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The symbiosis between the Hawaiian sepiolid squid *Euprymna scolopes* and its marine luminous bacterial partner *Vibrio fischeri* was developed as a model system by which to study the process of recognition and specificity in the establishment of animal-bacterial associations. These early studies on this system revealed that complex, redundant systems ensure the fidelity of the relationship between these two partners. Using routine techniques of cell biology, biochemistry and molecular biology, we determined that specific receptor-ligand interactions are required during colonization, and that proteins thus far only associated with pathogenic associations are recruited into the function of modulating this cooperative symbiosis. In addition, comparative studies with different host and symbionts species, obtained from various locations around the globe, demonstrated that the squid-vibrio relationships have coevolved, and determined that the host-symbiont specificity is determined during the early recognition phases of the association.

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FINAL REPORT

GRANT #: N00014-91-J-1357

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INSTITUTION: University of Hawaii

GRANT TITLE: The biochemical and molecular mechanisms mediating recognition and specificity in a squid/luminous bacteria symbiosis

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OBJECTIVE: To determine the biochemical and molecular bases of host-symbiont recognition and specificity in the mutualistic association between the cephalopod *Euprymna scolopes* and its bacterial symbiont *Vibrio fischeri*. Specifically: 1) biochemically define receptor-ligand interactions between partners during the recognition process; 2) characterize those aspects of the host-created environment surrounding the symbiont that participate in specificity; and, 3) taking advantage of the ability to infect the host under experimental conditions, determine the fidelity of specificity among Indowest-Pacific and Mediterranean sepiolids and their bacterial partners.

APPROACH: We have used currently available tools of biochemistry and molecular biology to accomplish our research aims. To study receptor-ligand interactions, we have employed a wide array of cytochemical techniques coupled with confocal microscopy to determine specific types of interactions and define the precise cell types involved. In efforts to define the host-created environment, we have used 2-D gel electrophoresis to characterize the proteomes of aposymbiotic and symbiotic animals, as well as adult animals over the diel cycle. In addition, we have generated cDNA libraries of light organs that were used in this study to characterize the occurrence of a halide peroxidase in the symbiotic tissue. For comparative studies, we collected specimens from several locations. Molecular phylogenies were constructed for both the hosts and symbionts, and host habitat loyality was assessed by determining whether strains isolated from a given host were capable of infecting other host species, both alone and in competition with native strains. In addition, using GFP-labeled bacteria in infection experiments, coupled with confocal microscopy, we characterized the mechanism by which non-native strains are outcompeted.

ACCOMPLISHMENTS: We have determined that recognition and specificity in the squid-vibrio association involves complex, redundant mechanisms of the host and symbiont. Mannose adhesin-glycan interactions are required for infection, and bacteria-induced rearrangement of host cytoskeleton results in an increasing intimacy of the bacteria-host cell-cell interaction in the days following infection. We have determined that two host cell types are interacting with the bacterial symbionts: the epithelial lining of the crypt space, and a population of macrophages that are trafficked into the crypts. By transmission electron microscopy, we have observed bacterial cells within these host macrophages. We are presently defining whether these macrophages phagocytose *V. fischeri*, or nonspecific interlopers into the light organ crypts. Two-dimensional gel electrophoresis has revealed dramatic symbiosis-induced changes in the light organs of the host, both during

the onset of the symbiosis and during the progression of the symbiosis through its diel cycle. A halide peroxidase, related to the mammalian antimicrobial protein myeloperoxidase, occurs in high abundance in the light organ, and other tissues (accessory nidamental gland and intestine) that interact with bacteria in the squid host. The onset of symbiosis causes a lowering of the expression of this gene in the light organ. Molecular phylogenies of the partners of this symbiosis provide strong evidence for coevolution of host and symbiont. In cross-infection experiments, all squid symbionts are capable of infecting any squid host. However, the ability of a strain to compete successfully with other strains for dominance in the light organ varies; specifically, the strain that wins in a competition will always be the strain from the host most closely related to the native host. This pattern of symbiotic competence directly mirrors the molecular phylogenies. An enhanced ability to bind to host epithelia appears to be at least one mechanism by which native strains effect dominance in the light organ.

CONCLUSIONS: The *Euprymna scolopes-Vibrio fischeri* association is an excellent model for studying a variety of aspects of symbiotic associations, including specificity and recognition, development, and mechanisms by which a stable, persistent associations are created between animals and their bacterial partners.

SIGNIFICANCE: In general, these studies pioneered the development of the squid-vibrio model. Our specific findings show that: 1) multiple mechanisms are involved in the determination of specificity, including both receptor-ligand interactions and the creation of a biochemical environment in the crypt space in which only the specific symbiont can survive; 2) symbiosis drastically changes the biochemical and molecular signature of the associated tissues; 3) mechanisms thus far associated only with antimicrobial activity are involved in the modulation of a cooperative association; and, 4) determinants of coevolution between host and symbiont are expressed at the initial stages of the symbiosis and are mediated by differences in cell-surface molecules.

PUBLICATIONS AND ABSTRACTS (for total period of grant 2/91 - 1/97)

ARTICLES IN PRESS ACKNOWLEDGING SUPPORT FROM ONR -

1. McFall-Ngai, MJ (1998) Pioneering the squid-vibrio Model. **ASM News** 64:639-645.

2. McFall-Ngai, MJ, C Brennan, V Weis, and L Lamarcq. (1998) Mannose adhesin-glycan interactions in the *Euprymna scolopes-Vibrio fischeri* symbiosis. In: New Developments in Marine Biotechnology (Y Le Gal and H Halvorson, eds). Plenum Press, NY, pp. 273-276.

3. Nishiguch, MK, EG Ruby, and MJ McFall-Ngai. (1998) Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in sepiolid squid-vibrio symbioses. **Appl. Env. Microbiol.** 64:3209-3213.

4. Lamarcq, LH and MJ McFall-Ngai (1998) Induction of a gradual, reversible morphogenesis of its host's epithelial brush border by *Vibrio fischeri*. **Infect. Immun.** 66:777-785

5. McFall-Ngai, MJ, and EG Ruby. (1998) Squids and vibrios: when first they meet. **BioScience** 48:257-265..

6. McFall-Ngai, MJ. (1998) The development of cooperative associations between animals and bacteria: establishing détente between Domains. **Amer Zool** (in press)

7. Nishiguchi, MK, EG Ruby, and MJ McFall-Ngai. (1997) Phenotypic bioluminescence as an indicator of competitive dominance in the *Euprymna-Vibrio* symbiosis. In: *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons* (JW Hastings, LJ Kricka and PE Stanley, eds). John Wiley & Sons, NY, pp. 123-126.

8. Weis, VM, AL Small, and MJ McFall-Ngai. (1996) A peroxidase related to the mammalian antimicrobial protein myeloperoxidase in the *Euprymna-Vibrio* mutualism. **PNAS** 93:13683-13688.

9. Boettcher, K, EG Ruby and MJ McFall-Ngai. (1996) Diel rhythm in a bacterial symbiosis. **J Comp Physiol** 179:65-73.

10. McFall-Ngai, MJ. (1996) Our essential partners. **Science Spectra** 1(4):13-19.

11. Doino, JA and MJ McFall-Ngai. (1995) Transient exposure to competent bacteria initiates symbiosis-specific squid light organ morphogenesis. **Biol Bull** 189:347-355.

12. Gates, R, O Hoegh-Guldberg, MJ McFall-Ngai and L Muscatine. (1995) Free amino acids elicit photosynthate translocation in algal-cnidarian symbioses. **PNAS** 92:7430-7434.

13. Montgomery, MK and MJ McFall-Ngai. (1995) The inductive role of bacterial symbionts in the morphogenesis of a squid light organ. A contribution to a symposium: "The role of cell-cell interactions and environmental stimuli in the development of marine invertebrates." **Amer Zool** 35:372-380.

14. McFall-Ngai, MJ (1994) Evolutionary morphology of a squid symbiosis. A contribution to a symposium: "Evolutionary morphology of marine invertebrate larvae and juveniles." **Amer Zool** 34:554-561.

15. Montgomery, MK and MJ McFall-Ngai (1994) The effect of bacterial symbionts on early post-embryonic developmental of a squid light organ. **Development** 120:1719-1729.

16. Tomarev, SI, RD Zinovieva, VM Weis, AB Chepelinsky, J Piatigorsky, and MJ McFall-Ngai (1993) Abundant mRNAs in the bacterial light organ of a squid encode a protein with high similarity to mammalian antimicrobial peroxidases: Implications for mutualistic symbioses. **Gene** 132:219-226.

17. Montgomery, MK and MJ McFall-Ngai (1993) Embryonic development of the light organ of the sepiolid squid *Euprymna scolopes*. **Biol Bull** 184:296-308.

18. Weis, VM, MK Montgomery, and MJ McFall-Ngai (1993) Enhanced production of ALDH-like protein in the bacterial light organ of the sepiolid squid *Euprymna scolopes*. **Biol Bull** 184:309-321.

19. Ruby, EG and MJ McFall-Ngai (1992) [Invited] Minireview. A squid that glows in the night: Development of an animal-bacterialmutualism. **J Bact** 174:4865-4870.

PUBLISHED ABSTRACTS

1. Nyholm, SV and MJ McFall-Ngai (1996) The microenvironment surrounding the bacterial symbionts of the *Euprymna scolopes* light organ. **Amer Zool** 36(5):68A.

2. Foster, JS, S von Boletzky and MJ McFall-Ngai (1996) Variation in symbiotic light organ development among sepiolid squids. **Amer Zool** 36(5):69A.

3. Nishiguchi, MK, EG Ruby and MJ McFall-Ngai (1996) Coevolutionary relationships between sepiolid squid and their bioluminescent bacterial symbionts. **Amer Zool** 36(5):121A.

4. Nishiguchi, MK, L Lamarcq, EG Ruby and MJ McFall-Ngai (1996) Competitive dominance of native strain symbioitic bacteria measure by colonization and in situ hybridization. **Amer Zool** 36(5):41A

5. Doino, JA and MJ McFall-Ngai (1996) Morphogenesis of a squid light organ is accompanied by changes in protein synthesis patterns. **Amer Zool** 36(5):41A

6. Doino, JA and MJ McFall-Ngai (1995) A study of protein synthesis in the light organ of Euprymna scolopes immediately following bacterial colonization. **Amer Zool** 35(5):30A.

7. Foster, JS and MJ McFall-Ngai (1995) Bacteria-induced cell death in epithelial tissue during morphogenesis of the symbiotic organ of *Euprymna scolopes*. **Amer Zool** 35(5):112A.

8. Small, AL and MJ McFall-Ngai (1993) Changes in the oxygen environment of a symbiotic squid light organ in response to infection by its luminous bacterial symbionts. **Amer Zool** 33(5):61A.

9. Doino, JA and MJ McFall-Ngai (1993) Transient exposure to luminous symbiotic bacteria induces morphogenesis of the host squid light organ. **Amer Zool** 33(5):69A.

10. Hensey, S and M McFall-Ngai (1992) A surface peptide of the bacterium *Vibrio fischeri* plays a key role in specificity and recognition in the symbiosis with the squid Euprymna scolopes. **Amer Zool** 32(5):37A.

11. Weis, V, A Small, M Nguyen and M McFall-Ngai (1992) Peroxidase-like protein found in the symbiotic light organ of the squid *Euprymna scolopes*. **Amer Zool** 32(5):45A.

12. Weis, VM and M McFall-Ngai (1991) Immunolocalization of an aldehyde dehydrogenase-like protein in the bacterial light organ of the squid *Euprymna scolopes*. **Amer Zool** 31(5):56A.