circulating levels of CT (*13*), binding sites in other neural regions would predictably not be able to interact with circulating CT of peripheral origin. In view of our evidence for equivalent competition by mammalian CGRP and mammalian CT at CT binding sites, CGRP could serve as an endogenous ligand for the CT receptors demonstrated in the central nervous system. The CGRP might carry out central nervous system actions previously ascribed to CT (*12*), in addition to altering neural function itself.

No evidence of CGRP effects on adenylate cyclase was found in tissues containing high-affinity binding sites for CGRP, but lacking high-affinity binding sites for CT. Consequently, CGRP and CT may function via separate second messenger systems. Nevertheless, CGRP was capable of influencing the adenylate cyclase system apparently through the CT receptor in tissues containing predominantly high-affinity CT binding sites. Consequently, each peptide may initiate cyclic AMP-dependent or -independent events depending on the initial binding site with which it interacts.

Therefore, CT and CGRP appear to have close biosynthetic links related to a single genomic locus (*1*), to have discrete target-tissue receptors with the potential for heterologous interactions, and to have different second messenger systems.

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## Measuring Gene Expression with Light

Abstract. *Light is produced by recombinant* Escherichia coli *that contain* lux *genes cloned from the marine bacterium* Vibrio fischeri. *The bioluminescence phenotype requires genes for regulatory and biochemical functions, the latter encoded by five* lux *genes contained in a single operon. These* lux *genes were disconnected from their native promoter and inserted into the transposon mini-Mu. The resulting transposon, mini-Mu*lux, *could induce mutations by insertional inactivation of a target gene, and the* lux *DNA was oriented to align target gene transcription with that of the* lux *genes. Genes in* Escherichia coli *and* Vibrio parahaemolyticus *were mutagenized, and mutants containing transposon-generated* lux *gene fusions produced light as a function of target gene transcription. Light production offers a simple, sensitive, in vivo indicator of gene expression.*

Transposon mutagenesis and gene fusions have been used to study gene regulation. Transposon insertions result in a null mutant phenotype (total loss of function), are convenient to map, and physically associate a selectable marker such as a drug resistance gene with the target gene (*1*). Gene fusions, constructed by aligning indicator structural genes to the controlling elements of other genes, link the transcription of a given gene to that of indicator genes such as the *lac* operon. Thus, a manageable and selectable phenotype is substituted for one that may be difficult to analyze (*2*). By inserting *lac* genes into transposon Mu, Casadaban (*3*, *4*) combined the transposon and gene fusion technologies. One particular construction, transposon mini-Mu, causes mutation by insertional inactivation, fuses target gene transcription to the *lac* genes, carries a selectable marker, and can be transduced by helper phage Mu. We have used mini-Mu to construct a transposon that generates gene fusion mutants that produce light.

Seven *lux* genes contained on one DNA fragment from *Vibrio fischeri* encode the regulatory and biochemical activities necessary for light production in *Escherichia coli* (*5*) (Fig. 1A). The genes *luxR* and *luxI* control the transcription of one operon containing *lux* genes *I*, *C*, *D*, *A*, *B*, and *E*. The subunits of luciferase

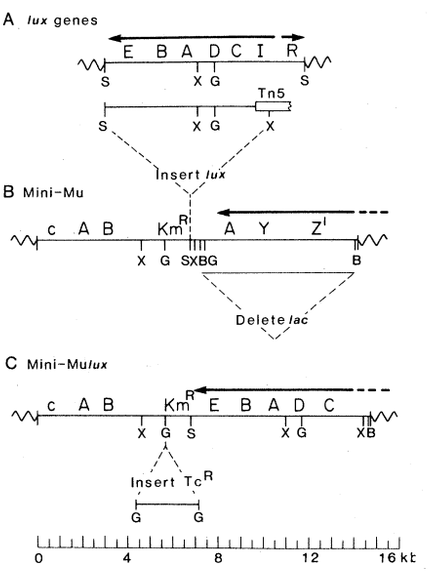


Fig. 1. Construction of mini-Mu*lux* transposons. Restriction sites are S, Sal I; B, Bam HI; X, Xho I; and G, Bgl II. Arrows indicate the extent and direction of transcription of operons. (A) The *lux* genes and operons in a Sal I fragment from *V. fischeri*. (B) The mini-Mu (MudII1681) which contains transposase genes (*A* and *B*), the gene for repressor of transposition (*c*, a temperature-sensitive allele), the kanamycin resistance gene ($Km^r$), and part of the *lac* operon with genes *Z'*, *Y*, and *A*. This particular mini-Mu generates protein fusions containing the functional carboxyl terminal portion of β-galactosidase and was chosen because the *lac* genes could be deleted by Bam HI digestion. Mini-Mu was transposed to plasmid pKO-1 to generate plasmid pJE101 with which the various recombinant DNA manipulations could be achieved. (C) After deletion of the *lac* genes, the Sal I–Xho I *lux* gene fragment was ligated into the Sal I site of mini-Mu producing mini-Mu*lux*($Km^R$). The DNA end produced by Xho I digestion ligated with the Sal I end of mini-Mu but could not be re-cleaved by Sal I or Xho I. A mini-Mu*lux*, mini-Mu*lux*($Tc^R$), which encodes tetracycline resistance, was constructed by addition of a Bgl II fragment from transposon Tn10.

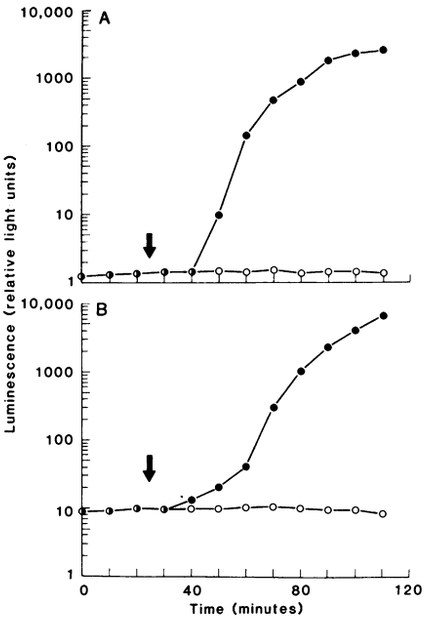


Fig. 2. Induction of light in fusion mutants. The mini-Mu*lux*($Km^R$) was transduced into $Lac^+$ $Ara^+$ *E. coli* strain AB259 with helper bacteriophage Mucts. To obtain transducing lysates, strain CSH4(*recA*) lysogenized with mini-Mu*lux*($Km^R$) and Mucts was induced by temperature shift. The *lac-lux* and *ara-lux* fusion mutants were recognized as white colonies on MacConkey agar containing kanamycin (40 μg/ml) (*11*) and either lactose or arabinose. (A) Induction of a *lac-lux* fusion mutant. (B) An *ara-lux* fusion mutant. The arrow marks the time at which the inducer of the *lac* operon (IPTG at a final concentration of 10 m*M*) or inducer of the *ara* operon (arabinose, at a final concentration of 1 percent weight/volume) was added. Growth was at 28°C in Luria broth. Induction experiments were performed on cultures (0.1 ml) of mutants (initial $OD_{600}$ of 0.2) in vials cycled continuously for the duration of the experiment in an LKB 1211 scintillation counter. To measure optical density, samples were removed from larger parallel cultures. Experiments with fusion strains were performed at or below 30°C since luciferase is inactivated at higher temperatures. Luminescence is reported in relative light units (scintillation counts per minute divided by $10^4$) normalized to 1 ml of culture at $OD_{660}$ of one. Luminescence can be converted to quanta per second by multiplying by $6 \times 10^4$. This conversion factor was obtained by comparing measurements of luminescent bacteria from our scintillation counter and from a calibrated photometer.

are encoded by *luxA* and *luxB* and *lux* genes *C*, *D*, and *E* specify activities for producing the long-chain aldehyde tetradecanal. Luciferase is a mixed-function oxidase that uses oxygen, the reduced form of riboflavin 5′-phosphate ($FMNH_2$), and tetradecanal as substrates for the light reaction (*6*). Construction of a transposon which would generate *lux* gene fusions required the insertion of a fragment containing *lux* genes *C*, *D*, *A*, *B*, and *E* (which did not contain the *lux* operon promoter) into a suitable restriction site in transposon mini-Mu (Fig. 1B). The *lac* genes were first removed from mini-Mu by deletion of a Bam HI restriction fragment. A fragment containing *lux* genes *C*, *D*, *A*, *B*, and *E* was then inserted into the Sal I site in mini-Mu. This fragment was obtained by Sal I and partial Xho I digestion of plasmid pJE347 (*7*) which has the transposon Tn5 inserted in the *luxI* gene. The arm of Tn5, IS50, provided the Xho I site necessary for subcloning a DNA fragment that contained the desired *lux* genes without the *lux* operon promoter. The structure of the resulting construction, mini-Mu*lux*($Km^R$) (Fig. 1C), was verified by restriction analysis. Approximately 900 base pairs (bp) of Tn5 DNA remained between the *lux* gene and the end of the transposon in mini-Mu*lux*. This interposing DNA did not prevent transcription of *lux* (see below). To allow mutagenesis of marine *Vibrio* species a DNA fragment (obtained by Bgl II digestion) containing the tetracycline resistance gene from transposon Tn10 was added to mini-Mu*lux*($Km^R$) to form mini-Mu*lux*($Tc^R$). Both mini-Mu*lux*($Tc^R$) and mini-Mu*lux*($Km^R$) retained the ends of Mu necessary for transposition.

The mini-Mu*lux* transposons were capable of transposing to a variety of loci. Mini-Mu*lux*($Km^R$) was transposed in *E. coli* to the *galK* gene in recombinant plasmid pDR740 by transduction with a Mu helper phage (the muduction technique) (*4*, *7*). The *galK* gene in plasmid pDR740 is transcribed from the *trp* promoter which can be induced by the addition of indoleacrylic acid. Like the parent transposon Mu, mini-Mu*lux*($Km^R$) could integrate in either of two orientations, and approximately 50 percent of the insertions in *galK* were aligned to fuse *galK* transcription with that of *lux*. Upon addition of indoleacrylic acid, light production increased by about 200-fold (*8*). Thus, mini-Mu*lux*($Km^R$) mutants produced light when the target gene was transcribed.

To mutagenize genes in the chromosome of *E. coli*, mini-Mu*lux*($Km^R$) was packaged by helper phage Mucts, and the phage lysate was then used to transduce mini-Mu*lux*($Km^R$) into recipient *E. coli* strains. The transposon mini-Mu*lux*($Km^R$), like Mucts, encodes a temperature-sensitive repressor of transposase genes. Temperature shift to 42°C induced vegetative growth of Mucts as well as the replication of both Mucts and mini-Mu*lux*($Km^R$), and the resulting lysate transduced mini-Mu*lux*($Km^R$) at high frequency. Libraries of strains with mini-Mu*lux*($Km^R$) insertions in nonessential genes were obtained by plating recipient bacteria on selective media containing kanamycin. Mutants with insertions in the *lac* or *ara* genes were identified by plating transductants on MacConkey indicator plates containing either lactose or arabinose. Measurement of the induction of the luminescence phenotype of typical $Lac^-$ or $Ara^-$ fusion mutants is shown in Fig. 2, A and B. The mini-Mu*lux* insertion was probably in the *lacZ* gene as the $Lac^-$ mutant did not produce β-galactosidase. The site of insertion in the $Ara^-$ mutant was not determined. Upon the addition of isopropyl-β-D-thiogalactopyranoside (IPTG), a nonmetabolizable inducer of the *lac* operon, light production in the *lac-lux* fusion mutant increased approxi-

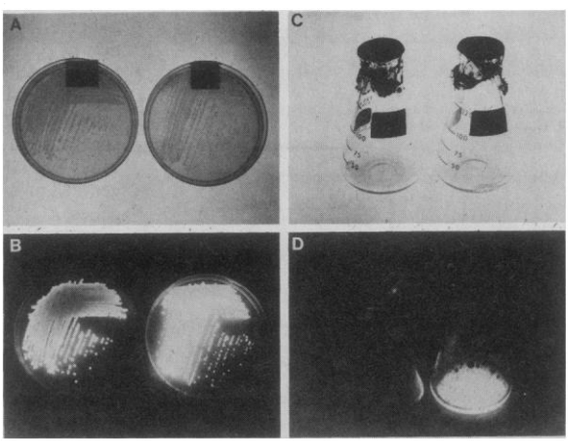


Fig. 3. *Lux* fusions with *V. parahaemolyticus*. Two mutants containing mini-Mu*lux*($Tc^R$) fusions were grown on solid medium (A and B) and liquid medium (C and D). The medium was Difco heart infusion broth supplemented with 2 percent NaCl. Solid medium contained 1.5 percent agar. Cultures were photographed with incident light (A and C) and by bioluminescence (B and D). The strain on the left in each panel was a *laf* (lateral flagella)-*lux* fusion mutant and produced light only when grown on an agar surface. The fusion strain on the right was constitutive for light production and is shown for comparison. To obtain transducing lysates for mutagenesis of *Vibrio*, strain MC4100 lysogenic for Plclr100CM and mini-Mu*lux*($Tc^R$) was temperature-induced (*9*). The $Lux^+$ phenotype of fusion strains was stable when the cells were grown at temperatures (≤30°C) which maintained the activity of the temperature-sensitive repressor of Mu transposition.

mately 1000-fold. A similar response (600-fold increase) was observed when arabinose was used to induce light in the *ara-lux* fusion mutant. The induction of light production in these fusion mutants was characteristic of *lac* and *ara* gene control in wild-type strains.

Fusion mutants of *Vibrio parahaemolyticus* were also isolated. Transducing phage Pl, specifically Plclr100CM, was used to mobilize mini-Mu*lux*($Tc^R$) into *Vibrio* bacteria. Bacteriophage Pl packages mini-Mu*lux*($Tc^R$) DNA and infects *V. parahaemolyticus* but does not replicate in this host (*9, 10*). Since this host was resistant to kanamycin, mutagenesis was performed with mini-Mu*lux*($Tc^R$). Mutants resistant to tetracycline and defective in lateral flagella function were collected. Mutants in lateral flagella were incapable of swarming over agar surfaces. Transcription of many lateral flagella genes (*laf*) takes place only when bacteria are propagated on the surface of nutrient agar plates and not when grown in liquid media (*9*). A typical *laf-lux* fusion mutant produced light only when the bacteria were grown on the surface of a nutrient agar plate (Fig. 3). As in the previous examples, light production mirrored the regulatory characteristics of the target gene.

Light was measured by means of x-ray or photographic film, a scintillation counter in chemiluminescence mode, or visual observation. It is convenient to identify luminescent colonies or to study the influence of physiological factors on gene expression in fusion strains by making exposures with x-ray film placed in close proximity to culture plates in a darkroom. The colonies were easily visible in a darkened room (Fig. 3). However, fully induced *ara-lac* fusion strains were barely visible. Several factors may influence the intensity of the light produced by *lux* fusions. The host bacterium supplies the energy source, $FMNH_2$, for the light reaction, and the amount of this reductant may vary with bacterial strain or physiological conditions. The stability or activity of luciferase and the associated *lux* enzymes and the capacity to transcribe or translate *lux* genes may also be influenced by the particular host. *Escherichia coli* and *V. parahaemolyticus* were suitable hosts for light production and for mini-Mu*lux* transposition. It is likely that other genera of bacteria can be used as well. Since mini-Mu*lux*, like phage Mu, integrates into the host genome at random sites, it should be possible to use mini-Mu*lux* to study the regulation of a great variety of gene systems. In contrast to fusions with *lacZ* as the indicator gene, use of mini-Mu*lux* fusions does not require that the host bacterium be $Lac^-$. Light can be measured simply, sensitively, and without perturbing the cell. Strains can also be marked with a traceable phenotype by transposing the *lux* genes into the genome.

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11. Approximately 30 to 40 percent of the $Lac^-$ or $Ara^-$ mutants produced detectable amounts of light. Fifty percent of mini-Mu*lux*($Km^R$) insertions would be expected to be $Lux^+$ if the orientation of insertion were random. Transposon-generated terminal deletions would reduce this frequency as would insertion in control regions or in regulatory genes such as *araC*; expression of *araC* would not be induced by arabinose.
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## Interspecific Morphogens Regulating Prey-Predator Relationships in Protozoa

Abstract. *The ciliate* Euplotes octocarinatus *and some close relatives of it are triggered by predator-released substances to undergo morphogenetic changes that inhibit their engulfment. The changes occur within a few hours and do not require cell division. They are perpetuated during reproduction so long as the concentration of the morphogen is maintained. The ability of* Euplotes *to respond to predator-produced signals by a defensive change in cell architecture probably provides an effective mechanism for damping population oscillations of both prey and predators and fosters coexistence. The signal-induced cell transformation merits study for its own sake because of its developmental implications.*

Although prey-predator relationships are often extremely complex, population geneticists elect to study them because they provide valuable insights into subtle processes of evolution. Particularly interesting are mechanisms that damp population oscillations of prey and predator and thus facilitate their coexistence. Heterogeneity of the prey population provides an example of one such mechanism. The ability of predators to switch to alternative food when the prey becomes scarce is another. A third mechanism, not yet well studied, is predator-induced defense. Many plants (*1*) and a few animals (*2–4*) mobilize inducible defenses in response to attack by consumers. Inducible defenses are produced only in response to stimuli from consumers and disappear shortly after the stimuli are withdrawn. The nature of the signals that trigger the defensive responses in the prey organisms is in most cases unknown. We now describe a newly discovered predator-induced defensive response occurring among protozoa. The system we studied provides favorable conditions for identifying the signal released by the predator, in this case most probably a polypeptide. It enables us, in addition, to study the development of the defensive response, a drastic change in cell sculpture that makes engulfment by the predator more difficult or even impossible. The signal-induced cell transformation is not only of ecological importance. It also provides an easy-to-handle model system to study morphological changes in cells. When the morphogen is present, the changes can be induced at will, under strictly controlled conditions, in large numbers of cells, in a synchronized fashion. This property could be useful for studying the process induced by the binding of the morphogen to specific cell receptors and the consequent morphogenetic events.

The ability of *Euplotes* to develop extended wings and ridges in response to factors released by predatory ciliates was discovered when we fed the hymenostome *Lembadion lucens* with the freshwater hypotrich *Euplotes octocarinatus*. Figure 1 shows *E. octocarinatus* cells before and after exposure to *Lembadion*-conditioned medium. Exposed cells develop prominent lateral wings, which give them an almost circular appearance, an extended ventral projection, and a particularly protuberant dor-