The Journal of Microbiology, September 2004, p.194-199 Vol. 42, No. 3

Coregulation of *lux* Genes and Riboflavin Genes in Bioluminescent Bacteria of *Photobacterium phosphoreum*

Nack-Do Sung[1] and Chan Yong Lee[2,*]

[1]*Department of Agricultural Chemistry and* [2]*Department of Biochemistry, Chungnam National University, Daejeon 305-764, Korea*

(Received April 8, 2004 / Accepted June 28, 2004)

Investigation of the expression of the riboflavin (*rib*) genes, which are found immediately downstream of *luxG* in the *lux* operon in *Photobacterium phosphoreum*, provides more information relevant to the evolution of bioluminescence, as well as to the regulation of supply of flavin substrate for bacterial bioluminescence reactions. In order to answer the question of whether or not the transcriptions of *lux* and *rib* genes are integrated, a transcriptional termination assay was performed with *P. phosphoreum* DNA, containing the possible stem-loop structures, located in the intergenic region of *luxF* and *luxE* (Ω_A), of *luxG* and *ribE* (Ω_B), and downstream of *ribA* (Ω_C). The expression of the CAT (Chloramphenicol Acetyl Transferase) reporter gene was remarkably decreased upon the insertion of the stem-loop structure (Ω_C) into the strong *lux* promoter and the reporter gene. However, the insertion of the structure (Ω_B) into the intergenic region of the *lux* and the *rib* genes caused no significant change in expression from the CAT gene. In addition, the single stranded DNA in the same region was protected by the *P. phosphoreum* mRNA from the S1 nuclease protection assay. These results suggest that *lux* genes and *rib* genes are part of the same operon in *P. phosphoreum*.

Key words: bioluminescence, *lux* operon, *Photobacterium*, riboflavin, transcription

The luminescent reaction ($FMNH_2 + O_2 +$ long chain aldehyde $\rightarrow FMN + H_2O +$ long chain fatty acid + light) in luminous marine bacteria is catalyzed by luciferase, whose two subunits are coded by two adjacent genes (*luxA* and *B* in Fig. 1) in the same operon (Hastings and Nealson, 1977; Meighen, 1988).

In *Photobacterium* species, the expression of bioluminescence occurs only at a late stage of bacterial growth, and is accompanied by an increase in the relative amount of luciferase in the bacteria. The fatty acid reductase complex coded by *luxC*, *D*, and *E* in the same operon is responsible for the aldehyde substrate in the luminescent reaction (Meighen, 1988; Meighen and Dunlap, 1993) (Fig. 1 and 2).

An additional gene, *luxF*, the specific function of which remains unknown, has been found in the *lux* operon of *Photobacterium phosphoreum* (Soly and Meighen 1988). In addition, the *luxG* gene, which codes for flavin reductase, is present in the downstream of *luxE* in *Vibrio* and *Photobacterium* (Swartzman *et al*, 1990). Strangely, the *luxL* gene, coding for the lumazine protein with the amino acid identity of riboflavin synthase, is located upstream of *luxC* in some *Photobacterium* species, and transcribed in the opposite direction to the *lux* operon (O'Kane *et al.*, 1991) (Fig. 1).

Open reading frames have been found in the downstream region of *luxG* in the *Photobacterium lux* operon (Lee and Meighen, 1992; Lee *et al.*, 1994; Lin *et al.*, 2001). These genes (*ribE*, *B*, *H*, and *A* in Fig. 1) are not only closely linked to the *lux* operon and transcribed in the same direction, but also exhibit the same organization and code for proteins which are homologous in sequence to the gene products of the second, third, and fourth genes of the *rib* operon of *Bacillus subtilis* (Lee *et al.*, 1994; Lin *et al.*, 2001).

The detection of the genes just downstream of *luxG*, corresponding in sequence to the key riboflavin synthesis genes, may be particularly relevant to the luminescence exhibited by *Photobacterium* species, as this genus produces the highest level of light intensities of any bioluminescent bacteria, with luciferase levels reaching up to 20% of soluble proteins (Wall *et al.*, 1984). As $FMNH_2$ is the substrate for the light-emitting reaction in bioluminescent bacteria, study on the riboflavin synthesis genes linked to the *lux* operon may provide important clues concerning the regulation of flavin supply for the light-emitting reaction.

It is of interest that a site capable of forming a *rho*-independent terminator does not appear to be present between *luxG* and *ribE* (Lee *et al.*, 1994). These results raise a question as to whether or not the *rib* genes are part of the same transcriptional unit as the *lux* genes. In order to

* To whom correspondence should be addressed.
(Tel) 82-42-821-5482; (Fax) 82-42-822-7548
(E-mail) cylee@cnu.ac.kr

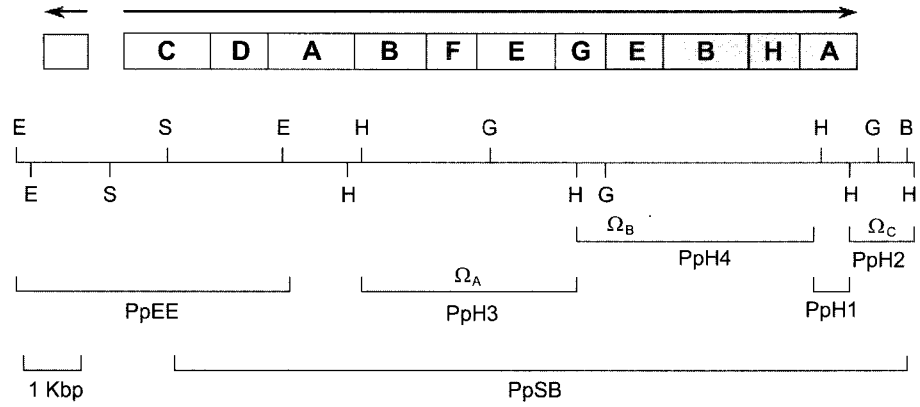

Fig. 1. Physical map of the *lux* operon of *Photobacterium phosphoreum*. The genes involved in the bioluminescence reaction are shown in the capital letter inside in the open box (*luxCDABFEG*) whereas the riboflavin genes are marked in the closed box (*ribEBHA*). The location of restriction fragment of *P. phosphoreum* for the S1 nuclease protection assay and the fragment inserts of *P. phosphoreum lux* DNA (PpH1, 2, 3, and 4) cloned into pMGM for the termination assay are also indicated. Restriction sites are labeled as follows: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hind*III; S, *Sac*I. Ω_A, Ω_B, and Ω_C denote the potent stem-loop structures shown in Fig. 3.

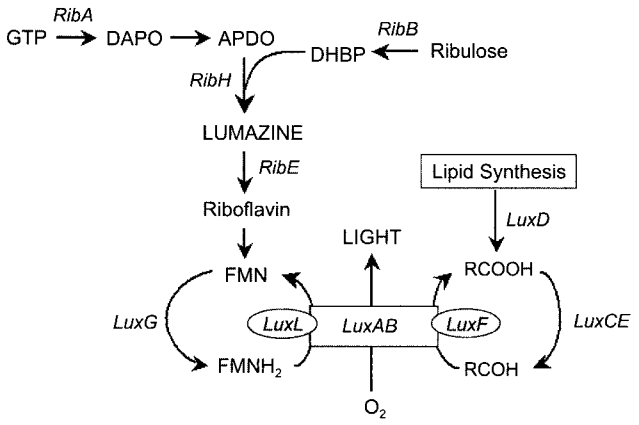

Fig. 2. Relationship between bacterial bioluminescence reaction and the genes and enzymes linked to the *lux* operon. APDO, 5-amino-6-ribitylamino-2,4-pyrimidinedione; DAPO, 2,5-diamino-6-(5'-phosphoribosylamino)-4-pyrimidineone; Cyt, Cytochrome; DHBP, 3,4-dihydroxy-2-butanone 4-phosphate; Lumazine, 6,7-dimethyl-8-ribityllumazine; Ribulose, ribulose 5'-phosphate. RCOOH, fatty acid; RCOH, fatty aldehyde; *LuxC*, *LuxD*, and *LuxE*, represent for acyl-CoA reductase, acyl transferase, and acyl protein synthetase, respectively. *luxAB*, *luxL*, *luxF*, and *luxG* code for α and β subunit of luciferases, lumazine protein, non-fluorescent flavoprotein, and flavin reductase, respectively.

answer this question, studies were carried out on the regulation of the riboflavin genes, and the data obtained from these studies are presented here.

Materials and Methods

Materials

Restriction enzymes, and T4 DNA ligase were purchased from Amersham Bioscience (Sweden). [^{14}C]-chloramphenicol (57 mCi/mmol) was obtained from Du Pont-New England Nuclear. Riboflavin, ampicillin, and kanamycin were obtained from the Sigma Chemical Company (USA). Authentic lumazine (6,7-dimethyl-8-ribityllumazine) was the gift of Professor H.C.S. Wood (University of Strathclyde). The bacterial strains used in these studies, were *Photobacterium phosphoreum* NCMB 844, *Vibrio harveyi* B392, *Escherichia coli* K38-1, and *E. coli* HB101. The plasmid of pMGM (pM), constructed from pKT230 (Miyamoto *et al.*, 1990), was used as a cloning vector for the transcriptional termination assay.

Transcriptional termination assay

The transcription termination assay was undertaken using an "*in vivo*" gene expression assay, as previously described (Miyamoto *et al.*, 1990). Four *Hind*III DNA fragments were inserted into the *Hind*III site of GH-pM, located between the *lux* promoter (GH) and the CAT gene (pM), and transferred by conjugation into *V. harveyi*. Expression of CAT activity was measured as previously described (Miyamoto *et al.*, 1990).

Cell growth and lysis

P. phosphoreum was grown at 4°C with limited aeration, in complex medium containing 5 g of Difco yeast extract, 2 ml of glycerol, 30 g of NaCl, 3.7 g of NaH_2PO_4, 1 g of KH_2PO_4, 0.5 g of $(NH_4)_2HPO_4$, and 0.1 g of $MgSO_4$ per liter. Extracts were prepared from cells grown to specific luminescence levels. The cells were harvested by centrifugation at 12,000 × g for 10 min, re-suspended in 1 mM β-mercaptoetanol (25% by volume of the original cell culture), and lysed by sonication at 4°C, to produce a uniform suspension. After the removal of cellular debris by centrifugation at 27,000 × g for 15 min, the supernatant was set to a concentration of 0.1 M in β-mercaptoethanol and 0.05 M in phosphate, pH 7.0, by the addition of 2 M β-mercaptoethanol, 1 M phosphate, pH 7.0. Phosphate buffers were prepared by mixing appropriate amounts of

K_2HPO_4 and NaH_2PO_4. Bacterial growth and luminescence *in vivo* were followed by an increase in A_{660} and in light units (LU) per ml of culture medium, respectively, where 1 LU is equivalent to 9×10^9 quanta/sec, based on the standard offset by Hastings and Weber (Hastings and Weber, 1963). One unit of absorbance at 660 nm is equivalent to approximately 5×10^8 cells/ml. A constant amount of cells was collected at different intervals (ml of culture $\times A_{660} = 20$) as growth proceeded, and was stored frozen, at -20°C.

S1 nuclease protection assay

S1 nuclease protection assay was performed by procedures described previously (Swartzman *et al.*, 1990), except that 100 μg of RNA was used in the hybridizations. A *Hind*III 3.6 kbp double-stranded DNA fragment (PpH4 in Fig. 1), as well as an *Eco*RI 4.6 kbp DNA fragment (PpEE in Fig. 1), were co-precipitated with *P. phosphoreum* mRNA, and the pellets were re-suspended in hybridization buffer (80% formamide, 40% PIPES, pH 6.6, 0.4 M NaCl, 1 mM EDTA), heated to 75°C for 10 min, then slow cooled to 50°C for 12~16 h. The hybrid mixture was then diluted to a final volume of 0.3 ml, with 30 μl of 10 × S1 nuclease buffer (0.3 M NaOAc, pH 4.6, 1 M NaCl, 10 mM $Zn(OAc)_2$, 50% glycerol) and H_2O. S1 nuclease reactions were carried out for 30 min at 37°C with the indicated amount of enzymes. Reaction products were phenol-extracted, precipitated with ethanol, re-suspended in 10 μl of 50 mM NaOH-1 mM EDTA solution, and added 2 μl of 6 × alkaline load buffer (300 mM NaOH, 6 mM EDTA, 18% Ficoll (type 400 Pharmacia), Bromophenol blue, 0.25%).

Southern transfer

The samples were applied to 1% agarose gel, and were run at 25 V for 4 h in running buffer (50 mM NaOH-1 mM EDTA). The gels were soaked in TBE solution containing ethidium bromide, after neutralization in a solution of 1 M Tris pH 7.6-1.5 M NaCl with stirring, and then transferred to 10 × SSC (0.15 M sodium citrate (pH7.5), 1.5 M NaCl) by blotting with Hybond-Nylon, at room temperature, for 16 h. Hybridization was carried out with *P. phosphoreum Sal*I-*Bam*HI DNA fragments (PpSB) labelled with [γ-^{32}P]CTP (0.1 pmol, 1.2×10^6 cpm/pmol) at 65°C overnight.

Enzyme assay

Riboflavin synthase activity was determined by the conversion of lumazine to riboflavin, by measuring the specific fluorescence of the product at 530 nm (excitation at 480 nm) using a Hitachi F3010 fluorescence spectrophotometer. Fifty μl of 6 mM lumazine substrate was added to 450 μl phosphate buffer, containing 1 mg of cell extract at 22°C and the fluorescence emission at 530 nm followed with time (Otto and Bacher, 1981). The amount of riboflavin produced was determined from a standard curve for the fluorescence of riboflavin in 50 mM phosphate buffer, at pH 7.0. The concentration of riboflavin was determined from its molecular extinction coefficient of 12,000 M^{-1} cm^{-1} at 445 nm.

Results and Discussion

Four genes immediately downstream of *luxG* in the *P. phosphoreum lux* operon (*ribEBHA* in the Fig. 1) have been sequenced, and the functions of their gene products have been identified as riboflavin synthase (*RibE*), DHBP synthase (*RibB*), lumazine synthase (*RibH*), and GTP cyclohydrolase II (*RibA*) (Lee *et al.*, 1994; Lin *et al.*, 2001) (Fig. 2). It is very important to show that the *lux* gene and key *rib* genes are integrated in the same operon, since riboflavin is the direct precursor of riboflavin 5'-monophosphate (FMN), which in reduced form is the substrate of the bacterial bioluminescence reaction.

In order to ascertain the potent transcriptional terminator for the *lux* operon, the nucleotide sequence containing the *lux* and *rib* genes was carefully checked from our previous sequence paper (Soly and Meighen 1991; Lee *et al.*, 1994). Twenty nucleotides upstream of *luxE*, a potent secondary structure element (Fig. 3A) was identified, in an intergenic non-coding DNA segment of 83 base pairs separated from *luxF* and the initiation codon of *luxE* (Soly and Meighen, 1991). The stem-loop is separated from the initiation codon of *luxE* by a 28-nucleotide segment that is very A/T rich (89%). There is a relatively large intergenic region of 80 bp between *luxE* and the gene upstream region (*luxB* or *luxF)* in *Photobacterium* and *Vibrio* species. This region contains a relatively conserved palindromic species (Meighen and Dunlap, 1993) that could allow the transcribed mRNA to form a strong hair-pin loop and also to stabilize the upstream mRNA against 3'-exonuclease digestion (Miyamoto *et al.*, 1985; 1988).

The first riboflavin gene, *ribE,* starts 168 bp after the *lux*G in *P. phosphoreum.* In the intergenic regions, it was initially reported that a sequence capable of forming a strong stem-loop structure was not present (Lee *et al.*, 1994). Careful examination revealed that a secondary structure capable of stem-loop structure, as shown in Fig. 3B, was present 40 nucleotides upstream of *ribE.* In contrast, a sequence capable of forming a strong stem-loop structure immediately preceding a T-rich region, is downstream of *ribA* (Fig. 3C), a result which is consistent with the presence of a *rho*-independent termination site in the *lux* operon in *V. harveyi* (Miyamoto *et al.*, 1985, 1988). Recently, functional analysis illustrated that the specific segments lay behind the *ribH* and *ribA* gene, respectively. The stem-loop structure located in the intergenic region between *ribH* and *ribA* functions as an mRNA stability loop or/and for sub-regulation by alternative modulation, whereas the stem-loop structure in the downstream region of *ribA* could be the transcriptional terminator of the *lux*

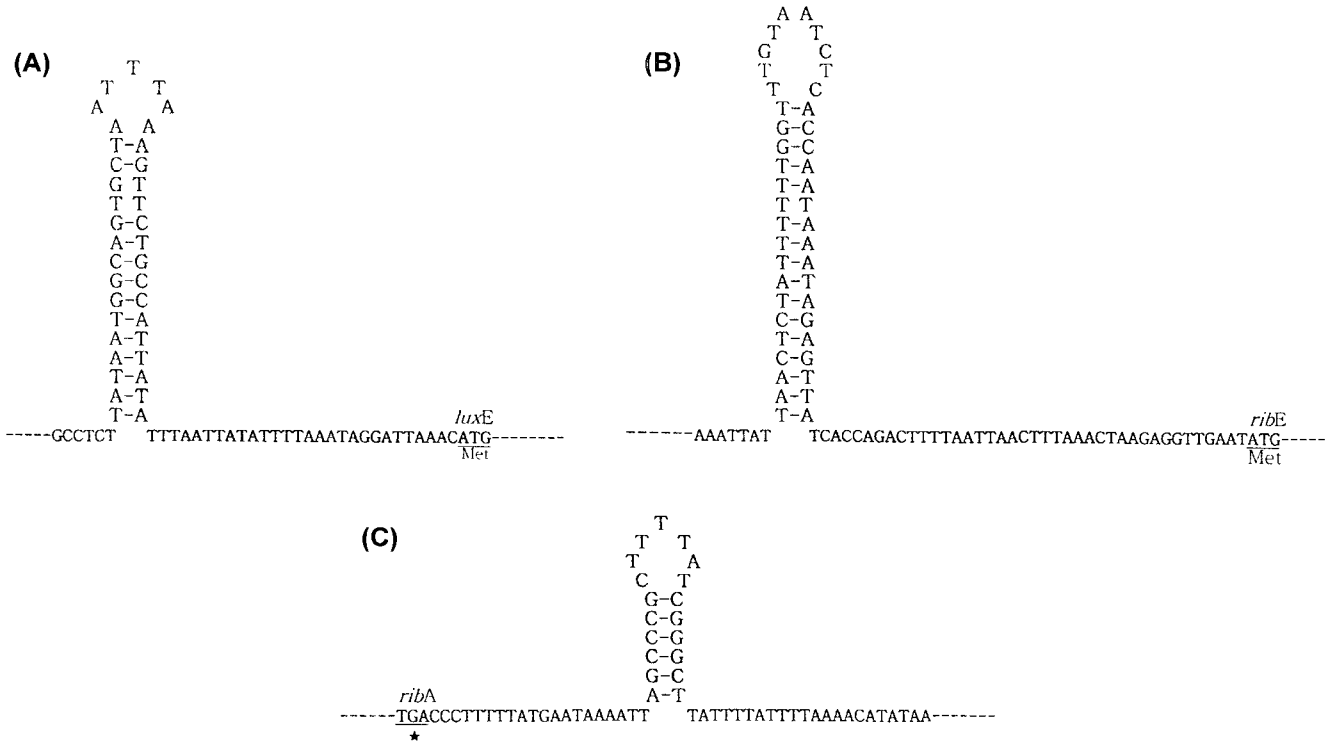

Fig. 3. Stem and loop structures in the intergenic DNA sequence located between *luxF* and *luxE* in PpH3 (A), between *luxG* and *ribE* in PpH4 (B), and the downstream of *ribA* in PpH2 (C) from *P. phosphoreum*. The original DNA sequences were obtained from the previous papers (Soly and Meighen, 1991; Lee *et al.*, 1994).

operon from *P. leiognathi* (Lin *et al.*, 2001).

In order to determine whether or not these segments work as transcriptional terminators, an *in vivo* termination assay was performed with the CAT reporter gene, using conjugational methods. The *P. phosphoreum* DNAs (PpH3, 2.5 kbp; PpH4, 3.6 kbp; and PpH2, 1.1 kbp, respectively) each containing the possible stem-loop structures shown above (Fig. 3A, B, and C, respectively), as well as the 0.4 kbp DNA fragment (PpH1) located inside the coding region of *ribA*, were inserted between a strong *lux* promoter and a reporter gene (chloramphenicol acetyl transferase, CAT) in the pMGM plasmid (Fig. 4) and transferred by conjugation into *V. harveyi*. The CAT gene in the recombinant pMGM plasmid (pM) could not be expressed without the appropriate DNA containing promoter activity, which is shown in lane 1 of Fig. 5. Insertion of PpH1 DNA fragment between *lux* promoter and CAT gene produced the same CAT activity (GH/H1/pM; lane 2) as those with the strong *lux* promoter (GH/pM; lane 6). Similarly, the cases of the insertion of the PpH3 and the PpH4 DNA fragments did not significantly decrease (lanes 4 and 5), compared to the value obtained using the strong *lux* promoter (lane 6). However, the insertion of the PpH2 DNA fragment, containing the stem-loop structure preceding a T-rich region, present in the downstream of *ribA*, produce a drastic decrease in CAT expression (up to 14%) (lane 3) compared to that from the strong *lux* promoter

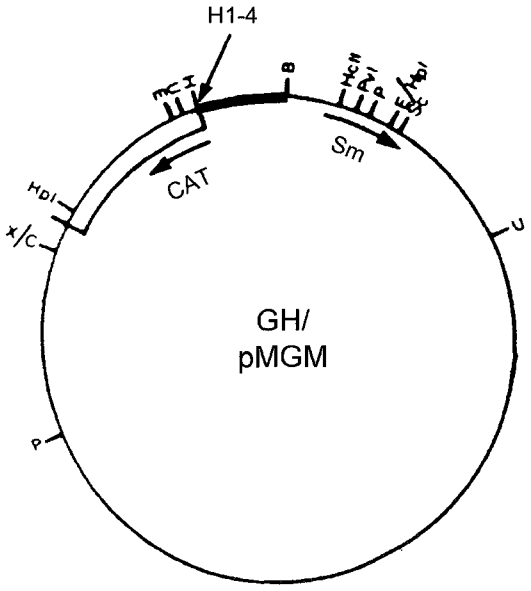

Fig. 4. Restriction map of the vector pMGM (pM) derived from pKT 230. The size of plasmid is 13.3 kbp. CAT, chloramphenicol acetyl transferase; Sm, gene conferring resistance to streptomycin; B, *Bam*HI; C, *Cla*I; E, *EcoR*I; H, *Hind*III; HcII, *Hinc*II; Hp, *Hpa*I, P, *Pst*I; PvI, *Pvu*I; Sc, *Sac*I; U, *Pvu*II; X, *Xho*I. The *V. harveyi* DNA digested into *Bgl*II-*Hind*III containing strong *lux* promoter was inserted into *Bam*HI-*Hind*III site yielding to GH-pMGM (GH/pM). Arrow with H1-4 indicates the site at which *P. Phosphoreum Hind*III DNA fragments (H1, 2, 3, 4) were inserted.

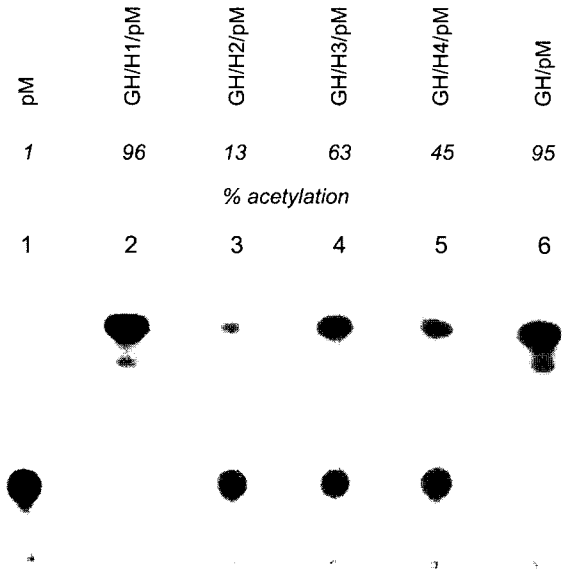

Fig. 5. Fluorography of the ^{14}C-labelled substrates and products in the CAT assay resolved by thin layer chromatography. CAT activity was assayed from the each extracts of *V. harveyi*, containing pMGM itself (pM) (lane 1), pMGM with strong *lux* promoter (GH/pM) (lane 6), and GH-pMGM with insertion of the *P. phosphoreum HindIII* DNA (lane 2, PpH1(GH/H1/pM); lane 3, PpH2 (GH/H2/pM); lane 4, PpH3 (GH/H3/pM); lane 5, PpH4 (GH/H4/pM) fragments between the *lux* promoter and CAT reporter gene in pMGM. All samples were collected from the same amount cells around at $OD_{660} = 3.5$. The CAT activity was shown as % acetylation from extracts of *V. harveyi* containing pMGM. CAT assay was performed according to the previously described procedure (Lee and Meighen, 1992; Swartzman *et al.*, 1990).

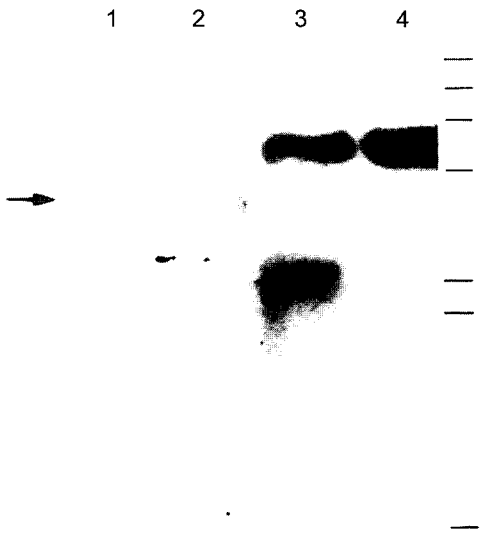

Fig. 6. S1 nuclease protection assay of *P. phosphoreum*. The single strand DNA containing *luxCD* and upstream of *luxC* (PpEE) and the DNA containing the intergenic region of *luxG* and *ribE* (PpH4) were tested for RNA protection by S1 nuclease. After S1 nuclease digestion and loaded alkaline agarose gel, the hybrid transferred to nitrocellulose filter followed by hybridization with ^{32}P-labelled PpSB DNA. PpH4 (lane 1) and PpEE (lane 3) hybridized with *P. phosphoreum* mRNA; PpH4 (lane 2) and PpEE (lane 4) hybridized with tRNA. The position of the each DNA fragment of λ *HindIII* DNA markers (23.7 kb, 9.5 kb, 6.7 kb, 4.3 kb, 2.3 kb, 1.9 kb, and 0.6 kb, respectively) is shown on the right. Further experimental details for S1 nuclease mapping are as described in reference (Swartzman *et al.*, 1990).

(lane 6). The possibility could not be ruled out that the PpH3 and PpH4 DNA fragments function to control the amount of mRNA in the gene, after the possible stem-loop structures. However, combined with the result that the CAT gene was not expressed in an analogous construct missing the *lux* promoter region in *P. leiognathi* (Lee and Meighen, 1992), it can be surmised that PpH3 and PpH4 do not contain any recognizable promoters or terminators.

It would be necessary to directly demonstrate that a polycistronic mRNA extends across the *lux*G-*rib*E region. Unfortunately, an RNase-minus mutant of *P. phosphoreum* does not exist, and Northern blots with *lux* probes have demonstrated the presence of a set of polycistronic mRNAs (Mancini *et al.*, 1988) that do not encompass all the *lux* genes. In order to confirm the above results from the conjugational termination assay, the S1 nuclease experiments were performed, and the data are presented in Fig. 6. As shown in lane 3, the partially labeled 2.6 kbp DNA fragment was generated by the protection of 5'-mRNA for the *lux* operon. The DNA fragment contains *luxC* and *luxL*, which transcribe in opposite directions to one another. This result indicates that the mRNA of *P. phosphoreum* for the transcription of the *lux* operon begins in front of the *luxC* region, according to the size of the labeled DNA band. In contrast, a single strand DNA of 3.6 kbp fragment extending across the *luxG/ribB* boundary (PpH4 in Fig. 1) was apparently fully protected by *P. phosphoreum* mRNA from S1 nuclease digestion (lane 1 in Fig. 6). The differences in the intensity of the labeled DNA may be due to the amount of mRNA, based on the fact that the center of the *lux* operon, including the *luxAB* gene, was highly expressed (Mancini *et al.*, 1988). The result supports the notion that the *rib* genes are part of the same transcriptional unit as are the *lux* genes.

Introduction of the polar mutation into the *lux* operon is also restricted, due to the lack of genetic studies on *P. phosphoreum*, and the difficulty in transferring DNA into this bacterial species. Currently, DNA can only be transferred into *P. phosphoreum* by trans-conjugation, with a very low efficiency, and has not yet been accomplished by transduction or transformation. Therefore, as an alternative method, we checked the riboflavin synthase activity in the extracts of *P. phosphoreum*. Measurement of the fatty acid reductase in extracts during the growth and

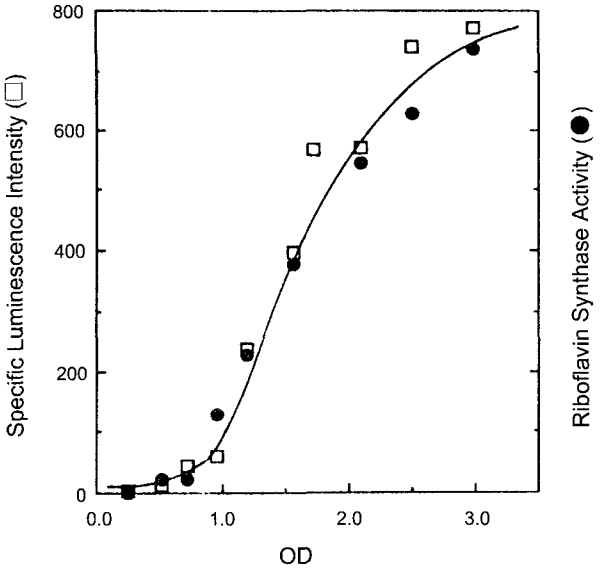

Fig. 7. Coinduction of riboflavin synthase during the development of bacterial bioluminescence. Specific luminescence intensity was represented as a value of light unit per optical density and riboflavin synthase activity were shown to nmol/mg/h × 100, respectively.

development of the luminescent bacteria showed that the fatty acid reductase activity is coinduced with luciferase (Riendeau and Meighen, 1980). Fig. 7 shows that riboflavin synthase and specific luminescence occur in the same fashion as does the growth of the bacteria of *P. phosphoreum*. This indicates that the riboflavin synthase and the luciferase activities are coinduced.

In conclusion, it can be surmised that *lux* genes and *rib* genes are part of the same operon, and are under common regulation in *Photobacterium* species.

Acknowledgements

This work was supported by a grant (No. R11-2002-100-03002-2) from the ERC program of the Korea Science and Engineering Foundation.

References

Hastings, J.W. and G. Weber. (1963) Total quantum flux of isotropic sources. *J. Opt. Soc. Am.* 53, 1410-1415.

Hastings, J.W. and K.H. Nealson. (1977) Bacterial bioluminescence. *Annu. Rev. Microbiol.* 31, 549-595.

Lee, C.Y. and E.A. Meighen. (1992) The *lux* genes in *Photobacterium leiognathi* are closely linked with genes corresponding in sequence to riboflavin synthesis genes. *Biochem. Biophys. Res. Commun.* 186, 690-697.

Lee, C.Y., D. O'Kane, and E.A. Meighen. (1994) Riboflavin synthesis genes are linked with the *lux* operon of *Photobacterium phosphoreum*. *J. Bacteriol.* 176, 2100-2104.

Lin, J.W., Y.F. Chao, and S.F. Weng. (2001) Riboflavin synthesis genes *rib*E, *rib*B, *rib*H, and *rib*A reside in the *lux* operon of *Photobacterium leiognathi*. *Biochem. Biophys. Res. commun.*, 284, 587-595.

Mancini, J.A., M. Boylan, R.R. Soly, A.F. Graham, and E.A. Meighen. (1988) Cloning and expression of the *Photobacterium phosphoreum* luminescence system demonstrates a unique *lux* gene organization. *J. Biol. Chem.* 263, 14308-14314.

Meighen, E.A. (1988) Enzymes and genes from the *lux* operons of bioluminescent bacteria. *Annu. Rev. Microbiol.* 42, 151-176.

Meighen, E.A. (1991) Molecular biology of bacterial bioluminescence. *Microbiol. Rev.* 55, 123-142.

Meighen, E.A. and P.V. Dunlap. (1993) Physiological, biochemical and genetic control of bacterial bioluminescence. *Adv. Microbiol. Physiol.* 34, 1-67.

Meighen, E.A. (1994) Genetics of Bacterial bioluminescence. *Annu. Rev. Genet.* 28, 117-139.

Miyamoto, C.M., A.F. Graham, M. Boylan, J.E. Evans, K. Hasel, and E.A. Meighen. (1985) Polycistronic mRNAs code for polypeptides of the *Vibrio harveyi* luminescence system. *J. Bacteriol.* 161, 995-1001.

Miyamoto, C.M., M. Boylan, A.F. Graham, and E.A. Meighen. (1988) Organization of the *lux* structural genes of *Vibrio harveyi*. Expression under the T7 bacteriophage promoter, mRNA analysis and nucleotide sequence of the *luxD* gene. *J. Biol. Chem.* 263, 13393-13399.

Miyamoto, C., E.A. Meighen, and A. Graham. (1990) Transcriptional regulation of *lux* genes transferred into *Vibrio harveyi*. *J. Bacteriol.* 172, 2046-2054.

O'Kane, D.J., B. Woodward, J. Lee, and D.C. Prasher. (1991) Borrowed proteins in bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA* 88,1100-1104.

Otto, M.K. and A. Bacher. (1981) Ligand binding studies on light riboflavin synthase from *Bacillus subtilis*. *Eur. J. Biochem.* 115, 511-517.

Riendeau, D. and E.A. Meighen. (1980) Coinduction of fatty acid reductase and luciferase during development of bacterial luminescence. *J. Biol. Chem.* 255, 12060-12065.

Swartzman, E., S. Kapoor, A.F. Graham, and E.A. Meighen. (1990) A new *Vibrio fischeri lux* gene precedes a bidirectional termination site for the *lux* operon. *J. Bacteriol.* 172, 6797-6802.

Wall, L., A. Rodriguez, and E. Meighen. (1984) Differential acylation *in vitro* with tetradecanoyl coenzyme A and tetradecanoic acid (+ATP) of three polypeptides shown to have induced synthesis in *Photobacterium phosphoreum*. *J. Biol. Chem.* 259, 1409-1414.