon$_L$ transcription are mediated by LuxR binding this sequence. In addition, a unique regulatory response was discovered in which the LuxR protein is capable of bidirectional stimulation of transcription of the two oppositely oriented *lux* operons through interaction with a single DNA binding site (Fig. 4A).

There are several possible mechanisms for bidirectional stimulation of transcription of the *lux* operons. Stimulation of both operons occurs as a result of LuxR interacting with the *lux* operator. LuxR bound to the operator may act as a transcriptional activator in the strict sense in that it contacts RNA polymerase directly and facilitates open complex formation at the promoter. Another possibility is that LuxR bound to the operator may relieve an overlapping LuxR-independent repression mechanism. We have demonstrated by deletion analysis that sequences present in the *luxR* coding region repress transcription of both *lux* operons in a manner not requiring LuxR (1, 21). Therefore, stimulation of transcription by LuxR may involve two components, relief of the observed repression and subsequent transcriptional activation. Finally, LuxR-dependent stimulation of operon$_L$ transcription may involve CAP, whereby LuxR could act in

TABLE 3. Luciferase activity in vivo from *E. coli* LE392 (pPD749) carrying operon$_L$ reporter plasmids harboring *lux* operator mutations

Operon$_L$ reporter plasmid	Operator mutation	Light units/ml[a]	
		− IPTG, − autoinducer	+ IPTG, + autoinducer
pGS122	Deletion of central 12 bp	6	5.5
pGS129	Position 3 C to T	3.5	4.5

[a] Values given represent the peak light emitted from 1 ml of culture at an OD_{600} of 1.5. Autoinducer and IPTG were added at an OD_{600} of 0.3 to final concentrations of 0.5 μM and 1 mM, respectively, where indicated.

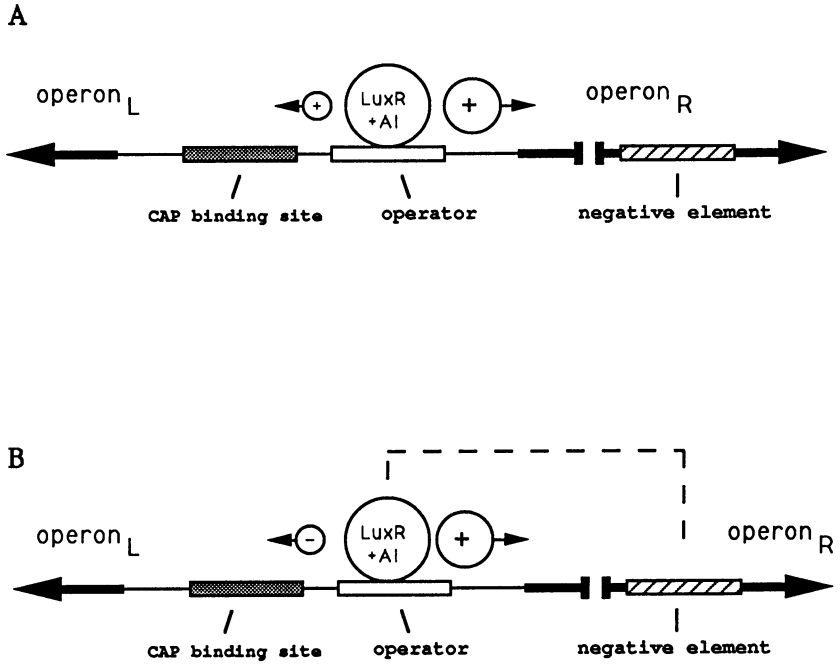

FIG. 4. Proposed interactions involved in the LuxR-autoinducer-dependent regulation of *V. fischeri lux* transcription. Symbols: +, stimulation; −, repression. Arrows indicate the directionality of the response (affecting either operon$_L$ [←] or operon$_R$ [→] transcription). The relative magnitude of the response is described qualitatively by the size of the circle encompassing the plus or minus sign. The CAP binding site is shown to emphasize its proximity to the *lux* operator. (A) Bidirectional stimulation of transcription resulting from the interaction of LuxR with the *lux* operator in the presence of autoinducer. (B) Repression of operon$_L$ transcription resulting from the interaction of LuxR with the *lux* operator in the presence of autoinducer and upstream operon$_R$ sequences. Dashed lines indicate a hypothetical interaction occurring between a negative element in operon$_R$ and the *lux* operator which is required for operon$_L$ repression.

some fashion to enhance the ability of CAP to activate operon$_L$ transcription.

Another question of fundamental importance that remains unanswered concerns the mechanism by which the negative-acting sequences in operon$_R$ act to achieve repression of operon$_L$ transcription. The requirement for both LuxR and autoinducer for repression of operon$_L$ transcription was reported previously (7), and our results confirm this conclusion. However operon$_L$ repression was shown in this study to require two other components, the *lux* operator and negative-acting operon$_R$ sequences. The fact that repression of operon$_L$ transcription requires both the negative-acting operon$_R$ sequences and a functional operator suggests an interaction between these two elements to establish repression (Fig. 4B). Most cases of repression at a distance involving an operator sequence near the regulated promoter have evoked a looping mechanism whereby regulatory proteins bound at two distantly separated sites are able to interact, forming a loop from the intervening DNA (for a review, see reference 15). The protein molecules bound in the looped conformation or bound at the primary operator more efficiently as a result of loop formation are then capable of negatively affecting either RNA polymerase or a function of some other regulatory protein at the promoter. We have not demonstrated the existence of a looping mechanism which functions to achieve repression of operon$_L$ transcription, but an interaction of this type between the *lux* operator and an operon$_R$ sequence mediated by LuxR is one possibility. It is tempting to think of this type of interaction as being modulated by binding of autoinducer which appears to constitute a molecular switch from an operon$_L$ stimulatory mechanism at low concentrations to an operon$_L$ repression mechanism that operates at high concentrations of autoinducer. It is interesting that all known chromosomal examples of repression at a distance in procaryotes involve operons which are regulated in some fashion by CAP (*lac*, *ara*, *gal*, *deo*, and *cdd*) (15). The *lux* system is likewise regulated by CAP at the level of operon$_L$ transcription and extends this intriguing correlation.

The cause of the repressive effect at the operon$_L$ promoter is unknown. The CAP binding site has been located in the *lux* regulatory region by DNA sequence analysis and DNase I footprinting (3, 21) and is positioned in close proximity to the *lux* operator (Fig. 4). As a result of this positioning, an interaction between LuxR, the *lux* operator, and the upstream operon$_R$ sequences may adversely affect the ability of cAMP-CAP to activate operon$_L$ transcription. Another possibility is that these interactions are directly affecting RNA polymerase function at the promoter. Regardless of the mechanism, in all reports of LuxR-negative autoregulation, repression of operon$_L$ transcription was observed only when LuxR was overproduced with the *luxR* gene supplied in *trans* to a low-copy-number reporter vector harboring an intact operon$_R$ (*luxICDABE*) (6, 7; this report). The requirement for such high levels of LuxR brings into question the significance of this response when LuxR is being produced from a single copy of the *luxR* gene on the chromosome of *V. fischeri*. Even without overproduction, with LuxR being produced from the *luxR* gene on a low-copy-number plasmid under control of the natural operon$_L$ promoter, we observed an autoinducer-dependent stimulation of operon$_L$ transcription both in the presence and absence of the negative-acting operon$_R$ sequences. These observations suggest that stimulation of operon$_L$ transcription is the physiologically signif-

icant response when LuxR is produced at lower levels from a single-copy gene on the chromosome. It is possible that the levels of LuxR required for negative autoregulation of operon$_L$ transcription are achieved only at the very high cell densities reached by cultures of *V. fischeri* in a fish light organ. Thus, under these environmental conditions, the negative regulation may be a significant factor. This point is discussed further in the context of the autoinduction response in the next section.

The mechanism of autoinduction, through which the expression of bioluminescence in *V. fischeri* is regulated in a cell-density-dependent fashion, involves a unique transcriptional positive feedback circuit mediated by the LuxR protein and a diffusible small molecule called the autoinducer. At relatively high cell densities, a rapid increase in luminescence is observed which is attributed primarily to the positive feedback induction of operon$_R$ transcription. This transcriptional positive feedback circuit results from the interaction between the LuxR protein and the *lux* operator sequence located in the regulatory region and subsequent activation of transcription by an autoinducer-dependent mechanism (4). This mechanism, like any positive feedback system, presents a regulatory problem since it potentially lacks a maximal response. This problem is apparently solved in the *lux* system through a compensatory negative regulatory circuit through which the LuxR protein limits its own synthesis. By limiting the accumulation of LuxR protein, the mediator of the positive response, this repression mechanism could inhibit continued positive feedback. Such a mechanism, however, does not constitute an off switch for operon$_R$ transcription, but rather could function to cap the system at a defined level of transcriptional activity allowing the maintenance of a constant rate of synthesis of the enzymes required for luminescence. The maintenance of high levels of luminescence at high cell densities is observed behavior for *V. fischeri* located in the light organs of fish.

The ability of relatively low amounts LuxR to stimulate transcription of its own gene would be a contributing factor affecting both the initial rate and the timing of operon$_R$ induction. By increasing operon$_R$ and operon$_L$ transcription bidirectionally, the LuxR protein coordinately increases the synthesis of both molecules required for positive feedback activation of operon$_R$ transcription. This would allow the *lux* system to respond more quickly to lower concentrations of autoinducer than would be possible in the absence of stimulation of transcription of *luxR*, allowing induction of luminescence at a lower cell density and under conditions of low levels of cAMP.

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