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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The lux genes of Vibrio fischeri encode the ability of this marine bacterium to produce light. V. fischeri occurs at high density in specialized light-emitting organs of certain marine fish, where the light produced is used by the fish. V. fischeri is also found in seawater, where it exists as a member of the bacterioplankton. In the planktonic habitat light-production is not useful and in fact V. fischeri possesses a genetic control mechanism which enables light production when the bacteria exist in the symbiotic state but does not allow synthesis of the light-emitting system when V. fischeri is in the planktonic habitat. This regulatory phenomenon is termed autoinduction, and the aim of this research is to fully elucidate the mechanism of autoinduction. Specific objectives of this research effort include a structure/function analysis of the sensory receptor; the LuxR protein, purification of the LuxR protein and development of a defined in vitro assay for studying lux gene transcription.

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PRINCIPAL INVESTIGATOR: Everett P. Greenberg

INSTITUTE: University of Iowa

GRANT TITLE: Regulation of *lux* Genes in *Vibrio fischeri*: Control of a Symbiosis-related Gene Expression System in a Marine Bacterium

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OBJECTIVE: To investigate the mechanism of autoinduction of the *Vibrio fischeri lux* genes and to understand some of the physical factors that affect autoinduction. Specific objectives include developing an understanding of the nature of the autoinducer interaction with the receptor, LuxR, and the nature of the LuxR interaction with *lux* DNA.

ACCOMPLISHMENTS (last 12 months): We have completed our 5' deletion analysis of *luxR* (see enclosed preprint). Our previous point mutational analysis (see enciosed reprint) indicated that amino acid residues 79-127 of the 250-residue LuxR protein form an autoinducer-binding region, and residues 184-230 include the DNA-binding region. The deletion analysis demonstrates that as is true of the wild-type LuxR, truncated LuxR proteins with N-terminal deletions through the autoinducer-binding region are capable of activating luminescence gene transcription. Unlike the wild-type protein, the N-terminal deletion proteins are autoinducer independent. In fact, proteins containing the C-terminal 30% only of LuxR appear to be fully active as transcriptional actvators. These data support a model whereby the N-terminal region of LuxR serves to mask the activity of an independently folded C-terminal domain that is the *lux* gene activator. According to this model, autoinducer binds to the N-terminal domain and alters it in such a way as to unmask the activity of the C-terminal domain. In related experiments it was demonstrated that the N-terminal region of LuxR is required for a second function. In addition to masking the activity of the C-terminal domain the N-terminal region is nessasary for negative autoregulation of *luxR* transcription.

Attempts to study the activity of purified LuxR in vitro have stalled while we test the hypothesis that this protein is membrane bound. If indeed the suggestion that LuxR is membrane bound proves to be correct, it would have a major influence on our approach to in vitro studies. Although *luxR* does not code for an N-terminal signal peptide, the LuxR protein does contain two potentially membrane spanning hydrophobic stretches. Therefore we have applied Tn*phoA* fusion technology in hopes of obtaining information about the cellular location of LuxR. To date we have one Tn*phoA* fusion that in fact directs *E. coli* to synthesize active alkaline phosphatase and we have demonstrated that the fusion protein fractionates with membranes. The location of this fusion (alkaline phosphatase is fused to LuxR residue 139) is in a region that should reside on the periplasmic side of LuxR assumming a hypothetical model based on the location of the two potential membrane spanning regions. One must be cautious with interpretations based on a single fusion. We are currently generating ten additional fusions by site-directed mutagenesis.

We have also continued to study *V. fischeri* ES114, a specific symbiont from the light organ of the squid, *Euprymna scolopes* (see enclosed abstract). This strain does not produce light in laboratory culture unless autoinducer is added to the medium, and we are interested in why it does not produce autoinducer. We have cloned the ES114 luminescence gene cluster and we have demonstrated that 1) the genes are organized in a fashion similar to those in autoinducer synthesizing strains, 2) the *luxI* gene directs *E. coli* to synthesize autoinducer, and 3) even when *luxI* transcription is activated in ES114, this strain makes very little autoinducer in laboratory culture. Thus ES114 is either incapable of producing a substrate for autoinducer synthase (which could be provided by the squid), there is posttranscriptional control such that ES114 contains little or no synthase, or there is an inhbitor of synthase activity in ES114 grown under standard laboratory conditions.

SIGNIFICANCE: The 5'-deletion analysis supports a strctural model of LuxR in which there are two modules. A C-terminal transcriptional activator module, and an N-terminal module that can mask the activity of the C-terminal module. Apparently the binding of autoinducer to the N-terminal module inhibits the masking activity. The N-terminal module is also required for negative autoregulation of *luxR*. The module concept has implications rogarding the evolution of LuxR and other members of the LuxR

family (also refered to as the FixJ family or the MalT family). The identification of an independently folded C-terminal domain suggests a number of other experiments that should yield a greater understanding of the structure and function of LuxR. The application of Tn*phoA* fusion technology will allow us to establish the cellular location of LuxR. At this time it appears that LuxR may be compartmentalized to the cytoplasmic membrane. Finally the studies of the squid symbiont allow us to begin to study the development of the symbiosis at a molecular level.

WORK PLAN (next 12 months): We plan to complete the Tn*phoA* mutagenesis studies. We will also embark on a 3' deletion analysis of *luxR* that should complement our 5' deletion analysis. We will determine the specific defect in ES114 leading to the inability of this strain to synthesis significant quantities of autoinducer, and we will initiate experiments to determine the significance of the ES114 *lux* genes to infection of squids by ES114.

INVENTIONS (last 12 months): No inventions.

PUBLICATIONS AND REPORTS:

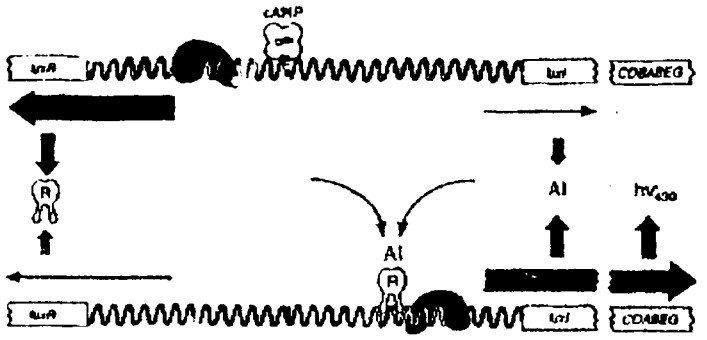
1. Slock, J., VanRiet, D., Kolibachuk, D., Greenberg, E. P. (1990) Critical regions of the *Vibrio fischeri* LuxR protein defined by mutational analysis. J. Bacteriol. *172,3974-3979.*

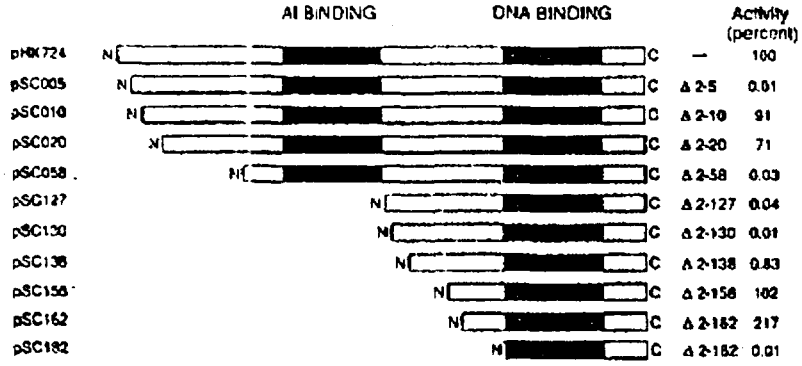
2. Dunlap, P. V., Greenberg, E. P. (1991) The role of intercellular chemical communication in the *Vibrio fischeri*-Monocentrid fish symbiosis. In Press.

3. Choi, S. H., Greenberg, E. P. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent *lux* gene activating domain. Proc. Natl. Acad. Sci. (USA) In Press

4. Choi, S. C., Greenberg, E. P. (1991) Activation of the *Vibrio fischeri* luminescence genes by truncated LuxR proteins is autoinducer independent. Abst. Annu. Mtg. Amer. Soc. Microbiol. *91* .

5. Gray, K. M., Greenberg, E. P. (1991) Cloning and characterization of the luminescence gene cluster from *Vibrio fischeri* ES114, the light organ symbiont of the sepiolid squid *Euprymna scolopes.* Abst. Annu. Mtg. Amer. Soc. Microbiol. *91* .





OBJECTIVES

-Determine the function of the N-terminal arm of the LuxR protein

-Determine the cellular location of LuxR

-Analyze the *lux* genes cloned from *Vibrio fischeri* ES114, the specific bacterial symbiont of the squid, *Euprymna scolopes* in an attempt to understand why this strain does not produce autoinducer in laboratory culture

ACCOMPLISHMENTS

-Completed 5'-deletion analysis of *luxR*. Demonstrated that the C-terminal 30% of the LuxR protein forms an autoinducer-independent transcriptional activator of the luminescence genes. The N-terminal region of LuxR masks the activity of the C-terminal region in the abscence of autoinducer. The N-terminal region is also required for negative autoregulation of *luxR*.

-Obtained preliminary evidence that LuxR is a membrane protein using Tn*phoA* fusion technology

-Demonstrated that ES114 contains a *luxI* gene that is fully functional in *E. coli*. This gene shows a similar organization in the *luxICDABEG* operon as other strains of *V. fischeri*. The inability of ES114 to synthesize autoinducer is not due to a defective *luxI* or a lack of *luxI* transcription

SIGNIFICANCE

-A C-terminal module of LuxR that serves to activate transcription of luminescence genes and an N-terminal module that blocks the activity of the C-terminal module in the absence of autoinducer have been identified.

-LuxR localization studies will allow a rational design for development of in vitro LuxR activitiy assays.

-Studies of the ES114 *lux* genes improves understanding of the symbiotic relationship between this bacterium and the host squid.

E. P. Greenberg, Univ. Iowa; 1991