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Short Sequence-Paper

The gene convergent to *luxG* in *Vibrio fischeri* codes for a protein related in sequence to RibG and deoxycytidylate deaminase [1]

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The nucleotide sequence of a convergent gene with the same bidirectional transcriptional terminator as the *Vibrio fischeri lux* operon has been determined This gene codes for a polypeptide of 147 amino acids which is related in sequence to the polypeptide coded by the first gene (*ribG*) of the *rib* operon of *Bacillus subtilis* as well as deoxycytidylate deaminase of T4 bacteriophage and *Saccharomyces cerevisiae* These results raise the possibility of a linkage between the regulation of the *lux* genes and riboflavin synthesis in *Vibrio fischeri*

The bioluminescence reaction in bacteria involves the oxidation of reduced riboflavin phosphate ($FMNH_2$) and long chain fatty aldehyde resulting in the emission of blue-green light [1–3] The *Vibrio fischeri lux* regulon contains two operons [4] The right operon (*luxICDABEG*) contains the *luxI* regulatory gene, *luxAB* coding for luciferase, *luxCDE* coding for proteins involved in aldehyde synthesis, and *luxG*, whose gene product is related in sequence to proteins involved in flavin reduction [5–8] The left operon, immediately upstream of *luxI*, contains one regulatory gene, *luxR*, transcribed in the opposite direction [2–4] Recently, genes coding for proteins with sequence homology to the riboflavin synthesis genes in *Bacillus subtilis* and *Escherichia coli* have been found to be closely linked and transcribed in the same direction as the *lux* operons in *Photobacterium leiognathi* and *Vibrio harveyi* [9,10] As the transcriptional terminator of the *lux* operon after *luxG* of *V fischeri* is unusual in that it also functions as a terminator for a convergent open reading frame [6], it raised the possibility that the function of its gene product may be related to bioluminescence as expression of this latter gene and the *lux* operon may be coregulated as overlapping mRNAs are generated

In order to identify the convergent gene, an *Sna*BI-*Hin*dIII DNA fragment downstream of *luxG* was subcloned from a recombinant plasmid pBR322 containing *V fischeri* DNA [11] into M13 mp18 and M13 mp19, and the sequence determined in both directions by the dideoxy termination method [12] The nucleotide sequence and deduced amino acid sequence of the convergent gene is given in Fig 1 This open reading frame (ORF) codes for a protein of 147 amino acids with a molecular mass of 16 6 kDa The intergenic sequence between *luxG* and the convergent gene is also shown with the location of the bidirectional terminator and the ends of the overlapping mRNAs (Fig 1)

Analyses of the primary structure of the ORF showed that the gene product has considerable sequence identity with the amino terminal of the gene product of the first structural gene (*ribG*) [13] of the *rib* operon of *B subtilis* A computer search of the SWISS-PROT and EMBL data libraries also revealed that the gene product has sequence similarity with deoxycytidylate deaminases (CD) from Bacteriophage T4 [14] and yeast *Saccharomyces cerevisiae* [15] (Fig 2) Although the function of *B subtilis* RibG is not defined, complementation of *B subtilis* riboflavin auxotrophs suggested that this gene is involved in the deamination of a pyrimidine ring during riboflavin synthesis [16] Alignment of this sequence with deoxycytidylate deaminases (CD) supports this proposed function As shown in Fig 2, the gene product of the *V fischeri* convergent gene and the amino-terminal

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[1] The sequence data reported in this paper have been deposited to the EMBL data library under the accession number X70289

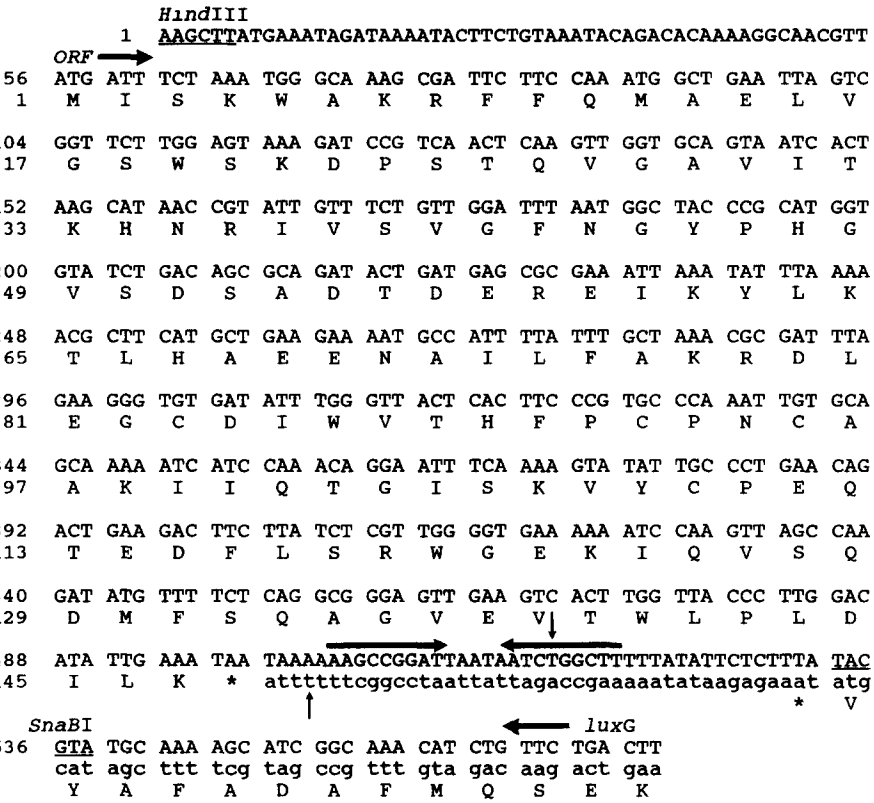

Fig 1 The complementary nucleotide sequence and corresponding translated amino acid sequences of the DNA downstream of *luxG* The nucleotides are numbered from the first nucleotide at the *Hin*dIII site The converging arrows represent the stem and loop structure The vertical arrow indicates where the mRNAs for the *lux* G and convergent gene terminate according to 3′ S1 nuclease mapping

region of RibG can be aligned with the deoxycytidylate deaminase of Bacteriophage T4 and the carboxy-terminal half of the yeast deoxycytidylate deaminase Although there are some gaps, the deaminases share 35–45% sequence identity with the region extending between amino acids 28 and 138 of the *V fischeri* open reading frame A highly conserved region corresponding to a pyrimidine binding site found in a variety of dTTP-binding proteins [15] is indicated by asterisks As the T4 bacteriophage deaminase function is encoded in a protein of 192 amino acids, it is likely that both yeast deaminase and RibG may have other functional do-

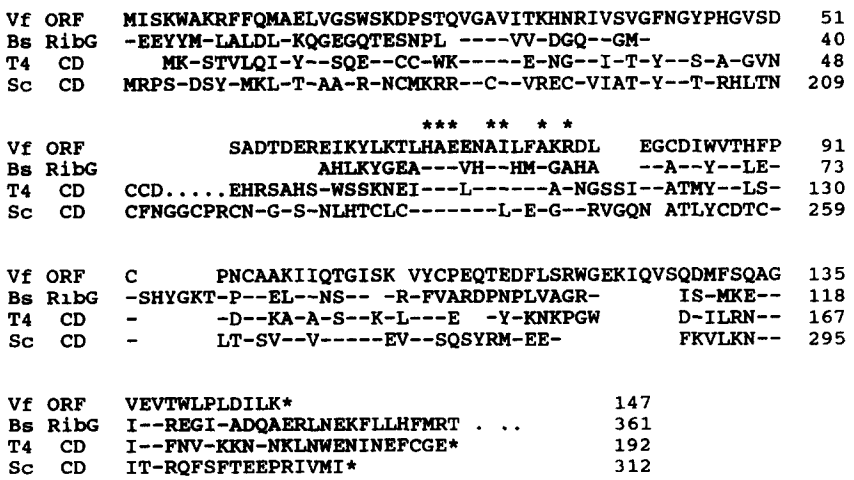

Fig 2 Amino acid sequence comparison of the proteins encoded by the ORF of *V fischeri* with those of RibG and dCMP deaminase From top to bottom, the sequences are given in the following order ORF of *V fischeri*, RibG of *B subtilis* [13], dCMP deaminase of T4 bacteriophage [14], and dCMP deaminase of *S cerevisiae* [15] Horizontal dash indicates an amino acid identical to the *V fischeri* ORF 39 acids of T4 dCMP deaminase were deleted ( ) in order to improve the alignments A putative pyrimidine binding site is shown by asterisks

mains, while the *V fischeri* ORF would encode only a 147 amino acid protein with a single function

Synthesis of riboflavin is of importance in bioluminescent bacteria as reduced riboflavin monophosphate ($FMNH_2$) is the substrate for the luminescence reaction Recently, riboflavin-related genes have been shown to linked to the *lux* genes from other species of luminescent bacteria The *luxL* gene coding for a lumazine-binding protein has been found just upstream of *P phosphoreum luxC* transcribed in the opposite direction [17] This gene product has sequence identity with the α subunit of riboflavin synthetase [17] The *luxH* gene product which is present downstream of *luxG* in *V harveyi* has significant homology with the 3,4-dihydroxy-2-butanone 4-phosphate synthetase required for riboflavin synthesis in *E coli* [10] In addition, three open reading frames immediately downstream and transcribed in the same direction as *luxG* in *P leiognathi* were shown to share identity with the gene products coded by the second, third and fourth genes of the riboflavin synthesis operon of *B subtilis* [9]

In the current work, it was unexpected to find a convergent gene whose mRNA overlaps the *V fischeri lux* mRNA and which yet corresponds in sequence to a *rib* gene product that had not previously been linked to the *lux* operons in luminescent bacteria Its sequence similarity with RibG of *B subtilis* and deaminases from yeast and bacteriophage may implicate this gene in a pyrimidine deamination step in riboflavin synthesis

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