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13. ABSTRACT (Maximum 200 words)  <p>The general objective of the proposed research is to fully elucidate the mechanism of quorum sensing and response in bacteria by continuing investigations of the most well-developed model for this phenomenon, autoinduction of <i>lux</i> genes in <i>Vibrio fischeri</i>. This research should continue to reveal general rules governing regulation of bacterial genes used specifically in symbiotic associations with marine animals. This research program also has recently provided and should continue to provide insights into how bacteria interact with eukaryotic hosts in a more universal way. Little is known about synthesis of the autoinducer, the sensory signal, other than that it is catalyzed by the <i>luxI</i> gene product. Thus, an analysis of the structure and function of LuxI was initiated. This analysis involved the construction of point and deletion mutations in <i>luxI</i> and studies of the activity of the mutant proteins encoded by these defective genes. This analysis also involved studies of autoinducer synthesis in <i>luxI</i>-containing <i>E. coli</i> amino acid biosynthesis mutants, and studies of the biochemistry of purified enzymes.</p>				
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INSTITUTION: University of Iowa

GRANT TITLE: Quorum Sensing in *Vibrio fischeri*: Cell Density-dependent Activation of Symbiosis Genes in a Marine Bacterium

AWARD PERIOD: 1 November 1994-31 March 1998

OBJECTIVE: Our general objective is to investigate the mechanism of autoinduction of the *Vibrio fischeri* lux genes. The specific goal was to understand the biochemical basis of acylhomoserine lactone, autoinducer signal synthesis.

APPROACH: Three basic approaches have been used to elucidate the mechanism of acylhomoserine synthesis in *V. fischeri* and other bacteria. I. We have studied the influence of amino acid biosynthesis mutations on autoinducer synthesis in recombinant *E. coli*. II. We have studied the activity of purified fusion and native autoinducer synthases in vitro. II. We have analyzed the activity of mutant forms of autoinducer synthases.

ACCOMPLISHMENTS Our research program over the past three years has led to a reasonably comprehensive view of the mechanism of autoinducer synthesis in gram-negative bacteria. The I proteins are synthase enzymes, which use as substrates acyl-ACP and SAM. The synthase catalyzes the formation of an amide bond and the intermediate, acyl-SAM, which then cyclizes to form acyl-homoserine lactone. There is a well conserved active site corresponding to residues 25-104 of the 193-amino acid LuxI protein. In contrast to previous proposals the reaction mechanism does not involve acyl group transfer to an active site cysteine or in fact to any other amino acid residue. A detailed analysis of the kinetics of pure, native RhII indicates the synthesis of autoinducer proceeds via an ordered, sequential mechanism.

Several other important research findings evolved as side projects made possible by this grant. The protein AinS was studied in collaboration with Dr. Paul Dunlap at the University of Maryland. This is *V. fischeri* protein that directs the synthesis of an acylhomoserine lactone, but it is not a LuxI homolog. We purified an AinS-MBP fusion and this protein catalyzes the synthesis of octanoylhomoserine lactone from SAM and either octanoyl-ACP or octanoyl-CoA. AinS represents a second family of acylhomoserine lactone synthase enzymes. We also established details about the architecture of the luxI promoter region. The LuxR protein appears to function in much the same way as other ambidextrous transcriptional activators. We also established that at least in some bacteria, acylhomoserine lactone signals are involved in formation or dispersal of biofilms.

SIGNIFICANCE: There is a growing appreciation that quorum sensing is of general importance in bacterial gene regulation. It is important not only to luminous marine symbiotic bacteria, but it is important for virulence of a number of animal and plant pathogens. Our studies have established important basic information about the generation of

autoinducer signals, the activation of responsive genes and the role of quorum sensing in the ecology of gram-negative bacteria. Efforts are under way to commercialize quorum sensing technology and much of the work supported by the ONR forms the basis of intellectual property for these efforts.

PATENT APPLICATION One patent has been issued (An Autoinducer Molecule), another on cell signaling and biofilm formation has been filed. A third on signal synthesis and inhibitors of signal synthesis is in preparation.

AWARD INFORMATION During the period of this grant Dr. Greenberg was elected as an ASM Foundation Lecturer, he was appointed to the Editorial Board of Current Opinions in Microbiology, he continued as Editor of the Journal of Bacteriology and Associate Editor of Annual reviews of Microbiology, and he has served as an ad hoc reviewer for the DOE and the Cystic Fibrosis Foundation.

PUBLICATIONS (from November 1, 1994)

1. Stevens, A. M., K. Dolan, and **E. P. Greenberg** (1994) Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. Proc. Natl. Acad. Sci. USA **91**:12619-12623.
2. Hanzelka, B. L., and **E. P. Greenberg**. (1995) Evidence that the N-Terminal region of the *Vibrio fischeri* LuxR protein constitutes an autoinducer-binding domain. J. Bacteriol. **177**: 815-817.
3. Pearson, J. P., L. Passador, B. H. Iglewski, and **E. P. Greenberg**. (1995) A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA. **92**:1490-1494.
4. Parrish, K. D., and **E. P. Greenberg**. (1995) A rapid method for extraction and purification of DNA from dental plaque. Appl. and Environ. Microbiol. **61**:4120-4123.
5. Kolenbrander, P. E., K. D. Parrish, R. N. Andersen, and **E. P. Greenberg**. (1995) Intergeneric coaggregation of oral *Treponema* spp. with *Fusobacterium* spp. and intrageneric coaggregation among *Fusobacterium* spp. Infect. and Immun. **63**:4584-4588.
6. Poellinger, K. A., J. -P. Lee, J. V. Parales, Jr., and **E. P. Greenberg**. (1995) Intragenic suppression of a luxR mutation: characterization of an autoinducer-independent LuxR. FEMS microbiol. Lett. **129**:97-102.
7. Gray, K. M., J. P. Pearson, J. A., Downie, B. E. A. Boboye, and **E. P. Greenberg**. (1996) Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum*: autoinduction of stationary phase and rhizosphere-expressed genes. J. Bacteriol. **178**:372-376.
8. Schaefer, A. L., B. L. Hanzelka, A. Eberhard, and **E. P. Greenberg**. (1996) Quorum sensing in *Vibrio fischeri*: probing autoinducer-LuxR interactions with autoinducer analogs. J. Bacteriol. **178**: 2897-2901.
9. Smith, J. J., S. M. Travis, **E. P. Greenberg**, and M. J. Welsh. (1996) Cystic Fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell. **85**:229-236.
10. Fuqua, W. C., S. C. Winans, and **E. P. Greenberg**. (1996) Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annual Reviews of Microbiology. **50**:727-751.

11. Schaefer, A. L., D. L. Val, B. L. Hanzelka, J. E. Cronan, Jr., and **E. P. Greenberg**. (1996) generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. Proc. Natl. Acad. Sci. USA. **93**: 9505-9509.
12. Hanzelka, B. H., and **E. P. Greenberg**. (1996) Quorum sensing in *Vibrio fischeri*: evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis. J. Bacteriol. **178**: 5291-5294.
13. Stevens, A. M., and **E. P. Greenberg**. (1997) Quorum sensing in *Vibrio fischeri*: Essential elements for activation of the luminescence genes. J. Bacteriol. **179**: 557-562.
14. Bassler, B., A. M. Stevens, and **E. P. Greenberg**. (1997) Cross-species induction of the quorum sensing bacterium *Vibrio harveyi*. J. Bacteriol. **179**: 4043-4045.
15. Hanzelka, B. L., A. M. Stevens, M. R. Parsek, T. J. Crone, and **E. P. Greenberg**. (1997) A mutational analysis of the *Vibrio fischeri* LuxI polypeptide: critical regions of an autoinducer synthase. J. Bacteriol. **179**: 4882-4887..
16. Greenberg, E. P. (1997) Quorum sensing in gram-negative bacteria. ASM News. **63**: 371-377.
17. Parsek, M. R., A. L. Schaefer, and **E. P. Greenberg**. (1997) Analysis of random and site-directed mutations in *rhII*, a *Pseudomonas aeruginosa* gene encoding an acylhomoserine lactone synthase. Molec. Microbiol. **26**: 301-310.
18. Puskas, A., **E. P. Greenberg**, S. Kaplan, and A. L. Schaefer. (1997) A quorum sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. J. Bacteriol. **179**: 7530-7537.
19. Davies, D., M. Parsek, W. Costerton, J. Pearson, B. Iglewski, and **E. P. Greenberg**. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. **280**: 295-298.
20. Fuqua, C., and **E. P. Greenberg** (1998) Self perception in bacteria: quorum sensing with acylated homoserine lactones. Current Opinions in Microbiology. **1**: 183-189.
21. Fuqua, C., and **E. P. Greenberg** (1998) Cell-to-cell communication in *Escherichia coli* and *Salmonella typhimurium*: they may be talking but who's listening. Proc. Natl. Acad. Sci. USA **95**: 6571-6572.
22. Stevens, A. M., and E. P. Greenberg (1998) Transcriptional activation by LuxR. In press.