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The goal of this project was to explore the sensory mechanisms which control expression of bioluminescence in the marine bacterium *Vibrio harveyi*. Genetic methods were used to discover a complex network of genes which encode functions for the production of extracellular, chemical signals (autoinducers) and for the synthesis of signal receptors (sensor kinases and response regulators). The genes and proteins defined by this study resemble elements of the phosphorelay paradigm known as two-component signal transduction. Therefore, the *Vibrio harveyi* system is considerably different from the quorum sensing mechanism used by other luminous bacteria.

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FINAL REPORT

GRANT #: N00014-93-1-0697

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GRANT TITLE: Sensory Control and Function of Bacterial Bioluminescence

AWARD PERIOD: 15 May 1993 - 14 May 1996

OBJECTIVE: The goal of this research was to explore the sensory mechanisms which control the expression of bioluminescence in the marine bacterium *Vibrio harveyi*. Examination of sensory control was focused on the genetic regulatory pathways which mediate the response to extracellular signaling molecules synthesized by the bacterium (autoinducers) and to other factors such as nutrient availability, oxygen tension and redox or other general indicators of the metabolic state of the cell.

APPROACH: Recombinant DNA methods and other genetic technologies were applied to the model marine bacterium, *Vibrio harveyi*, to investigate luminescence control and function. Transposon-directed, chemical, and allelic exchange mutagenesis were used to construct mutants which were then used to identify regulatory genes and to evaluate gene function. Luminescence genes (*lux*) were cloned using transposon-linked markers and complementation tests. Once cloned, the *lux* genes were sequenced and compared to other genes in the database to infer functional relationships. Reporter gene fusions and mRNA transcripts were also analyzed to identify regulatory connections and to map control circuits.

ACCOMPLISHMENTS: From analysis of the sequence of cloned lux regulatory genes and the phenotypes of mutants with lux defects constructed by allelic exchange mutagenesis, we developed a model for the molecular mechanism of density-dependent control of luminescence (also known as "quorum sensing") in *Vibrio harveyi*. Density-dependent control is mediated by two different autoinducers (bacterial pheromones) which are detected by two different sensory receptor systems. The LuxN protein is the receptor for one system and the LuxP and LuxQ proteins together function as the receptor for the second system. Signals from both signal transduction systems converge and are integrated by the LuxO protein which then activates expression of the *luxCDABEGH* operon encoding the luminescence enzymes. Because of the LuxN, Q and O proteins resemble proteins of a large family of adaptive response regulators, a few of which have been studied mechanistically, we infer that the biochemical process of signal transduction initiated by autoinducer binding consists of a series of phospho-transfer reactions which culminate in the modification of the LuxO protein resulting in the inactivation of its repressor activity. The LuxO protein could directly control (repress) *luxCDABEGH* transcription or it could function indirectly by controlling transcription of the *luxR* gene which encodes an activator of *luxCDABEGH* transcription.

We also identified three different genetic loci which stimulate light production in a reconstituted luminescence system in recombinant *E. coli* containing *luxR* and the *luxCDABEGH* operon. These loci contain candidates for genes which encode functions important for sensory control. Examination was focused on one gene, *luxT*, which was mapped and shown to activate transcription of *luxR*. DNA sequencing and mutagenesis for phenotype analysis was underway at the end of the award period.

CONCLUSIONS: Previous work with the light organ symbiont, *Vibrio fischeri*, revealed a signal-response mechanism, the *luxR-luxI* system, which controls density-dependent expression of luminescence. This regulatory paradigm has now been found in diverse genera of bacteria where it regulates a broad range of properties. Intercellular communication in *V. harveyi* appears to be more complex, involves multiple signals and sensors and employs a molecular mechanism remarkably different from that in *V. fischeri*. Part of the information processing network has already been identified, and further work should reveal how different inputs are channeled and integrated. It is clear that work on control of bacterial luminescence is yielding insights into novel control and communication mechanisms.

SIGNIFICANCE: These discoveries help us to understand how bacteria sense and adapt to changing circumstances in the marine environment and can also be extrapolated to understand communication and control in other non-marine bacteria as well.

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PUBLICATIONS AND ABSTRACTS (for total period of grant):

1. Bassler, B., M. Wright, and M. Silverman. 1994. Sequence and function of LuxO, a negative regulator of luminescence in Vibrio harveyi. Mol. Microbiol. 12:403-412.
2. Bassler, B. L., M. Wright, and M. R. Silverman. 1994. Multiple signaling systems controlling expression of luminescence in Vibrio harveyi: Sequence and function of genes encoding a second sensory pathway. Mol. Microbiol. 13:273-286.
3. Bassler, B. L., and M. R. Silverman. 1995. Intercellular Communication in Marine *Vibrio* Species: Density-dependent Regulation of the Expression of Bioluminescence. in Two-Component Signal Transduction. J. A. Hoch, and T. J. Silhavy, editors. American Society for Microbiology, Washington, DC, pp. 431-445.