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Lateral Transfer of the *lux* Gene Cluster*

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The *lux* operon is an uncommon gene cluster. To find the pathway through which the operon has been transferred, we sequenced the operon and both flanking regions in four typical luminous species. In *Vibrio cholerae* NCIMB 41, a five-gene cluster, most genes of which were highly similar to orthologues present in Gram-positive bacteria, along with the *lux* operon, is inserted between VC1560 and VC1563, on chromosome 1. Because this entire five-gene cluster is present in *Photorhabdus luminescens* TT01, about 1.5 Mbp upstream of the operon, we deduced that the operon and the gene cluster were transferred from *V. cholerae* to an ancestor of *Pr. luminescens*. Because in both *V. fischeri* and *Shewanella hanedai*, *luxR* and *luxI* were found just upstream of the operon, we concluded that the operon was transferred from either species to the other. Because most of the genes flanking the operon were highly similar to orthologues present on chromosome 2 of vibrios, we speculated that the operon of most species is located on this chromosome. The undigested genomic DNAs of five luminous species were analysed by pulsed-field gel electrophoresis and Southern hybridization. In all the species except *V. cholerae*, the operons are located on chromosome 2.

Key words: *Photobacterium phosphoreum*, *Photorhabdus luminescens*, transfer of the *lux* operon, *Vibrio cholerae*, *Vibrio fischeri*.

The number of species of luminous bacteria identified to date is only about 20 (*1*), and will not increase drastically because almost all species seem to have been identified as they can be detected readily by the bioluminescence they emit. All luminous bacterial species possess the *lux* operon, although the genes composing the operon vary depending on the species. The luminous species are widely distributed among γ-Proteobacteria considering their number: most luminous species are classified in *Vibrionaceae*, but several are also in *Shewanellaceae*, and even in *Enterobacteriaceae* (*1*). This feature of the distribution of the operon among bacteria seems to indicate that the operon was transferred independently of phylogeny, and means that it is difficult to find the pathway through which the operon was transferred, using a typical phylogenetic analysis. A comparative analysis of the two flanking regions of the operon in the respective species may be an alternative way to solve this problem because the operon usually seems to be transferred accompanying flanking genes.

We can compare analytically genes flanking the *lux* operons of various species quite easily if the genomic sequences have been published. Genome sequencing in a variety of organisms has advanced dramatically in recent years. Now, genomic sequences of over 400 prokaryotic strains have been published and the number of completed genomic sequences increases every month. However, the genomic sequences of only two species of luminous bacteria, *Photorhabdus* (*Pr.*) *luminescens* TT01 (*2*) and *Vibrio fischeri* ES 114 (*3*), have been published. Among the non-luminous species in *Vibrionaceae*, however, the genomic sequences of five species have been published: the sequence of *V. cholerae* N 16961 appeared in the DDBJ/EMBL/GenBank database in 2000 (*4*), that of *V. vulnificus* CMCP6 in late 2002 (accession numbers, NC 004459 and NC 004460), those of *V. parahaemolyticus* O3:K6 RIMD 2210633 (*5*) and *V. vulnificus* YJ016 (*6*) in 2003, and that of *Photobacterium* (*P.*) *profundum* SS9 (NC 006370 and NC 006371) in 2005. The sequences of non-luminous species also provide valuable information, because we found the DNA sequence of the luminous strain *V. cholerae* NCIMB 41, to be quite similar to that of the non-luminous genome-sequenced strain *V. cholerae* N 16961 in overlapping regions (*7*). In this study, we started to sequence the regions flanking the *lux* operon in four species of luminous bacteria, *V. cholerae*

*The nucleotide sequences reported in this paper have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence database and are available under accession numbers AB104437, AB105805, AB107370, AB107988, AB119994, AB120061 and AB261992.

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MCIMB 41, *Shewanella hanedai* NCIMB 2157, *V. fischeri* ATCC 7744, and *P. phosphoreum* IFO 13896, aiming to find pathways of lateral transfer of the *lux* operon.

MATERIALS AND METHODS

Bacteria—Six luminous bacterial species were used in this study. *V. cholerae* NCIMB 41 and *S. hanedai* NCIMB 2157 were obtained from NCIMB; *V. fischeri* ATCC 7744 from ATCC; *P. phosphoreum* IFO (NBRC) 13896 from the Graduate School of Engineering, Osaka City University; *P. leiognathi* NCIMB 2193 from Ocean Research Institute, The University of Tokyo; and *V. harveyi* B 392 from the Research Institute for Microbial Diseases, Osaka University. The respective strains were cultured at 23°C overnight with shaking in Luria–Bertani (LB) medium, containing 1% NaCl in the case of *V. cholerae* and 3% otherwise, and cells were harvested by centrifugation.

PCR and DNA Sequencing—Genomic DNA of the respective species for sequencing was prepared using Genomic-tip 500/G (QIAGEN) according to the supplier's manual. PCR and DNA sequencing were performed as described previously (*8, 9*). A revised protocol for SUGDAT (Sequencing Using Genomic DNA As a Template) (*7*) was used. The *lux* operons as well as both flanking regions of four species were sequenced as follows, and the sequencing is summarized in Fig. 1.

In the case of *V. cholerae* NCIMB 41, we had already sequenced the *lux* operon with both flanking regions (AB115761), and then performed a further four rounds of SUGDAT downstream from the terminal of this sequence. The sequence in the fourth round showed 99% identity with that of VC1563 of strain N 16961 (AE004234) (*4*). Accordingly, to determine the further downstream sequence, we amplified a 2079-bp sequence by PCR, using non-degenerate primers, the reverse one designed referring to the sequence of VC1566 of strain N 16961 (AE004234), and sequenced the PCR product. The combined 3689-bp sequence downstream of the *lux* operon was registered as AB120061. On the other hand, because the *lux* operon seemed to be inserted between VC1562 and VC1563 in strain NCIMB 41, we tried to amplify a partial sequence of VC1562 using the genomic DNA of strain NCIMB 41 as a template by PCR, with non-degenerate primers, which were designed referring to the sequence of VC1562 of strain N 16961. This trial and then the next one to amplify a partial sequence of VC1561 were unsuccessful. However, we were finally successful in amplifying a partial sequence of VC1560 of strain NCIMB 41. Because we had already performed three rounds of SUGDAT upstream of the *lux* operon at this stage, we tried to amplify a sequence between the upstream edge and VC1560 using non-degenerate primers. We were successful in amplifying a 3,694-bp sequence and sequenced this PCR product. To sequence further upstream, we amplified a 2068-bp sequence using non-degenerate primers and sequenced the product. Finally, we determined a 7649-bp sequence (AB119994) upstream of the *lux* operon in this strain.

In the case of *S. hanedai* NCIMB 2157, two partial sequences of *luxC* and *luxA* were amplified using two pairs of degenerate primers, which were used to amplify respective sequences of *V. cholerae* NCIMB 41 (*7*), and then sequenced. A sequence between these ones was amplified by PCR and then sequenced. Two PCR products, which were amplified using primers designed either according to the sequence determined as above or referring to the sequence AB058949, were sequenced. We then performed 10 rounds of SUGDAT in both directions from the respective terminals and finally determined a 13,760-bp sequence (AB261992). Comparing our sequence with AB058949, we found seven nucleotide-replacements in the *lux* operon and a large discrepancy downstream of *luxG*.

In the case of *V. fischeri* ATCC 7744, starting from the tail of *luxR*, we performed 12 rounds of SUGDAT and determined a 5884-bp sequence (AB105805). Although our sequence contained the sequence reported as the *cnf1* gene (*10*), in the sequence (AF023157), four nucleotide-insertions, a nucleotide-deletion, and two nucleotide-replacements were found compared with our sequence, and because of this discrepancy *VfUp2* is rather longer than that previously reported (*cnf1*). To sequence downstream of the *lux* operon, we performed eight rounds of SUGDAT starting from the tail of *luxG* and determined a 7898-bp sequence (AB107370).

In the case of *P. phosphoreum* IFO 13896, because we have already determined the nucleotide sequence of *luxF* and both flanking regions (*11*), we performed 25 and 4 rounds of SUGDAT upstream and downstream from this region, respectively, and determined a 17,720-bp sequence in all (AB104437). Meanwhile, we performed 13 rounds of SUGDAT from the head of *hcp* toward the opposite direction, and determined a 7110-bp sequence (AB107988).

Pulsed-field Gel Electrophoresis (PFGE)—Five species of luminous bacteria were cultured as described earlier. Cells were harvested by centrifugation, embedded in 0.8% agarose gels, and processed prior to electrophoresis as described previously (*12, 13*). PFGE was carried out on a CHEF Mapper system (Bio-Rad) with 0.8% agarose gel in 1.0× TAE buffer, pH 8.0. After electrophoresis, DNA bands were stained with ethidium bromide. Chromosomes of *Hansenula wingei* and *Schizosaccharomyces pombe* were obtained from Bio-Rad and used as size markers.

Southern Hybridization Analysis—After electrophoresis, DNA bands were transferred onto a nylon membrane. A 461-bp sequence in *luxA* was selected to prepare probes for detection of the *lux* operon. The following five pairs of primers were used to amplify the sequences: 5′-TGTTTCTCATATCAGCCTCC-3′ and 5′-GGGTAAACG TTTACATCTGG-3′ for *P. phosphoreum*; 5′-TGTTTCTCA TACCAACCACC-3′ and 5′-GGATAAACATTGACATCTG G-3′ for *P. leiognathi*; 5′-TGTTTTTCGTATCAACCACC-3′ and 5′-GGATACACATCTACATTAGG-3′ for *V. fischeri*; 5′-GGATTCAGTTGGATTTTCGG-3′ and 5′-GGATTCAGTT GGATTTTCGG-3′ for *V. harveyi*; and 5′-TTACTAACCTA CCAACCACC-3′ and 5′-GGTTGGACTTTCACTTCAGG-3′ for *V. cholerae*. The corresponding sequence was amplified by PCR to prepare a template for the preparation of

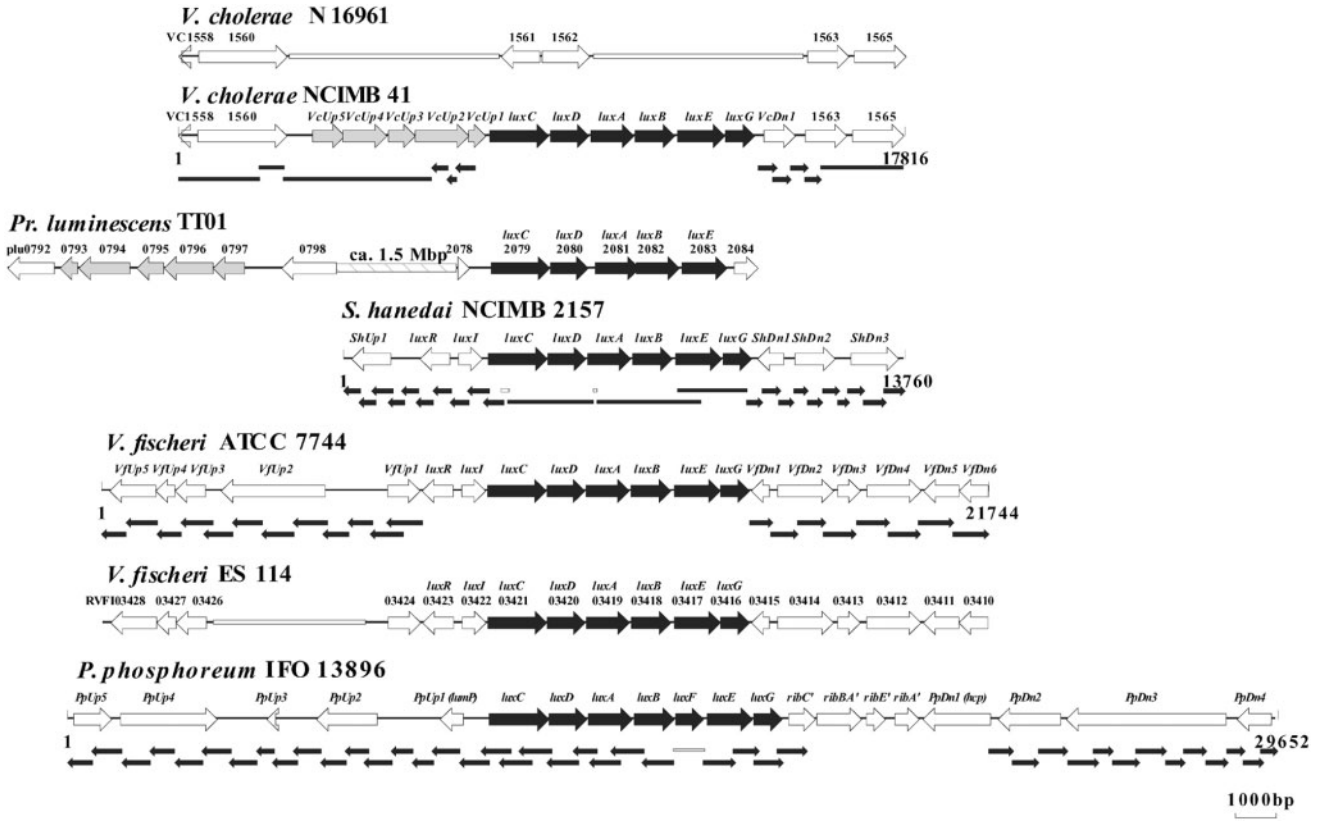

Fig. 1. **The *lux* operon and both flanking regions of four species, *V. cholerae* NCIMB 41, *S. hanedai* NCIMB 2157, *V. fischeri* ATCC 7744, and *P. phosphoreum* IFO 13896, along with the corresponding regions of the three genome-sequenced species, *V. cholerae* N 16961, *Pr. luminescens* TT01, and *V. fischeri* ES 114.** Arrangement of genes in the *lux* operon and both flanking regions of four species, *V. cholerae* NCIMB 41, *S. hanedai* NCIMB 2157, *V. fischeri* ATCC 7744, and *P. phosphoreum* IFO 13896, and the sequencing of the respective species are summarized. Solid and open bars indicate PCR products amplified using non-degenerate and degenerate primers, respectively. Solid thin arrows indicate sequences determined in these directions by SUGDAT. Arrangements of genes of the corresponding regions of three genome-sequenced species, *V. cholerae* N 16961, *Pr. luminescens* TT01, and *V. fischeri* ES 114, are also shown over or below the one of *V. cholerae* NCIMB 41, and below the one of *V. fischeri* ATCC 7744, respectively, for comparison. Thick black arrows indicate the *luxA* to *luxG* genes, while open or gray arrows indicate other genes, in the respective directions. Solid lines connecting genes indicate non-coding sequences, while open ones in *V. cholerae* N 16961 and *V. fischeri* ES 114, show that those sequences are missing in respective species. In *V. cholerae*, comparing the corresponding regions of two strains, NCIMB 41 and N 16961, in the latter, twelve genes including the *lux* ones in the former are replaced with only two genes, VC1563 and VC1564, which are placed tentatively at the center of this region. Comparing two strains of *V. fischeri*, ATCC 7744 and ES 114, in the latter, a 3-kbp sequence is missing upstream of the *lux* operon and owing to this deletion, *VfUp2* is omitted. Orthologues of five genes, *VcUp1* to *VcUp5*, upstream of the *lux* operon of *V. cholerae* NCIMB 41, were found as plu0793 to plu797, respectively, over 1.5 Mbp upstream of the *lux* operon in the opposite direction in the genome of *Pr. luminescens* TT01 and to show this arrangement, the respective genes were shaded and the compressed 1.5-Mbp sequence was hatched. Below the sequence of *P. phosphoreum*, a scale of 1000 bp is shown and this scale is applied for all sequences in this figure, except the compressed 1.5-Mbp sequence in *Pr. luminescens*.

a DIG-labelled probe in each species. The probe for the respective species was prepared with the above sequence using a PCR DIG labeling mix (Roche) according to the manufacturer's directions. DNA bands on the nylon membrane were hybridized with the probes and an image of the chemiluminescent bands was recorded on X-ray film.

RESULTS AND DISCUSSION

Sequence Analyses of both Flanking Regions of the lux *Operons of Four Species* V. cholerae *NCIMB 41,* S. hanedai *NCIMB 2157,* V. fischeri *ATCC 7744, and* P. phosphoreum *IFO 13896, and Comparison of these Sequences with the Corresponding Ones of the Three Genome-sequenced species* V. cholerae *N 16961,* Pr. luminescens *TT01, and* V. fischeri *ES 114*—In a previous study (*7*), we determined the nucleotide sequence of the *lux* operon of *V. cholerae* NCIMB 41. Because the genomic sequence of *V. cholerae* N 16961 had been published (*4*), we attempted to locate the operon in the genome. We sequenced both flanking regions of the operon as described earlier, and found that the operon is inserted between VC1560 and VC1563 (Fig. 1). Five genes, *VcUp1* to *VcUp5*, are present upstream of the operon, and by homology search using BLAST (*14*) (blastp) at the National Center for Biotechnology Information, USA (NCBI), we found that

the amino acid sequence deduced from the nucleotide sequence of *VcUp1* shows 56% identity with that of plu0793 of *Pr. luminescens* TT01 (*2*), and further, the amino acid sequence deduced from *VcUp2*, *VcUp3*, *VcUp4* and *VcUp5* shows 60%, 47%, 38% and 49% identity with that from plu0794, plu0795, plu0796 and plu0797, respectively. Five genes, plu0793 to plu0797, are present also in tandem in the genome of *Pr. luminescens*, although they are over 1.5 Mbp upstream of the *lux* operon, as shown in Fig. 1. By conducting BLAST analyses, we found that *VcUp4* or plu0796 is an uncommon gene and no other orthologues are present in the database, but orthologues of the other four genes are distributed widely among bacteria and even archaea, although mainly among Gram-positive bacteria. The amino acid sequences deduced from these four genes show higher similarity scores with those of orthologues found in Gram-positive bacteria but less with those of Gram-negative ones. Meanwhile, five lux proteins of *V. cholerae* NCIMB 41 show high similarity scores with those of *Pr. luminescens* (*7*).

Based on the earlier evidence, we deduced that the *lux* operon as well as the upstream cluster consisting of five genes was first transferred from a species of Gram-positive bacteria to *V. cholerae*. Thereafter, the five-gene cluster and the operon seem to have been transferred from *V. cholerae* to an ancestor of *Pr. luminescens*, although maybe indirectly, and further, either of the gene clusters may have been transferred to a new position 1.5 Mbp away from the original point in *Pr. luminescens* as shown in Fig. 2. The reversed transfer of the gene clusters seems to occur rarely because two gene clusters can hardly be transferred from two different positions on a genome just to neighboring positions on a different genome. On the other hand, a cluster of the *lux* operon and its upstream genes might have been first transferred into an ancestor of *Pr. luminescens* from a species of Gram-positive bacteria and next transferred into *Vibrio*, while the cluster might have been split in a descendant of *Pr. luminescens*, however, this scheme of transfer seems to be improbable for the following reasons. If the cluster was first transferred to an ancestor of *Pr. luminescens*, the cluster should have spread widely in *Enterobacteriaceae*, into which *Pr. luminescens* is classified. In reality, the *lux* operon is distributed mainly in *Vibrionaceae*. Moreover, in *Pr. luminescens* only, the *lux* operon is partially broken because the *luxG* gene is deleted from the operon, as we will discuss below.

For lateral gene transfer, two organisms must come into contact with each other. Because the habitats of strains of *V. cholerae* are unique among *Vibrio* species and terrestrial environments (*7*), two species of bacteria *V. cholerae* and *Pr. luminescens*, might have the chance to make contact with each other. We proposed that the function of the *lux* operon is to produce a halotolerant flavodoxin (*8, 15*) and even in *V. cholerae*, the operon should have the same function (*7*). However, because *Pr. luminescens* is classified in *Enterobacteriaceae* and all species in this family inhabit terrestrial environments, it is improbable that the operon has the same function. We speculate that the operon acquired a new function when it was transferred to *Photorhabdus* and for this reason, the *luxG* gene might be lost from the operon.

On the other hand, between the operon and VC1563, only one gene, *VcDn1*, is present (Fig. 1). Because the amino acid sequence deduced from the gene showed 47% identity with the sequences of two *Shewanella* species, ZP 00815380 and ZP 00905835, we expected an orthologue of this gene to be present downstream of the *lux* operon of *S. hanedai*, which had been reported to be luminous (*1*). We sequenced the operon with both flanking regions in *S. hanedai* NCIMB 2157 as previously described. We found three ORFs downstream of the operon but none of them was an orthologue of *VcDn1*. Meanwhile, upstream of the operon, we also found three genes, two of which, are present just upstream of the operon, are orthologues of genes designated as *luxI* and *luxR*, which were also reported to be present just upstream of the *lux* operon in *V. fischeri* (*16, 17*). Because we expected an orthologue of *ShUp1* to be present downstream of *luxR*, we sequenced both flanking regions of the *lux* operon of *V. fischeri* ATCC 7744 as previously described. However, we could not find an orthologue of *ShUp1* in the sequence determined and moreover, an orthologue is not present anywhere in the genome of *V. fischeri* ES 114. Except for *V. fischeri*, *luxI* and *luxR* were found in *S. hanedai* just upstream of the *lux* operon. This arrangement of genes indicates that the operon was transferred from *V. fischeri* to *S. hanedai*, and vice versa, although maybe indirectly. To show this

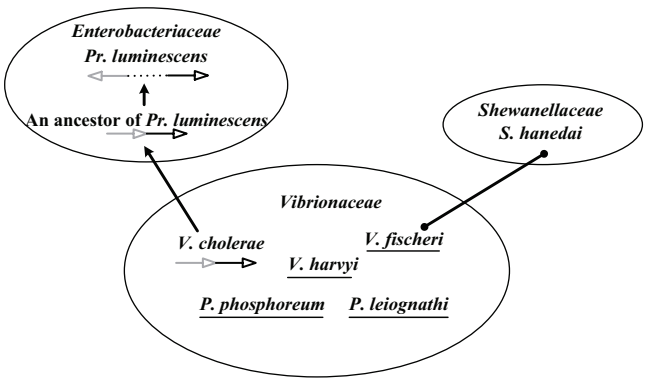

Fig. 2. **Transfer of the *lux* operon among luminous bacteria.** The *lux* operons are shown with open black arrows, whereas the upstream five-gene clusters are shown with open gray arrows in *V. cholerae*, *Pr. luminescence*, and its ancestor. The sequence intervening between the operon and the five-gene cluster in *Pr. luminescens* is shown with a dotted line. Species, in which the *lux* operons are located on chromosome 2, are underlined. The *lux* operon and the upstream gene cluster seem to have been transferred from *V. cholerae* to an ancestor of *Pr. luminescens*, and further to *Pr. luminescens*, in which either of the gene clusters might have been transferred to a new position 1.5 Mbp away from the original point. Therefore, respective species were combined with an arrow in the direction from the former to the latter. Meanwhile, because the operon seems to have been transferred from either *S. hanedai* or *V. fischeri* to the other, the two species were combined with a line without a direction. Pathways of transfer of the operon among other luminous species could not be determined in this study.

Table 1. **Sizes of chromosomes 1 and 2 along with total sizes of five luminous bacteria as well as five genome-sequenced *Vibrio* strains and one *Photobacterium* strain.**

Lane	Species (Strains)	References	Sizes (Mbp)		
			Chromosome 1	Chromosome 2	Total
1	*P. phosphoreum* IFO 13896		3.2	1.8	5.0
2	*P. leiognathi* NCIMB 2193		3.3	1.6	4.9
3	*V. fischeri* ATCC 7744		3.2	1.9	5.1
4	*V. harveyi* B392		3.5	2.7	6.2
5	*V. cholerae* NCIMB 41		3.1	1.6	4.7
	V. cholerae N 16961	(*4*)	3.0	1.1	4.0
	V. parahaemolyticus RIMD 2210633	(*5*)	3.3	1.9	5.2
	V. vulnificus CMCP6	AE016795 & AE016796	3.3	1.8	5.1
	V. vulnificus YJ016	(*6*)	3.4	1.9	5.3
	V. fischeri ES 114	(*3*)	2.9	1.3	4.2
	P. profundum SS9	NC006370 & NC006371	4.1	2.2	6.3

relationship, we have connected the two species with a line without an arrow in Fig. 2. In the case of *V. cholerae*, genes upstream of the *lux* operon show high similarity scores with orthologues present in Gram-positive bacteria as described earlier, whereas orthologues of the two genes, *luxI* and *luxR*, are widely distributed in Proteobacteria, regardless of bioluminescence, and consequently we speculate that the *lux* operon was first transferred by chance just downstream of *luxI* in a genome of a bacterium, very likely *V. fischeri* or *S. hanedai*, and further transferred to another bacterium, for example, *V. fischeri* or *S. hanedai*, along with *luxI* and *luxR*.

We compared the sequence of the *lux* operon along with both flanking regions of *V. fischeri* ATCC 7744 with that of the corresponding region of strain ES 114. Although we determined a 7862-bp sequence downstream of *luxR* (Fig. 1), the 1219 bp following *luxR* and the terminal 2725-bp sequence showed 87% and 95% identity with the corresponding regions of strain ES 114, respectively, and part of the intervening sequence of strain ATCC 7744 (3918 bp), showed only low identity with that of strain ES 114 (129 bp). In strain ES 114, owing to this reduction in the length of the sequence, *VfUp2*, which is present in strain ATCC 7744, is deleted (Fig. 1). In this strain, the deletion of genes seems to have occurred in many positions because the genome is about 1 Mbp smaller than that of strain ATCC 7744, as described subsequently (Table 1). After the lateral transfer of gene clusters, such as the *lux* operon, the genes brought together seem to be deleted if they are unnecessary for the host: they seem to first be damaged partially and then be discarded stepwise. For this reason, upstream of the laterally acquired gene clusters, rather long non-coding regions seem to arise. In all cases in this study, such long non-coding regions were found upstream of the *lux* operon (Fig. 1). On the other hand, the nucleotide sequence downstream of *luxG* of strain ATCC 7744, 5859-bp long, showed 92% identity with the corresponding sequence of strain ES 114.

Finally, we sequenced both regions flanking the *lux* operon in *P. phosphoreum* IFO 13896 as described earlier, to obtain information, to and from which species the operon was transferred. Upstream of the *lux* operon, five ORFs were found, and upstream of the *hcp* gene, three ORFs (Fig. 1). However, none of them were orthologues of genes found in the flanking regions of the operon of the species mentioned earlier. By BLAST analysis, we found that most of them are located on chromosome 2 in the genome-sequenced five *Vibrio* and one *Photobacterium* strains, and the operon seems to be present on chromosome 2.

Assignment of Loci of the lux *Operons of Five Luminous Bacteria to either of Two Chromosomes*—Okada *et al*. (*18*) reported that vibrios commonly possess two chromosomes. In *V. cholerae* NCIMB 41, the *lux* operon is suggested to be located on chromosome 1 because as shown above, the operon is inserted between VC1560 and VC1563, which are located on chromosome 1 in strain N 16961, while in *V. fischeri* ES 114, the operon is located on chromosome 2 (*3*). In *P. phosphoreum* IFO 13896, the *lux* operon is deduced to be located on chromosome 2 as described earlier.

We first confirmed that a two-replicon structure is common to the previous three species as well as two more typical luminous bacteria, *P. leiognathi* NCIMB 2193 and *V. harveyi* B 392. Intact genomic DNA of these five species was analysed using PFGE and two bands were found in all species examined, as shown in Fig. 3. The sizes of the respective bands were estimated by comparing them with standard size markers. Sizes of chromosomes 1 and 2 along with total sizes of these five species as well as six genome-sequenced *Vibrio* and *Photobacterium* strains are summarized in Table 1. Sizes of chromosome 1 of the respective species, except for *P. profundum*, are nearly the same. On the other hand, chromosome 2 varies in size over a wide range: that of *V. cholerae* N 16961 is 1.1 Mbp and much smaller than those of the others, whereas that of *V. harveyi* is 2.7 Mbp and far larger.

Because the chromosomes of all five species examined were composed of two replicons, we next determined on which chromosome the *lux* operon is located in the respective species by hybridization with probes of DIG-labeled partial DNA sequences of *luxA*s. As shown in Fig. 3, the operons of *P. phosphoreum* and *V. fischeri* were located on chromosome 2, as expected, and those of *P. leiognathi* and *V. harveyi* were also on the same

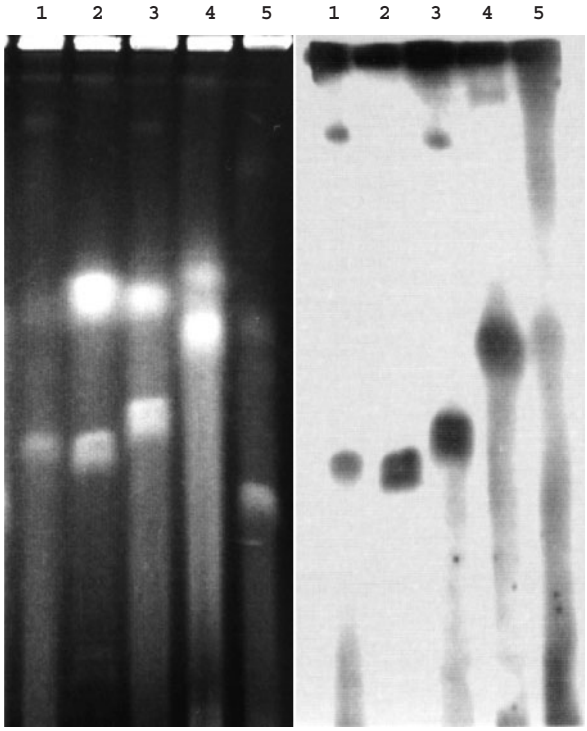

Fig. 3. **PFGE of intact genomic DNA of five species of luminous bacteria (left panel) and hybridization of the DNA with DIG-labeled DNA probes prepared based on partial *luxA* sequences (right panel).** Lane 1, *P. phosphoreum* IFO 13896; lane 2, *P. leiognathi* NCIMB 2193; lane 3, *V. fischeri* ATCC 7744; lane 4, *V. harveyi* B 392; and lane 5, *V. cholerae* NCIMB 41. Genomic DNA of five species of luminous bacteria was subjected to PFGE and stained with ethidium bromide as shown in the left panel. The respective DNA bands were transferred to a nylon membrane and hybridized with DIG-labeled probes, and the hybridized bands were detected by the chemiluminescence emitted on decomposition of CSPD as shown in the right panel. Although only one of two bands in the respective lane was hybridized with the probe, in lane 1–4 in the right panel, in lane 5, a long smear hybridized band was observed. However, note that the band of chromosome 1 in lane 5 is also hybridized.

chromosome. However, only the *lux* operon of *V. cholerae* NCIMB 41 was located on chromosome 1 consistent with the results obtained earlier.

As shown earlier, chromosome 1 of the respective species is similar in size, and Heidelberg *et al*. (*4*) suggested that chromosome 1 of *V. cholerae* contains a larger fraction of genes known to be essential for growth. On the other hand, chromosome 2 varies in size drastically depending on the species and contains a larger fraction of hypothetical genes or genes of unknown function, which are less similar to the genes found in γ-Proteobacteria (*4*). The evidence that the *lux* operon is present on chromosome 2 in major species of luminous *Vibrio* and *Photobacterium* is consistent with that the operon is an uncommon gene cluster even in *Vibrionaceae*. In the previous section, we proposed that the *lux* operon and the upstream five-gene cluster had been transferred from Gram-positive bacteria to *V. cholerae*. Although in *V. cholerae*, the operon is located on chromosome 1, it might have been transferred from this species to chromosome 2 of other luminous vibrios.

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