

# Developmental biology in marine invertebrate symbioses

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Associations between marine invertebrates and their cooperative bacterial symbionts offer access to an understanding of the roots of host–microbe interaction; for example, several symbioses like the squid–vibrio light organ association serve as models for investigating how each partner affects the developmental biology of the other. Previous results have identified a program of specific developmental events that unfolds as the association is initiated. In the past year, published studies have focused primarily on describing the mechanisms underlying the signaling processes that occur between the juvenile squid and the luminous bacteria that colonize it.

### Addresses

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### Abbreviation

AHL acylated homoserine lactone

### Introduction

Since its beginnings over 1 billion years ago, the process of metazoan evolution has taken place largely within the sea, the environment in which all known animal phyla first appeared. Only during the last third of this period did relatively few members of these phyla venture onto land and radiate into terrestrial habitats. In spite of these historical facts, because of our own phylogenetic position as land mammals, both a terrestrial bias and a vertebrate bias have come to dominate much of biological thought, including our ideas of how cooperative animal–bacterial associations evolve and function. The first of such symbiotic alliances are certain to have arisen within marine invertebrate taxa and, because marine invertebrates exhibit the greatest phyletic diversity in the current biosphere [1], it is likely that the greatest variety of animal–bacterial symbioses still occurs within this group. Thus, marine invertebrates and their associated microbiota provide a wealth of material for studies that can reveal much about both the diversity of host–bacterial associations and the origins of the mechanisms by which symbioses develop and persist [2,3]; for example, investigations of signaling pathways associated with animal–bacterial interactions have recently revealed that certain ancient mechanisms of host response, such as those mediated by the Toll receptor family [4•], are shared by both vertebrates and invertebrates, whose common ancestor arose in the marine environment. Comparative study of these types of host response pathways should reveal which components are essential ancient functions and which are specific to a given taxonomic group. Further, such analyses will also provide clues to the mechanisms by

which the cooperative communication between animals and their normal microbiota is subverted when chronic pathogenic infections develop and persist [5,6].

Because animal–bacterial interactions have been present since the origin of the metazoa, they are likely to be involved in shaping many aspects of host biology, including animal developmental programs. Even as metazoan body plans were gaining complexity and species were diverging, microorganisms were becoming incorporated into specific tissues and imposing selection pressure on the development of such diverse processes as digestion and immune competence [7,8••]. For these reasons, it has been proposed that the evolution of an animal can be more correctly viewed as the evolution of a community of closely interacting organisms that includes both the host and its associated microbiota [9]. Thus, when studying either the evolution of developmental mechanisms in general or the specific patterns of ontogenetic development expressed by successive generations of individuals, it is important to consider the roles played by each member of this community.

The above considerations suggest that, although underexplored, marine invertebrates and their associated microbiota provide a wealth of material for studies that can reveal much about both the diversity of host–bacterial associations and the origins of the mechanisms by which symbioses develop and persist. In this review, we examine three challenges that are encountered during the ontogenetic development of species-specific horizontally transmitted marine symbioses: preparation by the nascent host for the initial engagement; symbiont transmission to and early interactions with the target host tissues; and transformation of the association into a functioning unit. We will use the relatively well-understood light organ symbiosis between the squid *Euprymna scolopes* and the luminescent bacterium *Vibrio fischeri* [10••,11•] to illustrate some strategies that each partner has evolved to overcome these challenges. Where possible we will compare these strategies with what is known to occur in other marine associations.

### Preparation for the initial engagement

In a horizontally transmitted symbiosis, the nascent host is not exposed to symbionts throughout embryogenesis. Instead, upon hatching, it must acquire specific bacteria from the environment, which, in the case of marine associations, generally means the ambient seawater. Thus, to assure the fidelity of the association between generations, as well as to discourage opportunistic pathogenic infections, an array of morphological and biochemical features must be elaborated during embryogenesis (for a review, see [10••]). When the juvenile hatches, these features will be available to facilitate the initiation of the symbiotic interaction by the appropriate species of bacteria.

One goal of the newly hatched marine invertebrate is to increase its chances of becoming inoculated with the cells of potential symbionts present in the seawater. Terrestrial animals, such as termites and ruminants, often achieve this goal through a specific tending behavior of the adults that directly delivers a concentrated inoculum to the juvenile. In contrast, although adults of marine invertebrates may passively 'seed' the surrounding environment with bacterial symbionts [12,13], the initiation of the association rarely involves direct communication between generations. As a result, a juvenile must be equipped to obtain the necessary symbionts from the ambient seawater, in which they represent only a small fraction of the bacteria present.

Potential candidates for achieving such a goal include ciliary mucus currents, a mechanism by which many marine invertebrates obtain and process particulate food, including living microbial cells, as well as inhibit colonization by nonsymbiotic bacteria [1]. In the squid–vibrio association, ciliary mucus currents appear to be involved in colonization. A transient, superficial field of ciliated cells that is elaborated during embryogenesis [10••] and a mechanism that senses the presence of bacteria once the juvenile leaves the egg function together to facilitate symbiotic colonization [14••]. Interaction between bacteria in the ambient seawater and the juvenile squid induces the host to emit a stream of mucus-like material from a series of pores that open into the epithelia-lined crypts of the nascent light organ [11•]. Currents produced by the superficial ciliated tissue wind this material into a matrix that is suspended above these portals of colonization. *V. fischeri* cells become preferentially trapped in this mucus-like matrix and, after a few hours, migrate to the pores through which they enter and colonize the crypts. Once the symbiotic infection has been achieved, the host ceases to produce the mucus stream (SV Nyholm, personal communication), suggesting that this activity is linked to the development of the association. Similarly, after successfully promoting the harvesting of potential symbionts from the surrounding seawater, the ciliated tissue also undergoes regression [10••].

Because ciliary mucus currents are common features among marine invertebrates, it is likely that this mode of symbiotic colonization is not unique to the squid–vibrio system, although only fragmentary evidence is available from other associations; for example, juvenile chemoautotrophic tubeworms that obtain their symbiotic sulfur-oxidizing bacteria by horizontal transmission [15] bear ciliated patches of tissue associated with a transient mouth through which potential symbionts enter to colonize the host [16]. Once colonized, these ciliated patches disappear. Interestingly, in terrestrial animals the use of ciliary–mucus currents has become restricted to the moist surfaces of the respiratory and urogenital tracts, where they function to clear particles and inhibit bacterial migration to deeper tissues. These tissues are characteristically susceptible to chronic colonization by pathogenic bacteria, often in mucus-containing biofilms [6,17]. Thus, the ciliary

mucus currents in these types of chronic infections had their evolutionary origins in the behavior of particle capture in marine invertebrates. Understanding these more primitive systems may provide an insight into the mechanisms underlying the behavior of bacteria under these circumstances, and thereby, have implications for chronic disease treatments.

### Transmission and initiation of the symbiosis

Once potential symbionts are brought into the vicinity of the appropriate host tissues, they must find their way to the sites of colonization. Many marine symbioses are digestive-tract-associated and entry through the mouth serves to initiate symbiont inoculation. Usually, the gut and its associated bacterial symbioses remain in communication with the external environment throughout the life history of the host; for example, a number of marine fish species have light organ symbioses with luminous bacteria that are maintained in specialized diverticula of the gut [18]. The light organs remain connected to the digestive tract through ontogeny and bacterial symbionts are regularly shed into the gut from which they are eventually reintroduced into the surrounding seawater environment. In an interesting modification of this general pattern, the chemoautotrophic hydrothermal vent tubeworm, *Riftia pachyptila*, uses a transient digestive tract as an entry point through which the colonizing symbionts are believed to traverse from the ambient seawater to a specialized internal organ, the trophosome. These symbiotic bacteria are autotrophic and eventually support the host's nutritional needs so that exogenous sources of food are not required. As a consequence, once the bacteria have colonized the juvenile host and it begins to mature, the mouth is lost and the trophosome and its symbionts become isolated from the environment [16].

In most horizontally transmitted symbioses that have been examined, the bacterium plays an active role in the inoculation process. In particular, flagellar motility, perhaps coupled with chemotaxis, is required by several marine and estuarine *Vibrio* species to initiate either their cooperative or pathogenic associations [19]. Graf *et al.* [20] reported that non-motile mutants of *V. fischeri* were incapable of colonizing juvenile squid light organs, even when the bacteria were present in the ambient seawater at extremely high concentrations. The nature of this requirement became clearer with the discovery that the bacteria become aggregated in the host-derived mucus matrix described above, through which they must migrate to reach the light organ crypts [14••]. Recent progress toward understanding the genetics of chemotaxis in *V. fischeri* (AJ Wolfe, EJ Simel, KL Visick, *Am Soc Microbiol Abstr* 1999, 99:388) will facilitate an examination of the potential role of this behaviour in symbiosis initiation.

In a number of symbiotic associations, the bacterial cells stop elaborating flagella once they have successfully colonized their target tissue [19]. In the squid–vibrio symbiosis,

both flagellated and unflagellated cells occur in the crypts [11•,21]. The mechanisms by which the symbiotic bacterium recognizes its location and ceases expression of the dozens of genes involved in flagellar synthesis remain unknown, but analogous ones appear to operate in other marine symbionts [22•]. Interestingly, the recent recognition that the same signaling cascade that controls flagellation also coordinately regulates other colonization factors in certain pathogenic bacteria [19,23,24] focuses increased importance on this symbiotic regulatory response.

Once the appropriate bacteria arrive at the specific point of colonization, the host must present conditions that permit the symbionts to recognize this site and be retained there. This selective process is likely to be mediated by specific receptor–ligand interactions [25], as well as by the ability of the symbionts to withstand the biochemical environment created by the host cells [26•,27,28]. Only the squid–vibrio system and the mammalian intestine have been extensively studied in this respect and, in both instances, there is evidence that surface glycans function to recognize the specific bacterial symbionts and facilitate their maintenance [8••,29]. Recent mutational analyses of *V. fischeri* have implicated several surface attachment factors in the ability to persist in the association [11•].

### Transformation into the functional symbiosis

Once the appropriate bacteria have established themselves in their target tissues, both the host and the symbionts must change from an initiation mode to a persistence mode. These transformations establish the regulated exchange of the products that constitute the functional basis of the symbiosis, and promote the stable maintenance of the association. The resulting processes are often accompanied by dramatic changes in the morphology, biochemistry and molecular biology of the host tissue and less evident, but equally significant, changes in bacterial gene expression.

The symbiont's role in inducing tissue development in a marine host has been studied in only a few instances [9,12]. In the squid–vibrio associations, the bacteria induce significant remodeling of the tissues during the first few days that follow the onset of symbiosis. Bacteria-induced development includes loss of the juvenile squid's superficial ciliated field of cells [10••], the function of which seems to be confined to facilitation of light organ colonization by symbionts [14••]. Recent studies of this phenomenon indicate that part of this process involves apoptosis induced by the lipopolysaccharide of *V. fischeri* (JS Foster, MA Apicella, MJ McFall-Ngai, *Soc Dev Biol Abst* 1999, 210:337; see Now in press). In addition, the bacteria cause the epithelial cells with which they associate to elaborate more microvilli and the symbionts increase their contact with host microvilli [30]. Concomitantly, the bacteria also induce an edemic state in these host cells [31••]. Both the increase in microvillar density along the crypt epithelial brush border and the crypt cell swelling may be harbingers of enhanced signaling and/or nutrient exchange between the partners.

These early developmental events are triggered by the symbionts within hours of onset of the symbiosis [10••] and conspicuous morphological changes ensue over the first few days. In contrast, the elaboration of certain accessory tissues, which begins several days into the symbiosis, does not require the presence of *V. fischeri* [32]; however, although these dramatic morphogenetic changes of late development do not require interaction with symbionts, they are required for continued ultrastructural remodeling of the crypt epithelial cells. Further, bacteria-induced changes in host cell biochemistry and molecular biology continue for several days, if not weeks, after the initial interactions with symbionts [33]. These findings suggest that, similar to the legume–rhizobia symbioses [34,35], bacteria-induced development and maturation of the biochemical and molecular interchange between partners is a protracted dialogue between the two partners.

Turning to the symbiont's perspective, efforts to identify host-induced changes in bacterial gene expression have led to the emergence of a number of promoter-trap strategies [36•] and, more recently, microarray technologies. Application of these approaches to marine bacterial symbioses is just beginning, but already the results have provided insight into the conditions of the symbiont's environment. Preliminary data suggest that promoter activity for several *V. fischeri* genes has been reported to change as the bacterium adapts to its presence in the light organ; for example, the expression of a *V. fischeri* homologue of the *Escherichia coli* gene *brnQ*, which encodes a transporter of branched-chain amino acids, increases dramatically within 24 hours of colonization (KL Visick, EG Ruby, *Am Soc Microbiol Abstr* 1998, 98:277). This result is consistent with other studies suggesting that the host supports the growth of its symbionts by providing peptides and amino acids as nutrients [37], and that the proper regulation of nitrogen metabolism is critical [38•]. Continued examination of symbiosis-regulated gene expression, especially of novel loci with unknown function, may provide unexpected information about both nutrient exchange and signaling between the partners. An example of one such signaling process is quorum sensing, a mechanism by which symbiotic *V. fischeri* cells recognize and respond to their location in the light organ [39]. This genetic mechanism was discovered in *V. fischeri*, but has since been found to function in dozens of host-associated bacteria [40]. Briefly, these bacteria continuously secrete a small acylated homoserine lactone (AHL) molecule that will accumulate only if there is a large number of conspecifics in an enclosed space like a colonized tissue [41]. In *V. fischeri*, the synthesis of this AHL and the sensing of its concentration are controlled by the *luxI* and *luxR* genes, respectively; accumulation of AHL results in the induction of the genes responsible for luminescence and perhaps other phenotypes [42•]. Construction of null mutants in *luxI* or *luxR* results in strains that are symbiotically defective. Not only are these bacteria unable to persist normally in the light organ crypts, but they fail to

induce the characteristic swelling of the crypt epithelium [31\*\*]. Because these same two defects are also expressed by luminescence mutants of *V. fischeri*, the most parsimonious explanation is that the ability to produce light, the fundamental product of the symbiosis, is directly or indirectly responsible for the induction of normal host tissue development. In any case, we have just begun to understand the roles of bacterial AHLs in signaling the development and persistence of both cooperative and pathogenic host–bacterium associations [43,44].

## Conclusions and future perspectives

Although the vast array of marine symbioses remains largely unexplored, the recent proliferation of new molecular technologies promises to provide the opportunity to open and develop this frontier. For symbioses that involve one host and one microbial symbiont, such as the hydrothermal vent and squid–vibrio associations, the potential to sequence the entire genomes of bacterial partners like *V. fischeri* will provide invaluable information on the molecular basis for the ability to establish and maintain stable associations with animal hosts. Similarly, in the more common consortial symbioses, new molecular techniques, such as terminal restriction fragment length polymorphism (tRFLP) analyses [45], will allow biologists to determine how entire communities of digestive tract microbiota evolve within and between clades of invertebrates. From the host's perspective, the potential to build cDNA libraries and microarrays from increasingly smaller amounts of relevant host tissues, which often occur in rare animal species or in animals whose symbiotic tissues are in limited quantity, opens the horizon for the study of host response to the interaction with bacterial symbionts.

The past 12 years of research on the squid–vibrio system as a model marine symbiosis have revealed that the dialogue between a host and its associated symbiont can be extremely complex, resembling in some ways that of the well-studied legume–rhizobia symbioses [34]. Continued research on this system, as well as development of new marine models, promises to enhance greatly our understanding of how prokaryotic and eukaryotic cells make lasting alliances.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- \*\* of outstanding interest

1. Brusca RC, Brusca GJ: *Invertebrates*. Sunderland: Sinauer Associates Inc.; 1990.
  2. Douglas AE: *Symbiotic Interactions*. Oxford: Oxford University Press; 1994.
  3. Saffo MJ: *Invertebrates in endosymbiotic associations*. *Am Zool* 1992, **32**:557-565.
  4. Kopp EB, Medzhitov R: **The Toll-receptor family and control of innate immunity**. *Curr Opin Immunol* 1999, **11**:13-18. This article reviews the involvement of Toll receptors and the associated NF- $\kappa$ B signaling pathways in the functioning of the innate immune system. The components of this host response pathway are conserved among vertebrates, invertebrates and plants, suggesting an ancient mechanism by which metazoans communicate with microbes.
  5. Cossart P, Boquet P, Normark S, Rappouli R: *Cellular Microbiology*. Washington: ASM Press; 2000.
  6. Davies D, Parsek M, Costerton W, Pearson J, Iglewski B, Greenberg EP: **The involvement of cell-to-cell signals in the development of a bacterial biofilm**. *Science* 1998, **280**:295-298.
  7. Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM: **A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria**. *Science* 2000, **288**:2222-2226.
  8. Hooper LV, Falk PG, Gordon JI: **Analyzing the molecular foundations of commensalism in the mouse intestine**. *Curr Opin Microbiol* 2000, **3**:79-85.
- The authors summarize the present state of research on an emerging model system for the study of *Bacteroides thetaiotaomicron* colonization of the developing intestinal tract of the gnotobiotic mouse. Specifically, they describe the mechanism by which the bacteria induce fucosylation of receptors on the surface of enterocytes. This activity starts a feedback loop between enzymatic activity of the host and the nutrition of the bacteria.
9. McFall-Ngai MJ: **The development of cooperative associations between animals and bacteria: establishing détente among domains**. *Am Zool* 1998, **38**:593-608.
  10. McFall-Ngai MJ: **Consequences of evolving with bacterial symbionts: lessons from the squid–vibrio association**. *Annu Rev Ecol Syst* 1999, **30**:235-256.
- This review offers a comprehensive overview of findings in the squid–vibrio symbiosis throughout 1999. It describes the experimental evidence supporting the idea that specificity is controlled both by adhesin–glycan interactions between surface receptors of the host and symbiont, and by the host's creation of an oxidatively harsh environment in which only the native symbiont can persist. This article describes the influence of bacteria on host development, and the maintenance of the mature association.
11. Visick KL, McFall-Ngai MJ: **An exclusive contract: specificity in the *Vibrio fischeri*–*Euprymna scolopes* partnership**. *J Bacteriol* 2000, **182**:1779-1787.
- The current state of research into the development of the squid–vibrio symbiotic association is provided in this more abbreviated review. Particular attention is paid to the discussion of mechanisms by which the partners affect each other's development, and how specificity may be achieved and maintained.
12. Gros O, Frenkiel L, Moueza M: **Embryonic, larval, and post-larval development in the symbiotic clam *Codakia orbicularis* (Bivalvia: Lucinidae)**. *Invert Biol* 1997, **116**:86-101.
  13. Ruby EG, Lee K-H: **The *Vibrio fischeri*–*Euprymna scolopes* light organ association: current ecological paradigms**. *Appl Environ Microbiol* 1998, **64**:805-812.
  14. Nyholm SV, Stabb EV, Ruby EG, McFall-Ngai MJ: **Establishment of an animal–bacteria association: recruiting symbiotic vibrios from the environment**. *Proc Natl Acad Sci USA* 2000, **97**:10231-10235.
- This contribution describes a mechanism by which symbionts, which are low in number in the environment, are recruited from the water column during the onset of the squid–vibrio symbiosis. Environmental bacteria induce the host to secrete a mucus-like material from the pores of the juvenile light organ, the site through which symbionts colonize the host. This matrix is suspended above the pores by the activity of a juvenile-specific ciliated field of cells on the surface of the nascent light organ. After some residence time in the matrix, accumulated bacterial symbionts migrate to the pores, down ducts and into the light organ crypts, where they colonize throughout the host's life history.
15. Feldman RA, Black MB, Cary SC, Lutz RA, Vrijenhoek RC: **Molecular phylogenetics of bacterial endosymbionts and their vestimentiferan hosts**. *Mol Mar Biol Biotechnol* 1997, **6**:268-277.
  16. Jones ML, Gardiner SL: **Evidence for a transient digestive tract in Vestimentifera**. *Proc Biol Soc Wash* 1988, **101**:423-433.
  17. Tannock GW: *Medical Importance of the Normal Microflora*. Boston: Kluwer Academic Publishers; 1999.
  18. McFall-Ngai MJ, Toller W: **Frontiers in the study of the biochemistry and molecular biology of vision and luminescence in fishes**. In *The Molecular Biology and Biochemistry of Fishes*, vol 1. Edited by Hochachka P, Mommsen T. Amsterdam: Elsevier Science Publishers; 1991:77-107.

19. Ottemann KM, Miller JF: **Roles for motility in bacterial–host interactions.** *Mol Microbiol* 1997, **24**:1109-1117.
20. Graf J, Dunlap PV, Ruby EG: **Effect of transposon-induced motility mutations on colonization of the host light organ by *Vibrio fischeri*.** *J Bacteriol* 1994, **176**:6986-6991.
21. Ruby EG, Asato LM: **Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis.** *Arch Microbiol* 1993, **159**:160-167.
22. Millikan DS, Felbeck H, Stein JL: **Identification and characterization of a flagellin gene from the endosymbiont of the hydrothermal vent tubeworm *Riftia pachyptila*.** *Appl Environ Microbiol* 1999, **65**:3129-3133.
- This contribution is one of the first papers describing the use of molecular genetic analysis to investigate the function of symbiotic genes in as yet unculturable symbionts of chemoautotrophic invertebrates. The work illustrates that these symbionts, while unflagellated in the association, retain a functional flagellar structural gene.
23. Akerley BJ, Cotter PA, Miller JF: **Ectopic expression of the flagellar regulon alters development of the *Bordetella*–host interaction.** *Cell* 1995, **80**:611-620.
24. Schmiel DH, Young GM, Miller VL: **The *Yersinia enterocolitica* phospholipase gene *ypIA* is part of the flagellar regulon.** *J Bacteriol* 2000, **182**:2314-2320.
25. Ofek I, Doyle RJ: *Bacterial Adhesion to Cells and Tissues*. New York: Chapman and Hall; 1994.
26. Ruby EG, McFall-Ngai MJ: **Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*.** *Trends Microbiol* 1999, **7**:414-420.
- This paper reviews the lines of evidence for the involvement of reactive oxygen species in the molecular interplay between the squid host and its bacterial partner.
27. Small AL, McFall-Ngai MJ: **A halide peroxidase in tissues that interact with bacteria in the host squid *Euprymna scolopes*.** *J Cell Biochem* 1999, **72**:445-457.
28. Weis VM, Small AL, McFall-Ngai MJ: **A peroxidase related to the mammalian antimicrobial protein myeloperoxidase in the *Euprymna*–*Vibrio* mutualism.** *Proc Natl Acad Sci USA* 1996, **93**:13683-13688.
29. McFall-Ngai MJ, Brennan C, Weis V, Lamarcq L: **Mannose adhesin–glycan interactions in the *Euprymna scolopes*–*Vibrio fischeri* symbiosis.** In *New Developments in Marine Biotechnology*. Edited by Le Gal Y, Halvorson H. New York: Plenum Press; 1998:273-276.
30. Lamarcq L, McFall-Ngai MJ: **Induction of a gradual, reversible morphogenesis of its host's epithelial brush border by *Vibrio fischeri*.** *Infect Immun* 1998, **66**:777-785.
31. Visick KL, Foster J, Doino J, McFall-Ngai MJ, Ruby EG: ***Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ.** *J Bacteriol* 2000, **182**:4578-4586.
- Null mutations are created in three *lux* genes of *V. fischeri*, and the symbiotic performance of the resulting mutants is analyzed in juvenile squid. This study is the first to show that the capacity for bioluminescence is critical for normal cell–cell interactions between a bacterium and its animal host and presents the first examples of *V. fischeri* genes that affect normal host tissue development.
32. Montgomery M, McFall-Ngai MJ: **Late postembryonic development of the symbiotic light organ of *Euprymna scolopes* (Cephalopoda:Sepiolidae).** *Biol Bull* 1998, **195**:326-336.
33. Lemus JD, McFall-Ngai MJ: **Alterations in the proteome of the *Euprymna scolopes* light organ in response to symbiotic *Vibrio fischeri*.** *Appl Environ Microbiol* 2000, **66**:4091-4097.
34. Hirsch AM, McFall-Ngai MJ: **Fundamental concepts in symbiotic interactions: light and dark, day and night, squid and legume.** *J Plant Growth Regul* 2000, in press.
35. Stougaard J: **Regulators and regulation of legume root nodule development.** *Plant Physiol* 2000, **124**:531-539.
36. Chiang SL, Mekalanos JJ, Holden DW: ***In vivo* genetic analysis of bacterial virulence.** *Annu Rev Microbiol* 1999, **53**:129-154.
- The authors compile an informative comparison of several methods of identifying host-induced genes in bacterial pathogens, including *in vitro* expression technology and signature-tagged mutagenesis.
37. Graf J, Ruby EG: **Characterization of the nutritional environment of a symbiotic light organ using bacterial mutants and biochemical analyses.** *Proc Natl Acad Sci USA* 1998, **95**:1818-1822.
38. Graf J, Ruby EG: **Novel effects of a transposon insertion in the *Vibrio fischeri* *glnD* gene: defects in iron uptake and symbiotic persistence in addition to nitrogen utilization.** *Mol Microbiol* 2000, **37**:168-179.
- A defect in the synthesis of a regulator of nitrogen metabolism is shown to have an unexpected effect on *V. fischeri* cells. The data suggest that the regulator, GlnD, is required to properly modulate siderophore utilization and to maintain a normal symbiont number in the developing squid light organ.
39. Boettcher KJ, Ruby EG: **Detection and quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs.** *J Bacteriol* 1995, **177**:1053-1058.
40. Swift S, Williams P, Stewart GSAB: **N-acylhomoserine lactones and quorum sensing in proteobacteria.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington: ASM Press; 1999:291-314.
41. Fuqua C, Greenberg EP: **Self perception in bacteria: quorum sensing with acylated homoserine lactones.** *Curr Opin Microbiol* 1998, **1**:183-189.
42. Callahan SM, Dunlap PV: **LuxR- and acyl-homoserine-lactone-controlled non-*lux* genes define a quorum-sensing regulon in *Vibrio fischeri*.** *J Bacteriol* 2000, **182**:2811-2822.
- This paper uses the results of a comparison of two-dimensional protein gels to present the first evidence that the expression of *V. fischeri* genes other than those in the *lux* operon are controlled by the *luxIR* quorum-sensing system. The work opens up the possibility that such genes may be of direct importance to symbiotic colonization.
43. Fuqua C, Winans SC, Greenberg EP: **Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators.** *Annu Rev Microbiol* 1996, **50**:727-751.
44. Visick KL, Ruby EG: **The emergent properties of quorum sensing: consequences to bacteria of autoinducer signaling in their natural environment.** In *Cell–Cell Signaling in Bacteria*. Edited by Dunny GM, Winans SC. Washington, DC: ASM Press; 1999:333-352.
45. Lui WT, Marsh TI, Hans C, Forney LJ: **Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16s rRNA.** *Appl Environ Microbiol* 1997, **63**:4516-4522.

## Now in press

The work referred to in the text as (JS Foster, MA Apicella, MJ McFall-Ngai, *Soc Dev Biol Abst* 1999, 210:337) is now in press:

46. Foster JS, Apicella MA, McFall-Ngai MJ: ***Vibrio fischeri* lipopolysaccharide induces developmental apoptosis, but not complete morphogenesis, of the *Euprymna scolopes* light organ.** *Dev Biol* 2000, **126**:in press.