JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 7490–7493
0021-9193/92/227490-04$02.00/0
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Vol. 174, No. 22

The *luxR* Gene Product of *Vibrio harveyi* Is a Transcriptional Activator of the *lux* Promoter

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Received 29 April 1992/Accepted 3 September 1992

Expression of the *lux* operon from the marine bacterium *Vibrio harveyi* is dependent on cell density and requires an unlinked regulatory gene, *luxR*, and other cofactors for autoregulation. *Escherichia coli* transformed with the *lux* operon emits very low levels of light, and this deficiency can be partially alleviated by coexpression of *luxR* in *trans*. The *V. harveyi lux* promoter was analyzed in vivo by primer extension mapping to examine the function of *luxR*. RNA isolated from *E. coli* transformed with the *Vibrio harveyi lux* operon was shown to have a start site at 123 bp upstream of the first ATG codon of *luxC*. This is in sharp contrast to the start site found for *lux* RNA isolated from *V. harveyi*, at 26 bp upstream of the *luxC* initiation codon. However, when *E. coli* was cotransformed with both the *lux* operon and *luxR*, the start site of the *lux* mRNA shifted from −123 to −26. Furthermore, expression of the *luxR* gene caused a 350-fold increase in *lux* mRNA levels. The results suggest that LuxR of *V. harveyi* is a transcriptional activator stimulating initiation at the −26 *lux* promoter.

The generation of light from bioluminescent marine bacteria is influenced by a variety of factors (5) and is regulated by cell density-dependent autoinduction. Although genes responsible for light production have been cloned from several different species of bacteria (13), the best-characterized luminescent system is from *Vibrio fischeri*, in that all the genes required for producing a regulated luminescence phenotype have been isolated and expressed in *Escherichia coli* (7).

The light-generating reaction is catalyzed by the α and β subunits of luciferase (products of the *luxA* and *luxB* genes, respectively) and requires O_2, reduced flavin mononucleotide, and aldehyde as substrates. The aldehyde is provided by polypeptides of the fatty acid reductase complex, encoded by the *luxCD* and *luxE* genes (2). In *V. fischeri*, the genes are located within one operon in the order *luxICD ABEG*, where *luxI* is responsible for autoinducer production (8). Although the role of *luxG* in bioluminescence is uncertain (20), LuxG was found to be a member of the FNR (ferredoxin $NADP^+$ reductase) protein family (1). The autoinducer, identified as *N*-β-ketocaproyl homoserine lactone, is a freely diffusible molecule that accumulates in the growth medium as cell density increases (6, 11). It has been proposed that the autoinducer establishes a positive feedback loop by binding to a receptor protein and stimulating transcription of the *luxICDABEG* operon (9). The receptor protein is encoded by the *luxR* gene, which is located immediately upstream of the *lux* operon but is transcribed in the opposite direction (8). The operator for LuxR binding has been identified (4), although the mechanism of transcriptional activation has not yet been determined.

The *lux* operon of *Vibrio harveyi* has also been isolated and consists of *luxCDABEGH* (15, 21). *luxH* shares significant homology with the *htrP* or *ribB* gene of *E. coli*, whose gene product is involved with the biosynthesis of riboflavin (18). Unlike *V. fischeri*, *E. coli* expressing the *luxCDABEGH* genes of *V. harveyi* does not emit high levels of light in a cell density-dependent manner (14). It has been established that the regulatory genes analogous to *luxI* and *luxR* of *V. fischeri* are not linked to the operon (17). The autoinducer of *V. harveyi* has been isolated and identified as *N*-β-hydroxybutryl homoserine lactone (3) and, in view of its having a structure similar to that of the autoinducer of *V. fischeri*, may function in the same manner. A gene that is able to increase light production 10,000-fold in *E. coli* containing the *V. harveyi lux* operon has recently been cloned and is designated *luxR*, although there is no sequence homology to the *luxR* gene of *V. fischeri* (12, 19). This stimulation is not cell density dependent and does not require an autoinducer, yet *luxR* is required for light production in *V. harveyi*.

In order to better understand the regulation of the *V. harveyi lux* promoter in *E. coli*, primer extension mapping was performed with mRNA isolated from *E. coli* cells transformed with three different *V. harveyi lux* clones (Fig. 1). Bacterial strains, growth conditions, and primer extension mapping were as described previously (14, 21), except that a 31-mer synthetic oligomer complementary to nucleotides +4 to +33 of the *luxC* gene was used as the primer and hybridization was carried out at 45°C. Despite the variation in length of the 5′-noncoding regions, the transcriptional start sites map to the same location, at nucleotide −123, with respect to the initiation codon of the *luxC* gene (Fig. 2B). In contrast, the 5′ end of the *lux* mRNA isolated from *V. harveyi* maps to position −26 (Fig. 2A), a difference of 97 nucleotides. Although the transcriptional start site of the *V. harveyi lux* mRNA has been previously determined to be at position −28 (21), this site is likely to be more accurate because of the better resolution of the primer extension product. Several smaller transcripts are noticeable for the reactions seen in Fig. 2A and B; however, *lux* mRNA from *E. coli* recombinants was not observed to have a 5′ end at −26 (see Fig. 3, lane 3). The multiple transcripts could be due to partial RNA degradation, incomplete extension of the primer, or internal transcription initiation. Consequently, the largest primer-extended product of each reaction was used to map the 5′ end of the transcripts. There is some

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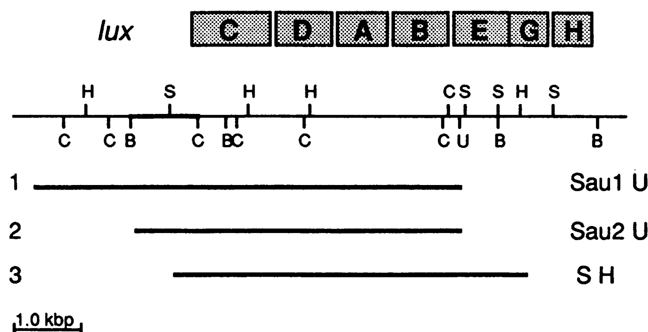

FIG. 1. Restriction map of the *V. harveyi lux* operon. Primer extension mapping was performed with RNA isolated from *E. coli* transformed with vectors 1 (pSau1U), 2 (pSau2U), and 3 (pSH) (15) containing the indicated inserts of *V. harveyi lux* DNA. Plasmids pSau1U and pSau2U are subclones of pVhSau1 and pVhSau2 (15), respectively, cut and religated at the *Pvu*II site. C, *Cla*I; H, *Hin*dIII; B, *Bam*HI; S, *Sac*I; U, *Pvu*II.

inconsistency in the levels of *lux* mRNA from the three different *E. coli* recombinants, as seen in Fig. 2B. These differences might be due to a variation of plasmid copy number or mRNA stability. Regardless, 10-fold more RNA and a 5-fold longer exposure time were required to visualize the primer-extended products of RNA from the *E. coli* recombinants in Fig. 2B compared with the RNA from *V. harveyi* in Fig. 2A. It has already been shown that the amount of *lux* mRNA increases with induction of luminescence (16, 19, 21). Accordingly, the low level of light produced by the *E. coli* recombinants can be attributed to the low abundance of *lux* mRNA.

Sequences upstream of the start sites were examined for consensus −10 (TATAAT) and −35 (TTGACA) *E. coli* promoter sequences (10) (Fig. 2C). The sequence TATAAT is located 7 bp upstream from the −123 start site, in complete agreement with the −10 hexamer with respect to position and sequence. The sequence TTACGA is located 34 bp upstream from the −123 start site, showing some homology to the −35 hexamer. The low level of transcription at this promoter in vivo may be a reflection of the imperfect −35 sequence. The −26 start site has the sequence ATT AAT 6 bp upstream from the start site, in good agreement with the −10 sequence, although no comparable −35 sequence could be located within this promoter.

Because *luxR* has been shown to stimulate luminescence in recombinant *E. coli* containing the *V. harveyi lux* operon (19), the effect of *luxR* on transcription of the *lux* promoter was tested in vivo. RNA isolated from *E. coli* transformed with both the *lux* operon (pSH) (15) and *luxR* (pMR1403) (19) in *trans* was analyzed by primer extension and was compared with RNAs from *V. harveyi* and *E. coli* containing just the *lux* operon. In Fig. 3, lane 1, the 5′ end of the *lux* RNA from *E. coli* recombinants containing both the *lux* operon and *luxR* maps to position −26, the same start site as for *V. harveyi* RNA (lane 2) and different from that seen for RNA isolated from *E. coli* containing just the *lux* operon (position −123; lane 3). Expression of *luxR* in *E. coli* is therefore responsible for the shift in transcriptional start site from −123 to −26. Moreover, it was determined by densitometry scanning that there was 350-fold more *lux* mRNA in lane 1 than in lane 3, indicating that the shift of start site by LuxR is responsible for increasing levels of *lux* mRNA in *E. coli*. We attempted to measure *lux* mRNA levels in a LuxR⁻ mutant that was generated by transposon mutagenesis in *V. harveyi* BB7 (12). However the oligomer, which is complementary to the *lux* DNA of *V. harveyi* B392, failed to hybridize to *lux* RNA from the mutant or wild-type BB7

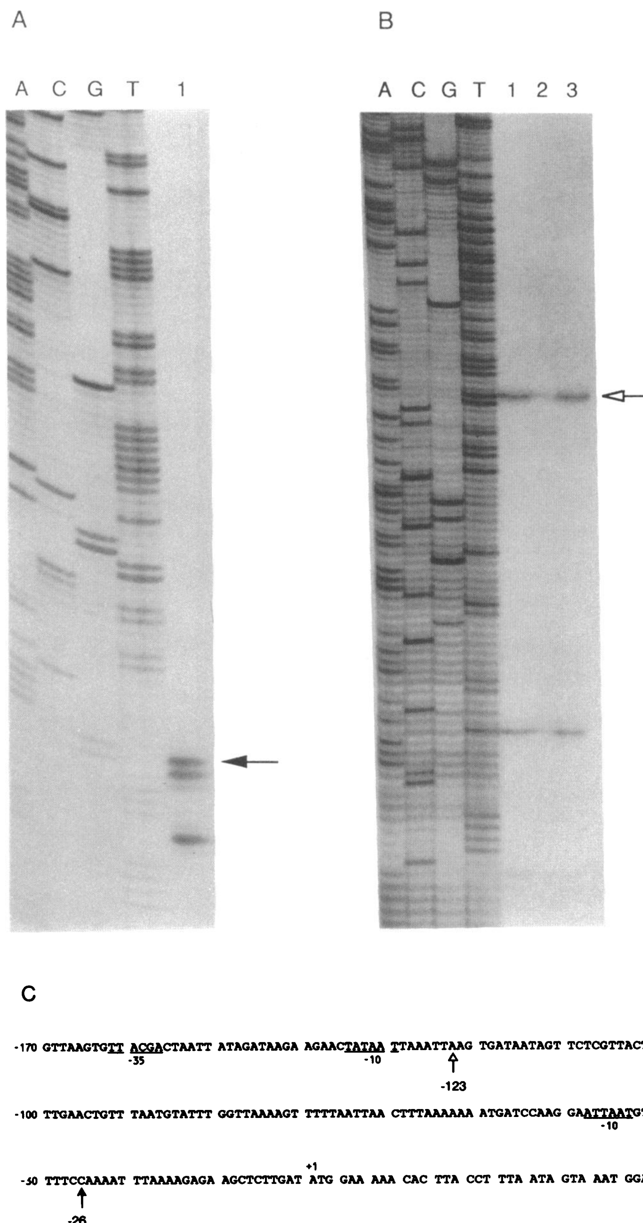

FIG. 2. Primer extension mapping of *lux* mRNA. (A) Primer extension product from 10 μg of RNA isolated from *V. harveyi* (lane 1). (B) Primer extension products from 100 μg of RNA isolated from *E. coli* transformed with pSau1U (lane 1), pSau2U (lane 2), and pSH (lane 3). The primer for both panels A and B was used to generate the sequence ladders (lanes A, C, G, and T) in order to accurately locate the 5′ end of the primer extension products. (C) Nucleotide sequence of the 5′ end of the *luxC* gene and upstream region. The first nucleotide of *luxC* is designated +1. The solid arrow indicates the 5′ end of *lux* mRNA isolated from *V. harveyi*, and the open arrow indicates the 5′ end of *lux* mRNA isolated from *E. coli* transformed with *lux* DNA. The underlined sequences correspond to the −10 and −35 promoter consensus sequences. The DNA sequence was obtained from Miyamoto et al. (14).

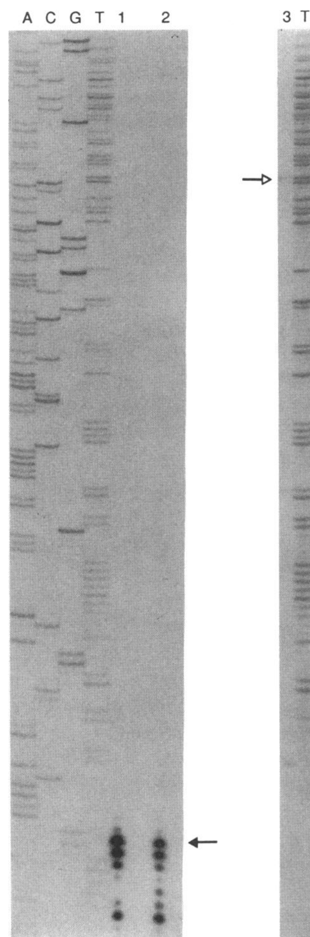

FIG. 3. Primer extension mapping of *lux* RNA with and without expression of *luxR*. Primer extension mapping was performed with 10 μg of RNA isolated from *E. coli* cotransformed with pSH and pMR1403 (18) (lane 1) 10 μg of RNA isolated from *V. harveyi* (lane 2), and 150 μg of RNA isolated from *E. coli* transformed with pSH (lane 3). The open arrow indicates the −123 start site, and the solid arrow indicates the −26 start site. Lanes A, C, G, and T contain the sequence ladder obtained by using the same primer as in the primer extension reactions, while only the T tract of the same sequence reaction is shown next to lane 3.

strain of *V. harveyi*. Since there are no sequence data for the *lux* operon of *V. harveyi* BB7, we can only speculate that there exists sufficient sequence disparity between the two strains to account for the inability of the oligomer to hybridize to the *lux* RNA from *V. harveyi* BB7. Although Northern (RNA) blot experiments might have been used to quantitate mRNA levels, in the past this technique has proven to be very inconsistent, particularly in assessing the amounts of full-length *lux* transcripts (15).

In order to determine how *luxR* functions as an activator, it was necessary to first understand why *E. coli* recombinants containing just the *lux* operon could not produce high levels of light. A comparison of 5′ ends of transcripts produced in vivo showed that the transcriptional start site used in the *E. coli* recombinant containing just the *lux* operon was at −123, almost 100 bp upstream from the start site used in *V. harveyi*. This difference is independent of the length of the 5′-untranscribed region, eliminating the possibility of missing *cis*-activating elements upstream of the *luxC* gene. Analysis of RNA isolated from *E. coli* recombinants containing both the *lux* operon and *luxR* revealed that *luxR* was essential for accurate transcription of the *V. harveyi lux* promoter. In addition to increasing levels of *lux* mRNA, the expression of *luxR* in *E. coli* shifted the transcriptional start site by almost 100 bp to the −26 promoter site seen in *V. harveyi*. The possibility that the difference in 5′ ends caused by LuxR is due to specific RNA processing seems unlikely, particularly because the deduced amino acid sequence of *luxR* was found to contain a Cro-like DNA-binding domain that is common to many prokaryotic transcription regulators (19), and no sequence homology to any RNases or RNA-binding proteins was found. There is also evidence that LuxR binds specifically to DNA sequences upstream of the *luxC* gene (unpublished data). Although the mechanism of activation remains elusive, it has now been established that LuxR of *V. harveyi* is required for transcription initiation at the −26 promoter.

This work was supported by grant MA-7672 from the Medical Research Council of Canada.

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