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Control of the *lux* Regulon of *Vibrio fischeri*

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Regulation of expression of bioluminescence from the *Vibrio fischeri lux* regulon in *Escherichia coli* is a consequence of a unique form of positive feedback superimposed on a poorly defined *cis*-acting repression mechanism. The *lux* regulon consists of two divergently transcribed operons. The leftward operon contains only a single gene, *luxR*, which encodes a transcriptional activator protein. The rightward operon contains *luxI*, which together with *luxR* and the 218 base pairs separating the two operons comprises the primary regulatory circuit, and the five structural genes, *luxC*, *luxD*, *luxA*, *luxB* and *luxE*, which are required for the bioluminescence activity. Transcription of *luxR* from P_L is stimulated by binding of the *E. coli crp* gene product to the sequence TGTGA-CAAAAATCCAA upstream of the presumed promoter. Binding of pure *E. coli* CAP protein in a cAMP-dependent reaction to the *V. fischeri lux* regulatory region has been demonstrated by *in vitro* footprinting. The *luxI* gene product is an enzyme which catalyses a condensation reaction of cytoplasmic substrates to yield the autoinducer, *N*-(3-oxo-hexanoyl) homoserine lactone. Accumulation of autoinducer, which is freely diffusible, results in formation of a complex with LuxR. The complex binds to the sequence ACCTGTAGGATCGTACAGGT upstream of P_R to stimulate transcription of the rightward operon. Increased transcription from P_R should yield increased levels of LuxI and higher levels of autoinducer which would further activate LuxR. The LuxR binding site is also a LexA binding site, as demonstrated by *in vitro* footprinting. Basal transcription from both P_L and P_R is repressed by sequences within the *luxR* coding region. Hence there appear to be at least two effects resulting from the interaction between LuxR: autoinducer and the control region DNA. One effect is to relieve the repression afforded by the sequences within *luxR* and the second is to stimulate transcription from P_R. Recent analysis of the rightward promotor by site-directed mutagenesis has suggested a different location for P_R than that which was implicated in earlier studies. Our results suggest that the -35 sequence is located at a position which overlaps the 3′ edge of the LuxR binding site by one base pair.

Keywords: Bioluminescence; *Vibrio fischeri*; positive feedback; transcriptional regulation; operator; LexA; LuxR

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INTRODUCTION

The mechanism through which luminescent marine bacteria regulate expression of bioluminescence has been a subject of inquiry for many years (Harvey, 1952). Kempner and Hanson (1968) ascribed the lag in appearance of luminescence following inoculation of a broth culture to metabolism of inhibitors in the medium. Nealson and his colleagues showed that the bacteria produce and secrete into the medium a compound responsible for induction of bioluminescence, and appropriately described this phenomenon as 'autoinduction' (Nealson *et al.*, 1970; Rosson and Nealson, 1981). Eberhard and his colleagues have isolated the autoinducer of *Vibrio fischeri* and determined its structure (Eberhard *et al.*, 1981: Fig. 1). This substance, *N*-(3-oxohexanoyl)homoserine lactone, has been synthesized and shown to function in a biological assay system (Eberhard *et al.*, 1981; Kaplan and Greenberg, 1985; Kaplan *et al.*, 1985). Investigation of the effect of synthetic autoinducer on expression of luminescence from a natural isolate of *V. fischeri* deficient in autoinducer synthesis confirmed that the autoinducer is both freely diffusible and effective at very low concentrations (Kaplan *et al.*, 1985).

The *lux* system of *V. fischeri* is under control of a complex regulatory network, the general features of which have been established due in large part to the efforts of Engebrecht and Silverman (Engebrecht *et al.*, 1983; Engebrecht and Silverman, 1984, 1986, 1987). The regulon consists of two divergently transcribed operons and at least seven structural genes (Fig. 2). The leftward operon (operon$_L$) has a single gene, *luxR*, while operon$_R$ includes the *luxICDABE* genes. The products of *luxR* and *luxI* appear to function together to stimulate transcription of operon$_R$. By having regulatory functions encoded in both operons of the regulon, both positive and negative feedback loops between the two operons are possible. Based on the work of Engebrecht and Silverman and of Greenberg and his colleagues (Dunlap and Greenberg, 1985; Dunlap and Greenberg, 1988; Engebrecht *et al.*, 1983), we have proposed a model describing the regulation of the *V. fischeri lux* system (Devine *et al.*, 1988; Fig. 3).

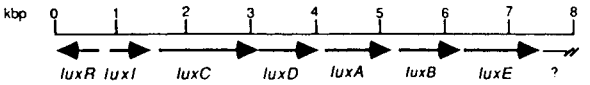

Figure 2. Physical map of the *lux* regulon from *V. fischeri*. The primary regulatory functions reside within *lux*R and *lux*I and the intergenic DNA. The *lux*CDABE region encodes enzymatic functions required for bioluminescence activity

Transcription of operon$_R$ is stimulated by binding of the positive regulatory protein: autoinducer complex to the operator region. Autoinducer is produced from cytoplasmic precursors by the action of the LuxI protein, the product of the first gene of operon$_R$. Therefore, when autoinducer accumulates to a level that allows even weak transcription (or transcription in only a few cells of the population) of operon$_R$, the first protein produced causes an increased production of autoinducer, which in turn stimulates even greater transcription of operon$_R$. Transcription of operon$_L$ is stimulated by cAMP-CAP and the operon$_L$ itself appears to be under control of a complex autoregulatory process. In addition to these complex autoregulatory loops and pleiotropic effectors, the *lux* system appears to be modulated by O_2 levels and possibly by *htpR* (σ^{32}) (Ulitzer and Kuhn, 1988). All of the regulatory processes discussed here have been proposed on the basis of genetic and physiological data; no molecular details are known, except for the nucleotide sequences (Devine *et al.*, 1988; Engebrecht and Silverman, 1987).

The purpose of this paper is to bring together information from several laboratories and to describe our recent efforts to understand the molecu-

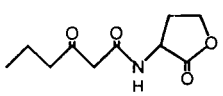

Figure 1. *Vibrio fischeri* autoinducer (Eberhard *et al.*, 1981)

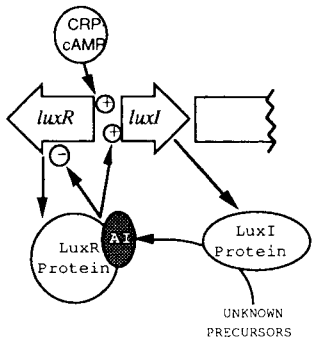

Figure 3. Model of the primary regulatory circuit of the *lux* regulon of *V. fischeri* (Devine *et al.*, 1988)

lar basis of regulation of the *lux* system. Based on our published nucleotide sequence of the *lux* regulatory region, we proposed several protein binding sites. We will present data here to confirm these earlier suggestions, and we will discuss the structure of the promoter of the rightward operon with respect to the function of the LuxR: autoinducer complex.

MATERIALS AND METHODS

Chemicals and enzymes

Restriction enzymes were purchased from New England Biolabs, Promega, or Boehringer Mannheim Biochemicals. Sequenase enzyme was obtained from United States Biochemicals. Radionucleotides were purchased from New England Nuclear. DNase I and cAMP were obtained from Sigma. All other reagents were of the highest quality available. Oligonucleotides were custom synthesized on an Applied Biosystems model 380B DNA synthesizer. *E. coli* CAP (cAMP activator protein) protein was a generous gift of Dr Michael Fried (University of Texas Health Science Center, San Antonio) and was provided as a 0.161 mg/ml solution which was estimated to be 3.7 μ mol/l dimer. *E. coli* LexA protein was kindly provided by Dr John Little (University of Arizona) and was supplied as a 2·6 mg/ml solution estimated to be 52 μ mol/l dimer.

Site-directed mutagenesis

The site-directed mutagenesis procedure of Kunkel (Kunkel *et al.*, 1987) was used with slight modifications. Uracil-containing single-stranded template DNA was purified from phagemid-infected cells.

Footprinting *in vitro*

Plasmid containing the control region from the *V. fischeri lux* regulon (8 μg of pGSS1) was digested at a unique SalI restriction site. The 5′-phosphate was removed by treatment with calf intestinal phosphatase and the 5′-end was subsequently radiolabelled using γ-P^{32} ATP and T4 polynucleotide kinase. The radiolabelled DNA was then digested with *Hind*III which yielded three restriction fragments of 2.9 kbp, 250 bp, and 20 bp. The 250 bp fragment contained the control region and was labelled at its 5′-end; the 20 bp fragment was also end-labelled. The 2.9 kbp fragment remained unlabelled and served as a source of competitor DNA. The sample was then precipitated and resuspended in 60 μl of TE (pH 8.0) to yield a stock of labelled fragment estimated to be 60 nmol/l in each fragment.

All DNaseI footprinting reactions (Galas and Schmitz, 1978) were done in a total volume of 25 μl containing 7 nmol/l of the stock DNA in transcription buffer (100 mmol/l KCl, 30 mmol/l TrisCl pH 7.5, 1 mmol/l EDTA, and 1 mmol/l DTT). DNase I was diluted from a 1.0 mg/ml stock solution to a final concentration of 40 μg/ml in each reaction after preincubation of the DNA and protein components at 37 °C for 10 minutes. The samples were allowed to incubate for an additional 5 minutes after which 25 μl of a stop solution (0.15 mol/l sodium acetate, pH 5.2, 0.75 mg/ml tRNA, 12 mmol/l EDTA) were added. The samples were precipitated with 100 μl of ethanol and the pellets were resuspended in tracking dye and applied to an 8% sequencing gel. The protected sequence was determined by sequencing the plasmid pGSS1 with a primer, the 5′-end of which corresponded to the labelled 5′-end of the SalI site and running the sequencing reactions on the same gel with the footprinting reactions. Footprinting reactions contained CAP at concentrations between 370 nmol/l and 740 nmol/l and cAMP concentrations from 1 to 2 mmol/l. Footprinting reactions contained LexA protein at concentrations between 4 and 8 μmol/l. The amount of LexA protein used in these experiments was significantly above the expected equilibrium dissociation constant. Titration experiments to determine the precise equilibrium dissociation constant of the LexA:DNA complex have not been done.

RESULTS AND DISCUSSION

We have completely determined the nucleotide sequence of the *lux* regulon of *V. fischeri* ATCC 7744 (Baldwin *et al.*, 1989). The nucleotide sequence confirmed the physical map presented in Fig. 2. The portion of the *lux* regulon that resides between the two operons, the regulatory region, appears to contain the two promoters, P_L and P_R, and binding sites for regulatory proteins. The sequence of this region is presented in Fig. 4.

```
5'   AGT  GTC  GTC  GGC  ATT  TAT  GTC  TTT  CAT  ACCCATC TCTTTATCCT TACCTATTGT
3'   TCA  CAG  CAG  CCG  TAA  ATA  CAG  AAA  GTA  TGGGTAG AGAAATAGGA ATGGATAACA
     Thr  Asp  Asp  Ala  Asn  Ile  Asp  Lys  Met
                                          ←luxR

TTGTCGCAAG TTTTGCGTGT TATATATCAT TAAAACGGTA ATAGATTGAC ATTTGATTCT AATAAATTGG
AACAGCGTTC AAAACGCACA ATATATAGTA ATTTTGCCAT TATCTAACTG TAAACTAAGA TTATTTAACC

    • cap binding site                                          lux Operator

ATTTTTGTCA CACTATTATA TCGCTTGAAA TACAATTGTT TAACATAAGT ACCTGTAGGA(x) TCGTACAGGT
TAAAAACAGT GTGATAATAT AGCGAACTTT ATGTTAACAA ATTGTATTCA TGGACATCCT(x) AGCATGTCCA

                                                        luxICDABE→
                                                          Met  Thr  Ile  Lys
TTACGCAAGA AAATGGTTTG TTATAGTCGA TTAAACGCAA  GGGAGGTTGG T ATG  ACT  ATC  AAA-3'
AATGCGTTCT TTTACCAAAC AATATCAGCT AATTTGCGTT  CCCTCCAACC A TAC  TGA  TAG  TTT-5'
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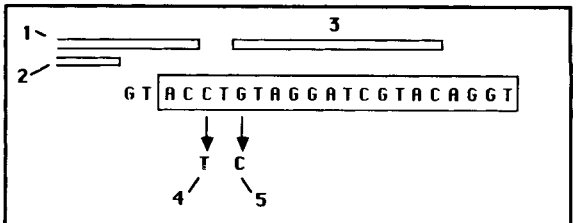
Figure 4. Sequence of the intergenic region between *lux*R and *lux*I of *V. fischeri* ATCC 7744. The CAP binding site and the *lux* operator region are indicated

Identification of the *lux* operator and discovery of a *cis*-acting repression mechanism

Analysis of the nucleotide sequence of the intergenic region which separates the two operons in the *V. fischeri* ATCC 7744 *lux* regulon revealed a 20 base pair palindromic sequence which was proposed to be the binding site for the LuxR:autoinducer complex (Devine *et al.*, 1988). In order to determine directly the location of the *lux* operator, a series of deletion studies were done (Devine *et al.*, 1989; see Table 1). Deletion of sequences 5′ of the proposed operator (from the 3′ end of *luxR*) did not abolish the autoinducer-dependent stimulation of rightward transcription when *luxR* was supplied *in trans*. However, a deletion which extended to the 5′ AC of the palindrome (see Fig. 4) did result in a decrease in the autoinducer-dependent response. These observations strongly supported the proposal that the palindrome was the LuxR:autoinducer binding site. To confirm our proposal, three operator variants were constructed by site-directed mutagenesis. The plasmids containing these variants retained the sequences from 5′ of the operator to just beyond the 3′ end of *luxR*. Two of the variants were point mutations, one C to T at position 3 in the palindrome and the other G to C at position 5 of the palindrome. The change at position 3 resulted in a loss of measurable response to autoinducer while the change at position 5 responded only weakly to autoinducer. The third change was a deletion of the central 12 base pairs of the operator which resulted in a complete loss of autoinducer-dependent stimulation of rightward transcription. The basal levels of transcription, monitored from the *luxAB* transcription reporter, of these deletions and point mutations are summarized in Table 1.

While analysing the operator-variant constructions, an effect on the basal level of transcription (without autoinducer) was observed. As shown in

Table 1. Effect of deletions and operator point mutation on transcription from P_R. The various constructions are described graphically in the top panel. The activity of P_R from these constructions was monitored by measuring the activity of luciferase encoded by the *V. harveyi lux*AB genes that were inserted downstream of P_R. Cell growth was at 28 °C. Optical density and bioluminescence were monitored throughout growth. For comparison in the table, the relative luminescence levels at O.D. 1 are presented



Construction	Basal Level of Transcription from P_R
Intact Control	2
Variant 1	20
Variant 2	20
Variant 3	2
Variant 4	2
Variant 5	2

Table 1, deletion of sequences extending from the 5′ edge of the operator to the 3′ end of the *luxR* gene result in a 10-fold increase in the basal level of rightward transcription. Hence there appears to be a *cis*-acting mechanism which is capable of repressing basal transcription of the rightward operon. It is apparent that the operator sequence is not directly involved in this repression mechanism since deletion of the central 12 base pairs of the operator as well as the introduction of the other operator variants discussed above had no effect on the basal level of transcription (Table 1). This repression also appears not to be mediated by the LuxR protein, since inactivation of LuxR expression, either through small deletions at the 3′ or 5′ end of the gene or by incorporation of a nonsense mutation, does not result in an increased basal level of transcription (Shadel, Devine and Baldwin, unpublished observations). When similar deletions in *luxR* were made but transcription was monitored from the leftward promotor, a similar effect on the level of transcription was observed (Baldwin *et al.*, 1989). Thus it appears that sequences exist within *luxR* which are responsible for maintaining a low basal level of transcription from both the rightward and leftward promoters. It remains unclear whether or not this repression mechanism is mediated through a protein-DNA interaction.

Characterization of protein binding sites by footprinting *in vitro*

Two features which became apparent upon inspection of the nucleotide sequence of the control region of the *V. fischeri* ATCC 7744 *lux* regulon were a sequence resembling a consensus *E. coli* CAP (cAMP activator protein) binding site located about midway between the start codons for the *luxR* and *luxI* genes and upstream of the proposed leftward promoter, and a twenty base pair palindrome located upstream of the proposed rightward promoter. Several experiments *in vivo* have shown an influence of the cAMP-CAP global regulatory system on transcription of the leftward operon (Dunlap and Greenberg, 1985, 1988; Friedrich and Greenberg, 1983; Nealson *et al.*, 1970, 1972) and our own experiments have demonstrated that the 20 base pair palindrome is the LuxR: autoinducer binding site as discussed above. The *lux* operator sequence was also found to resemble a consensus *E. coli* LexA protein binding site (Ulitzer and Kuhn, 1988). These experiments suggest a possible role for LexA in controlling the timing of induction of the rightward operon. In order to demonstrate directly the interaction of these *E. coli* proteins with the implicated sequence elements within the *lux* DNA, footprinting experiments were done *in vitro*.

Purified *E. coli* CAP protein was found to protect the *lux* DNA from DNase I digestion in a cAMP-dependent fashion (Fig. 5). Although some protection was observed in the absence of cAMP, sequence-specific protection was enhanced by addition of cAMP to the reaction. The region protected in the cAMP/CAP containing reaction (Fig. 5, lane 2 and 3) encompassed the sequence, TGTGA-CAAAAATCAA, identified previously as a possible CAP binding site. The precise locations of the regions of the *lux* DNA that were protected from

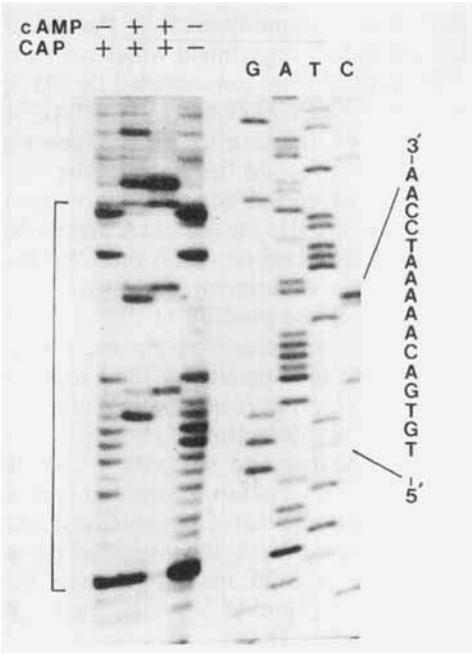


Figure 5. DNase I footprint of *E. coli* CAP protein on the *V. fischeri* control region. DNase I reactions are shown in lanes 1–4. All reactions contain 7 nmol/l labelled fragment as described in the 'Materials and Methods' with additions as follows: lane 1, 370 nmol/l CAP; lane 2, 370 nmol/l CAP and 1 mmol/l cAMP; lane 3, 740 nmol/l CAP and 2 mmol/l cAMP; lane 4, no additions. Lanes 5–8 were included to determine which sequences were protected. These lanes contain a sequencing ladder of the same strand that was end-labelled in the footprinting experiment using pGSS1 as sequencing template. Brackets indicate the boundary of the protected sequences and the sequence of the proposed CAP site on the *lux* DNA is indicated to the right of the sequencing ladder

DNase I by binding of cAMP/CAP were identified by sequencing of the DNA using a primer which had a 5′-end corresponding to the 5′-end of the labelled fragment which was used for footprinting. Use of DNase I for footprinting often results in overestimation of the DNA contact region in the protein:DNA complex, presumably because of the physical size of the nuclease. It is apparent from the results shown in Fig. 5 that sequences outside the presumed CAP binding site were protected from DNase I by binding of the CAP protein. These results show directly that the sequence identified by sequence analysis as a probable *E. coli* CAP binding site does in fact interact with *E. coli* CAP protein in a cAMP-dependent reaction. This observation supports previous results which implicated the cAMP-CAP system in affecting the expression of bioluminescence (Dunlap and Greenberg, 1988).

Binding of pure *E. coli* LexA protein to the *lux* operator region is demonstrated by the results of a DNase I protection experiment which are shown in Fig. 6. The protected region included the *lux* operator sequence ACCTGTACGATCCTACAGGT (read 5′ to 3′ on the labelled strand; see Fig. 4). Again, sequences beyond the 20 base pair operator sequence were protected, presumably overestimating the binding site size for the LexA protein for the same reasons described above for the CAP binding experiment. This experiment constitutes a direct demonstration of the binding of the *E. coli* LexA protein to the *lux* operator sequence, a sequence which is also the binding site for the LuxR:autoinducer complex (Devine *et al.*, 1989; Walker, 1984). The possibility of interaction of these two proteins at the same binding site suggests a role in the induction of the *lux* regulon *in vivo*, but such a role has yet to be demonstrated conclusively. Our results are consistent with the experiments which show an effect of SOS inducing agents on the timing of transcription of the rightward operon (Ulitzer and Kuhn, 1988).

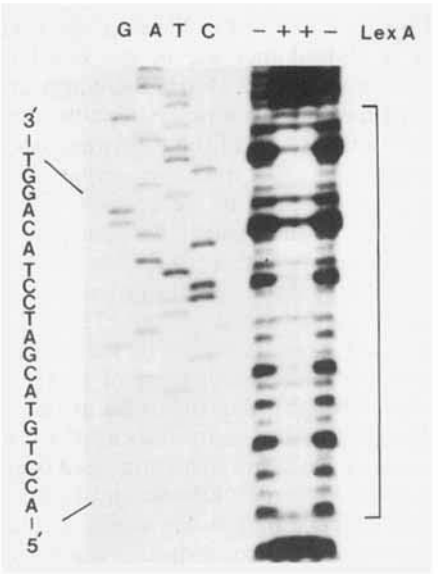


Figure 6. DNase I footprint of *E. coli* LexA protein binding to the *V. fischeri lux* operator. DNase I reactions are shown in lanes 5–8. All reactions contain 7 nmol/l labelled fragment as described in the Materials and Methods, with additions as follows: lanes 5 and 8, no additions; lanes 6, 4 μ mol/l LexA; lane 7, 8 μmol/l LexA. Lanes 1–4 are included to allow determination of the sequence which were being protected. These lanes contain a sequencing ladder of the same strand that was end-labelled in the footprinting experiment using pGSS1 as sequencing template. Brackets indicate the boundary of the protected DNA sequences and the sequence of the *lux* operator is indicated to the left of the sequencing ladder.

Location of the rightward promoter

The transcriptional start for the rightward operon of *V. fischeri* strain MJ1 has been mapped using the S1 nuclease protection assay (Engebrecht and Silverman, 1987). The promoter which was identified had a poor -10 sequence (TGTTAT) and no obvious -35 sequence. The consensus -35 and -10 hexamers are TTGACA and TATATT respectively. We have sequenced the control region of the *V. fischeri* strain ATCC 7744 and have tentatively identified a more conventional promoter element slightly downstream of the proposed MJ1 promoter. This putative promoter has a -35 sequence (TTTACG) separated by 16 base pairs from a -10 sequence (TATAGT) (Fig. 7). The published sequence of the control region of strain MJ1 differs from that of the ATCC 7744 strain at a point that overlaps what we propose to be the -35 sequence. This difference could explain why the -35 sequence was not observed in the MJ1 P_R. We have sequenced this region numerous times and are confident that our sequence is correct.

To determine whether the promoter elements we have identified function *in vivo*, several point mutations were created by site-directed mutagenesis. These mutations were designed to be either promoter 'up' or promoter 'down' mutations based on the *E. coli* consensus sequence (see Fig. 7). Both the -10 'up' and the -35 'up' mutations resulted in an

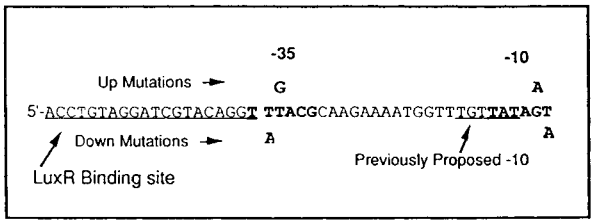


Figure 7. Design of promoter up and promoter down mutations. The LuxR binding site and the previously proposed -10 element are labelled and indicated by *underlining*. The -10 and -35 elements which we propose, and the mutations which we constructed and analysed to test our proposal, are indicated by **boldface** type

increased basal level of transcription, as expected. The characteristics of these 'up' mutations, while consistent with our predictions, do not offer firm support, since we could be creating new promoters by the mutations. The 'down' mutations offered stronger support for our proposed promoter, since a decrease in transcriptional activity would signal decreased efficiency of the promoter. The -35 'down' mutation behaved as predicted by decreasing the basal level of transcription. The -10 'down' mutation unexpectedly resulted in a slight *increase* in the basal level of transcription but the induced level of transcription was significantly decreased. The reason for the increase in transcriptional activity from a promoter that should be less active remains unclear. Even so, the results support our proposed location for the rightward promoter. Our proposal would place the 5′ base of the -35 sequence at a position which overlaps the 3′ edge of the *lux* operator by one base pair. The -35 element would then be separated by 16 base pairs from a -10 sequence that differs by one base from the consensus sequence. The promoter we describe here could initiate transcription from the general area indicated by the results of the S1 nuclease experiments, given the error intrinsic to the S1 technique.

CONCLUSION

Our analysis of the nucleotide sequence of the regulatory region of the *lux* regulon of *V. fischeri* ATCC 7744 revealed several interesting features. Perhaps the most obvious was a 20 base palindrome located within the control region separating the two divergently transcribed operons. Other features included a sequence resembling a consensus *E. coli* CAP protein binding site and a putative promoter element with a -35 sequence adjacent to and overlapping the 3′-edge of the 20 base pair palindrome by one base pair. This paper presents both a summary of earlier studies and the results of recent experiments which were designed to elucidate the role of these sequences *in vivo* and to demonstrate the direct interaction of *E. coli* proteins with these elements *in vitro*.

We have demonstrated through deletion analysis and site-directed mutagenesis that the 20 base pair palindrome is the binding site for the LuxR: autoinducer complex and is required for the autoinducer-dependent stimulation of transcription of the rightward operon (Devine *et al.*, 1989). This operator sequence was also observed to be a consensus *E. coli* LexA protein binding site and the binding of purified *E. coli* LexA protein to this operator sequence was demonstrated *in vitro* by DNase I footprinting (Fig. 6). During the identification of the operator sequence it was noticed that deletion of sequences upstream of the operator extending past the 3′-end of the *luxR* gene resulted in an increase in the basal level of rightward transcription. Another set of deletions within *luxR* were shown to have a similar effect on the transcription of the leftward operon indicating the existence of a *cis*-acting repression mechanism whereby sequences in the *luxR* gene maintain a low basal level of transcription of both operons. The influence of the cAMP-CAP global regulatory system of *E. coli* on the transcription of the *luxR* gene has been demonstrated *in vivo* (Dunlap and Greenberg, 1988). The results of DNase I footprinting *in vitro* presented in this paper demonstrate the direct interaction of purified *E. coli* CAP protein in a cAMP- dependent fashion with the CAP site identified in the *lux* DNA sequence, further emphasizing the role of cAMP-CAP in the regulation of bioluminescence. Analysis of promoter mutations which altered the level of transcription in a predictable fashion have implicated a different location for the rightward promoter than was postulated by other investigations (Engebrecht and Silverman, 1987).

Acknowledgements

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REFERENCES

Baldwin, T. O., Devine, J. H., Heckel, R. C., Lin, J.-W. and Shadel, G. S. (1989). The complete nucleotide sequence of the *lux* regulon of *Vibrio fischeri* and the *lux*ABN region of *Photobacterium leiognathi* and the mechanism of control of bacterial bioluminescence. *J. Biolumin. Chemilumin.*, **4**, 326–341

Devine, J. D., Countryman, C. and Baldwin, T. O. (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.

Devine, J. D., Shadel, G. S. and Baldwin, T. O. (1989). Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC 7744. *Proc. Natl. Acad. Sci. USA*, **86**, 5688–5692.

Dunlap, P. V. and Greenberg, E. P. (1985). Control of *Vibrio fischeri* luminescence gene expression in *Escherichia coli* by cyclic AMP and cyclic AMP receptor protein. *J. Bacteriol.*, **164**, 45–50.

Dunlap, P. V. and Greenberg, E. P. (1988). Control of *Vibrio fischeri lux* gene transcription by a cyclic AMP receptor-LuxR protein regulatory circuit. *J. Bacteriol.*, **170**, 4040–4046.

Eberhard, A., Burlingame, A., Eberhard, C., Kenyon, G. L., Nealson, K. H. and Oppenheimer, N. J. (1981). Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry.*, **20**, 2444–2449.

Engebrecht, J. and Silverman, M. (1984). Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA*, **81**, 4154–4158.

Engebrecht, J. and Silverman, M. (1986). Regulation of expression of bacterial genes for bioluminescence. *Genetic Eng.*, **8**, 31–44.

Engebrecht, J. and Silverman, M. (1987). Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. *Nucleic Acids Res.*, **15**, 10455–10467.

Engebrecht, J., Nealson, K. and Silverman, M. (1983). Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell.*, **32**, 773–781.

Friedrich, W. F. and Greenberg, E. P. (1983). Glucose repression of luminescence and luciferase in *Vibrio fischeri*. *Arch. Microbiol.*, **134**, 87–91.

Galas, J. and Schmitz, A. (1978). DNAase footprinting: A simple method for the detection of protein-DNA binding specificity. *Nucleic Acids Res.*, **5**, 3157–3170.

Harvey, E. N. (1952) *Bioluminescence*, Academic Press, New York.

Kaplan, H. B. and Greenberg, E. P. (1985). Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.*, **163**, 1210–1214.

Kaplan, H. B., Eberhard, A., Widrig, C. and Greenberg, E. P. (1985). Synthesis of *N*-[3-oxo-(4,5-$^3H_2$)-hexanoyl] homoserine lactone: biologically active tritium-labelled *Vibrio fischeri* autoinducer. *J. Labelled Compounds Radiopharm.*, **22**, 387–395.

Kempner, E. S. and Hanson, F. E. (1968). Aspects of light production by *Photobacterium fischeri*. *J. Bacteriol.*, **95**, 975–979.

Kunkel, T. A., Roberts, J. D. and Zakour, R. A. (1987). Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.*, **154**, 1367–1382.

Nealson, K. H., Platt, T. and Hastings, J. W. (1970). The cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bacteriol.*, **104**, 313–322.

Nealson, K. H., Eberhard, A. and Hastings, J. W. (1972). Catabolite repression of bacterial bioluminescence: functional implications. *Proc. Natl. Acad. Sci. USA*, **69**, 1073–1076.

Rosson, R. A. and Nealson, K. H. (1981). Autoinduction of bacterial bioluminescence in a carbon-limited chemostat. *Arch. Microbiol.*, **129**, 299–304.

Ulitzer, S. and Kuhn, J. (1988). The transcription of Bacterial Luminescense is Regulated by Sigma 32, *J. Biolumin. Chemilumin.*, **2**, 81–93.

Walker, G. C. (1984). Mutagenic and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol. Rev.*, **48**, 60–94.