CHAPTER 9

Host-Microbe Symbiosis:
The Squid-Vibrio Association—A Naturally Occurring, Experimental Model of Animal/Bacterial Partnerships

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Abstract

Many, if not most, animals have specific symbiotic relationships with bacterial partners. Recent studies suggest that vertebrates create alliances with highly complex consortia of hundreds to thousands of prokaryotic phylotypes. In contrast, invertebrates often have binary associations, i.e., relationships with a population of a single bacterial species. In this chapter, the association between the Hawaiian sepiolid squid *Euprymna scolopes* and the marine luminous bacterium *Vibrio fischeri* is highlighted. This symbiosis offers a relatively simple, yet naturally occurring, association that can be experimentally manipulated. Studies of this system are providing insight into the precise mechanisms by which a beneficial animal-bacterial symbiosis can be established and maintained.

Introduction—The Context

Researchers in biomedicine are becoming increasingly aware that an understanding of evolutionary and ecological principals can provide great insight into the underlying dynamics of human health and disease. Humans, as all animals, are products of their evolutionary history, a basic feature that will be reflected in all aspects of their biology. This newfound awareness is likely to influence few groups of biomedical researchers as profoundly as those who study the relationships of microbes to their host animals. The evolution of animals occurred relatively late in earth's history as a patina over the continued evolution of the microbial world. Specifically, all animal body plans evolved at Cambrian explosion 540 million years ago in the context of marine environments with millions of bacterial cells in each milliliter of seawater. As such, from the beginning through the present day, animals have been interacting with microbes in a variety of ways. Thus, it is not surprising to find that the responses of present-day animals to microbes can be ancient responses, highly conserved over evolutionary history.

An example of this conservation can be found in the form and function of the immune system. Recent studies of innate immunity have demonstrated that all three major subkingdoms of the kingdom Animalia, i.e., the Deuterostomia, (e.g., vertebrates, sea squirts, urchins), Ecdysozoa (e.g., fruit fly and nematode worm) and the Lophotrochozoa (e.g., snails, squids, marine worms), share orthologous pattern-recognition receptors and elements of response pathways specific to interacting with the microbial world.[1] The invertebrates are highly diverse and evolutionarily successful, yet

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are thought to rely chiefly on the activity of the innate immune system to interface with microbes.[2] On the other hand, the gnathostome vertebrates, the divergence of which dates back to the very early diversification of the animals in the Paleozoic, have not only the innate immune system but also an adaptive immune system, the major components of which are conserved throughout all gnathostome classes of vertebrate, from bony fishes to mammals. Invertebrates are highly successful, have as a group every known life history strategy and show no higher morbidity or mortality to pathogenic infection than vertebrates. These traits suggest that the vertebrate adaptive immune system is not a 'better' interface with the microbial world, but rather an alternative strategy.

The differences in the immune systems of invertebrates and vertebrates have some reflection in trends of occurrence of their interactions with microbes. All animal cells, of course, have mitochondria, but the invertebrates very often have other binary, intracellular or extracellular symbioses. For example, approximately 11% of all insects have bacteriocyte symbioses wherein a monoculture of intracellular bacteria in the fat bodies (the liver equivalent) provides essential nutrients to the host;[3] also the widely studied associations of hydrothermal vent animals with sulfur oxidizing bacteria and coral hosts with their unicellular algae offer other examples of ecologically important binary, intracellular symbioses.[4] In contrast, vertebrates rarely, if ever, have beneficial binary, intracellular symbioses and extracellular binary alliances occur rarely and only among the fishes (e.g., the luminous bacteria-light organ symbioses in several families of marine fishes). Recent evidence suggests that vertebrates instead harbor highly complex consortia, a condition that appears to occur rarely in the invertebrates (exceptions—termites, cockroaches and their relatives)[5] and that the consortia of the vertebrate gut may profoundly affect the activity of the adaptive immune system.[6]

Despite these differences between the invertebrates and vertebrates, they share enough conserved elements in their interaction with microbes to render broad comparative studies of animal-microbe interactions compelling. If biologists are to obtain a reasonably accurate picture of the very basic mechanisms underlying the dynamics of animal-microbe relations, they must have a similar strategy to that employed by the developmental biologists, wherein a variety of models have been exploited (e.g., mouse, chick, frog, zebrafish, fruit fly, nematode, urchin, etc.). Each model has key features that have provided and continue to provide, the pieces of a complex set of puzzles. In the frontier of the field of animal-microbe interactions, a wide variety of model systems have been and are being developed. Among the vertebrates, most notable are the germ-free and gnotobiotic systems. The zebrafish and mouse are particularly powerful models in this group, as the genetics of host responses can be studied.[7-9] However, these systems are naturally consortial with hundreds to thousands of microbial partners; thus, genetic approaches on the microbes are likely to be limited in their ability to inform about the dynamics of the intact set of communities.

As simpler, binary associations, invertebrate symbioses ought to offer limitless opportunities, as they are diverse and abundant. However, many of these alliances are so tight that one or the other partner cannot be cultured outside of the symbiosis. This problem renders many of the invertebrate systems intractable as experimental models. However, recently several invertebrate symbioses, such as the nematode,[10-12] leech[13] and earthworm[14] associations with specific bacterial partners, are emerging experimental systems that hold great promise. Comparisons among these associations and comparisons of these systems with the vertebrate consortial symbioses should provide great insight not only into what is basic or conserved in animal-microbe associations but also into what processes create the diversity of symbioses.

The remainder of this chapter focuses on the squid-vibrio symbiosis. The intent is to provide an example of what is known to date about the degree complexity that can underlie the establishment, development and maintenance of a binary association. Studies of this system have been aimed at understanding how the symbionts are harvested from the environment, how specificity is achieved, how partner development is affected by their reciprocal interaction and how stability is achieved once a mature association is established. In addition, a key question asked in this system has been: how does the language of beneficial interaction differ from that of pathogenesis?

The Monospecific Squid-Vibrio Symbiosis as an Experimental System

The symbiosis between the Hawaiian sepiolid squid *Euprymna scolopes* and the marine luminous bacterium *Vibrio fischeri* has been studied for over 15 years as an unusually tractable experimental model for the study of animal-bacterial interactions at the interface of epithelial tissues and their associated, colonized lumina.[15] The partnership is highly specific in that only one bacterial species, against a background of thousands of other species in the ambient seawater, is capable of forming a stable relationship; i.e., in the absence of *V. fischeri*, no other environmental bacteria colonize host light-organ tissues. The binary nature of this symbiosis provides the opportunity to define the precise dialogue that occurs between the partners over the trajectory of their long-term relationship. As such, this symbiosis offers a complement to the studies of the dynamics of symbiotic associations that occur between mammalian hosts and their diverse and complex consortial microbiota.

As mentioned above, while many animals have binary associations with bacteria, rarely are both the partners easily culturable outside of the symbiosis.[4] Most often, the association is nutritionally based and either or both partners cannot withstand the aposymbiotic state (i.e., occurring in the absence of the native symbiont). In the squid-vibrio system, the light produced by *V. fischeri* is the principal product benefiting the host; no evidence exists that the bacterial symbionts provide nutrients to the host animal. The morphology of the light organ suggests that the host uses the light of the bacteria in antipredatory behavior called counterillumination, in which the host emits light as a camouflage. Thus, under laboratory conditions where no predators are present, the absence of the symbiont does not negatively affect the fitness of the host.

Several other characteristics of the host animal render it a suitable subject for studies of symbiosis. Male and female adult squids are easily obtained from the field and maintained in either running or recirculating seawater systems. A colony of 12-15 adult squids produces in excess of 50,000 juvenile squid/year that can be used for the experimental analyses of this symbiosis. Recently, an EST database of nearly 14,000 unique clusters has been created from juvenile light organ tissue and the cDNAs have been arrayed. These resources have expanded the studies of the host animal to the arena of genomic analysis.

The microbial light organ symbiont *V. fischeri*, a member of the gamma proteobacteria subgroup of Gram-negative bacteria, is among the best-understood marine bacteria due to its roles as a model both for bacterial light production and for symbiotic relationships with animals.[16,17] These two general facets of the biology of *V. fischeri* have spurred active research programs for almost three decades and the resulting studies have, perhaps surprisingly, converged in many respects with the field of pathogenic microbiology. For example, the phenomenon of quorum sensing, also known as autoinduction, whereby bacteria induce expression of particular genes only after achieving a critical cell-density, was first discovered through studies of *V. fischeri* luciferase regulation.[18] Subsequent to its discovery in *V. fischeri*, quorum sensing systems were found in several pathogenic bacteria, including *Pseudomonas aeruginosa*, *S. typhimurium*, *Vibrio cholerae* and *Helicobacter pylori*, where, at least in some cases, they contribute to the regulation of virulence factors.[19,20] Similarly, the interactions between *V. fischeri* and its animal hosts display several morphological and mechanistic parallels to host-pathogen associations.[21,22]

V. fischeri has also been well-studied, in part, because it is amenable to laboratory manipulations. It grows rapidly in liquid or solid culture (optimally doubling in <30 min), is prototrophic, tolerates a wide range of oxygen levels and is amenable to conjugally-or electrochemically-mediated transformation. Because genetic manipulations, particularly mutant analyses, constitute a powerful tool for dissecting the bacterial attributes that contribute to the squid-vibrio symbiosis, bacteriologists have developed a number of molecular and genetic tools for use with *V. fischeri*.[23-26] In additional, the recent sequencing and annotation of the *V. fischeri* genome has provided researchers not only with the information of the full complement of genes in the symbiont, but with a valuable source by which to compare genomes of *V. fischeri* with that of the pathogenic *Vibrio spp.*, such as *V. cholerae*.

In addition to the above-described favorable characteristics of each partner, several aspects of the symbiosis itself make it ideal for experimental analysis. These include the following characteristics—

(i) The time course of development of the symbiosis is relatively brief.[27] The animal host is colonized by the symbiont within hours of hatching and the symbiosis matures within a few days following the initial inoculation of host tissues. (ii) The infected and uninfected (aposymbiotic) animals can be compared directly. (iii) The extent of bacterial colonization can be quantified non-invasively and repeatedly, on the same animal by measuring light emission.[28] (iv) The light organ is accessible to dissection and observation during development. The size and anatomical relationships of the light organ tissues render real-time analysis of the progression of symbiosis by confocal microscopy an ideal approach (e.g., see Fig. 1). (v) The pores on the surface of the light organ extend into the light organ crypts, allowing experimentally introduced solutes (e.g., purified LPS, PGN, proteins, antibiotics or fluorochromes) to diffuse to the site of infection.[29-33] (vi) The juveniles are large enough to allow molecular analysis of the host's symbiotic tissues. And, (vii), the symbiosis can be studied intact, so that all naturally interacting systems (e.g., the epithelia and the innate immune system) are functional; therefore, the association provides a powerful complement to studies of host-bacterial interactions in cell culture.

Colonization of Host Tissues by *Vibrio fischeri* and Subsequent Symbiont-Induced Host Development

In the mature symbiosis, *V. fischeri* resides extracellularly within deeply invaginated epithelial crypts of the host squid's light organ.[34] Surrounding the bacteria-rich epithelial core are tissues that serve to direct and diffuse the bacterial luminescence, as well as control the intensity of the emitted light. As in most coevolved animal-bacterial symbioses, including those of humans, the squid-vibrio association is horizontally transmitted between generations, i.e., the host acquires the symbiont population anew each generation. During embryogenesis, the host animal develops a set of tissues that prepares it for immediate interaction with environmental *V. fischeri* cells when it hatches from the egg (Fig. 1).[35] Specifically, a bilaterally symmetrical nascent light organ is developed that bears, on each side, a complex, superficial, ciliated epithelium. This tissue is involved in potentiating the colonization of host tissues by the symbionts.[36] In the middle of each ciliated field, at the base of two extended epithelial appendages, are three pores, the sites of eventual entry of the *V. fischeri* cells. During colonization, the bacteria enter these pores, travel up ducts and invade three independent crypt spaces. The population of *V. fischeri* cells that has entered the crypts then grows to fill the crypt spaces within twelve hours.[37] Restricted to these anatomical sites throughout the life history of the host, the bacterial symbionts interact with two cell types: the crypt epithelia and migrating phagocytes, or hemocytes, which sample the crypt spaces.[37,38] The host controls the symbiont population by a daily venting of 90-95% of the bacterial culture from the light organ pores out into the surrounding seawater; growth of the remaining 5-10% of the population over the subsequent 12 h fully recolonizes the organ each day.[38,39]

Studies of the colonization process in the squid-vibrio symbiosis have revealed that hatching into environmental seawater induces the cells of this ciliated epithelium to shed mucus that is focused, by the activity of the cilia, into masses above the light organ pores[36] (Fig. 1B and C). The symbionts aggregate in this mucus and, after some residence time as an aggregate (2-3h), migrate to pores on the surface of the organ, through the ducts and into their final place of residence in the crypt spaces (Fig. 1D-G). As *V. fischeri* aggregates in the mucus, a 'winnowing' occurs during which ever-increasing specificity results in competitive dominance of *V. fischeri*.[15] Specifically, whereas both Gram-positive and negative bacteria are capable of inducing host mucus secretion, only Gram-negative bacteria adhere to this mucus and only living Gram-negative bacteria form tight aggregations. In the absence of *V. fischeri* other Gram-negative bacteria will aggregate in the mucus, but when *V. fischeri* cells are present at their normal ratio to other environmental bacteria (i.e., 1:10,000), after a 3-h incubation period, the aggregate contains *V. fischeri* cells exclusively.[40] These data suggest that *V. fischeri* is, by some means, a competitive dominant in the host-secreted mucus. Analyses of this phenomenon have indicated that this facility of the symbiont is most likely due to an enhanced ability to occupy sites in the mucus (e.g., to better adhere to the matrix or resist antimicrobial substances in the mucus), rather than competitive dominance for a resource; growth

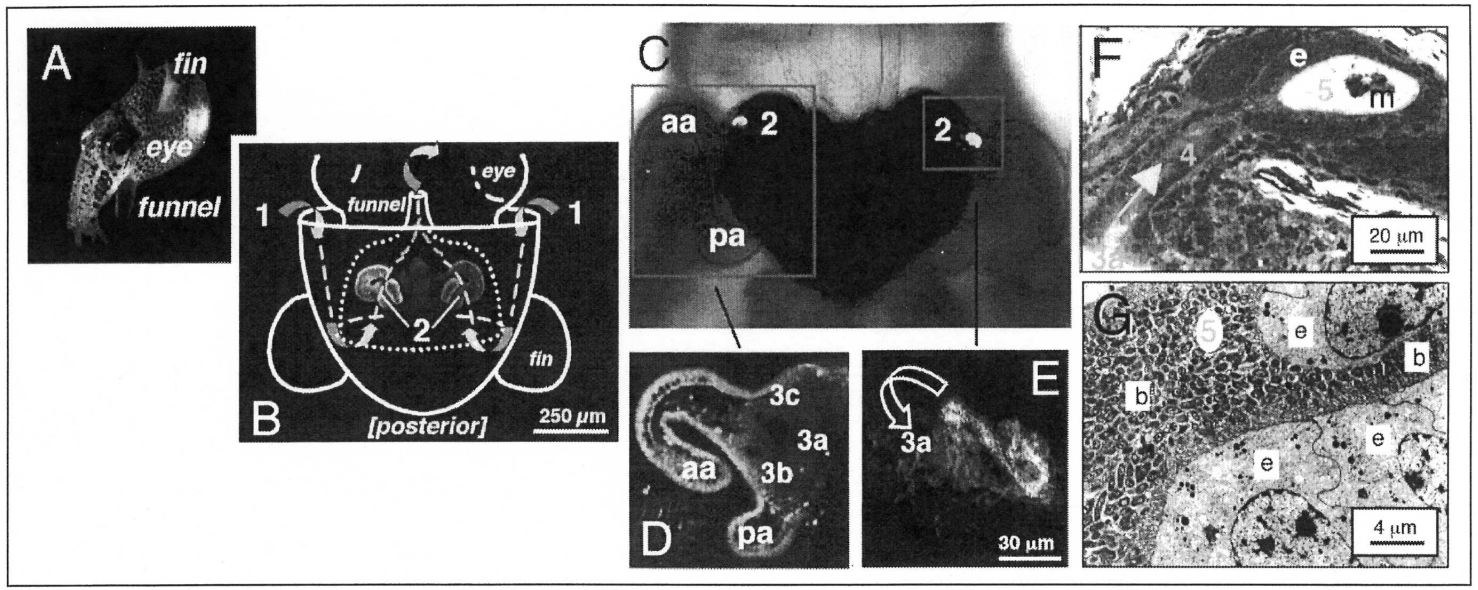

Figure 1 The pathway of colonization of the *E. scolopes* light organ by *V. fischeri*. A) The host squid E. scolopes, which as a newly hatched juvenile averages 3 mm in total length, is colonized by *V. fischeri* cells within hours following emergence from the egg. B-G) The path of the symbiotic bacteria (designated 1-5) that brings them from the surrounding environmental seawater into their eventual colonization site in the host epithelium-lined crypts. B) A diagram of the host's body cavity viewed ventrally. A confocal micrograph of the light organ is projected in this cutaway. Seawater that contains potential symbionts (1) is brought to the site of susceptible host tissues by the normal ventilatory movements of the host (arrows/dashed lines). Symbionts gather (2) in a biofilm of host-derived mucus, the production of which is stimulated by exposure of the host to peptidoglycan from environmental bacteria. C) A differential-interference-contrast image of the light organ, following a 2-3 h exposure of the host to water containing GFP-labeled *V. fischeri* (grey aggregates to the left and right of '2'). The activity of two lateral, superficial fields of ciliated epithelial cells, with their anterior (aa) and posterior appendages (pa) serves to suspend the symbiont-mucus aggregate above the organ surface (2). D) A confocal image of one-side of the light organ showing the three pores (3 a-c) through which the bacteria will enter host tissues. E) After a residence time in the aggregates of a few hours, the symbionts migrate (arrow) toward the pores (at arrowhead) and into the ducts leading to the internal crypt spaces. In this confocal image-GFP-labeled *V. fischeri* (bright regions) are seen in association with the host-shed mucus, which has been labeled with a fluorescent lectin (dimmer grey). F) A light micrograph of a cross section through the uncolonized juvenile organ. Symbionts travel into each pore (3a depicted here) and through each long duct (4, arrow), into crypt spaces (5), where the bacterial cells encounter the crypt epithelial (3) and host-macrophage-like cells (m) with which they will interact throughout the life of the host. G) A transmission electron micrograph of *V. fischeri* (b) colonizing the crypt spaces. [For review see Nyholm & McFall-Ngai, 2004].

of the bacterial population is minimal in the aggregates.[40] Following the migration of an aggregate into the crypts (Fig. 1E and F), subsequent aggregates are formed in a similar fashion and continue to form and migrate into the crypts over the first 24 h post hatching.[41] However, between 24 and 36 h following colonization, *V. fischeri* cells in the crypts cause the secretion of mucus from the superficial epithelium to cease and no additional aggregates are formed.

Within hours of the initial crypt colonization, the symbiont cells induce a series of developmental changes both in the crypt cells with which they directly interact and in the remote superficial epithelial fields of the organ, which have facilitated colonization (Fig. 2); for review see.[15] The most dramatic of the developmental changes is the complete regression of the superficial field of epithelial cells.[27] This process is characterized by a hemocyte trafficking into the blood sinuses of the ciliated epithelia and apoptosis of the cells of this field. The ducts change both anatomically and biochemically in response to interaction with the symbionts. Nitric oxide production, which is high in the ducts of aposymbiotic animals, is attenuated with the onset of the symbiosis and changes in the actin cytoskeleton of the ducts results in their constriction. The epithelial cells that line the crypts, i.e., those cells that will interact with the symbionts persistently, exhibit an increase in the density of their microvilli, as well as swell 4-fold in cytoplasmic volume, in response to the direct interactions with *V. fischeri*.

Microbe-Associated Molecular Patterns of *V. fischeri* and Host Responses to These Molecules During the Early Stages of the Symbiosis

The conserved molecules of the bacterial envelope, particularly components of the lipopolysaccharide (LPS) of the outer membrane and the peptidoglycan (PGN) of the cell wall, signal play a critical role in the early stages of the squid-vibrio symbiosis. The activities of this class of bacteria-specific molecules, examples of microbe-associated molecular patterns (MAMPs; 53, 62), are most often associated with and best understood in the onset and progression of bacteria-induced disease.[42,43] In the pathogenesis, LPS and PGN can work either alone or in concert[44-47] and are known to play central roles in host response (e.g., in the mediation of septic shock).[48,49]

The influence of MAMPs begins immediately upon hatching of the juvenile host. Mucus secretion by cells of the nascent light organ is induced by the exposure of the animal to the PGN that has been shed by the Gram-positive and Gram-negative environmental bacteria.[41] In addition, the morphogenetic process that results in the loss of the superficial ciliated epithelium is due to the synergistic activity of *V. fischeri*-shed PGN and LPS derivatives.[31] Beginning at about 2 following initial exposure to *V. fischeri*, i.e., coincident with aggregation of symbiont cells in host-secreted mucus, the migration of hemocytes into the blood sinues of the superficial epithelial field of cells occurs in response to a *V. fischeri*-shed PGN fragment, specifically the tetrapeptide fragment of PGN that has been most widely called 'tracheal cytotoxin' or 'TCT'. TCT was first described in *Bordetella pertussis* infection where it causes the epithelial cell disruption characteristic of that infection (Koropatnick and McFall-Ngai, Luker et al., 1993[50a]). At about 6 h following initial exposure of the host squid to environmental *V. fischeri*, when the symbionts are traveling through the ducts, the first apoptosis events triggered in the cells of the superficial epithelium in response to exposure to *V. fischeri* lipid A, a component of LPS.[32] Early characterizations of this phenomenon showed that the numbers of hemocytes trafficking into this field, as well as the numbers of epithelial cells undergoing apoptosis, peak at ~12 h, but the processes continue throughout the regression process. Around this 12 h time point, the lipid A and TCT shed by *V. fischeri* in the crypts send an irreversible signal that results in the full regression of the superficial field, a process that requires 4 d to complete. Specifically, when animals are cured of symbionts before 12 h, or exposed to TCT and LPS for less than 12 h, the field does not regress; however, if they are cured at or after 12 h of exposure, the full 4-d program continues unabated.[31,33]

The recognition and response system to MAMPs is also highly conserved among animals responding to pathogens and recent studies of the squid-vibrio system suggest that these same

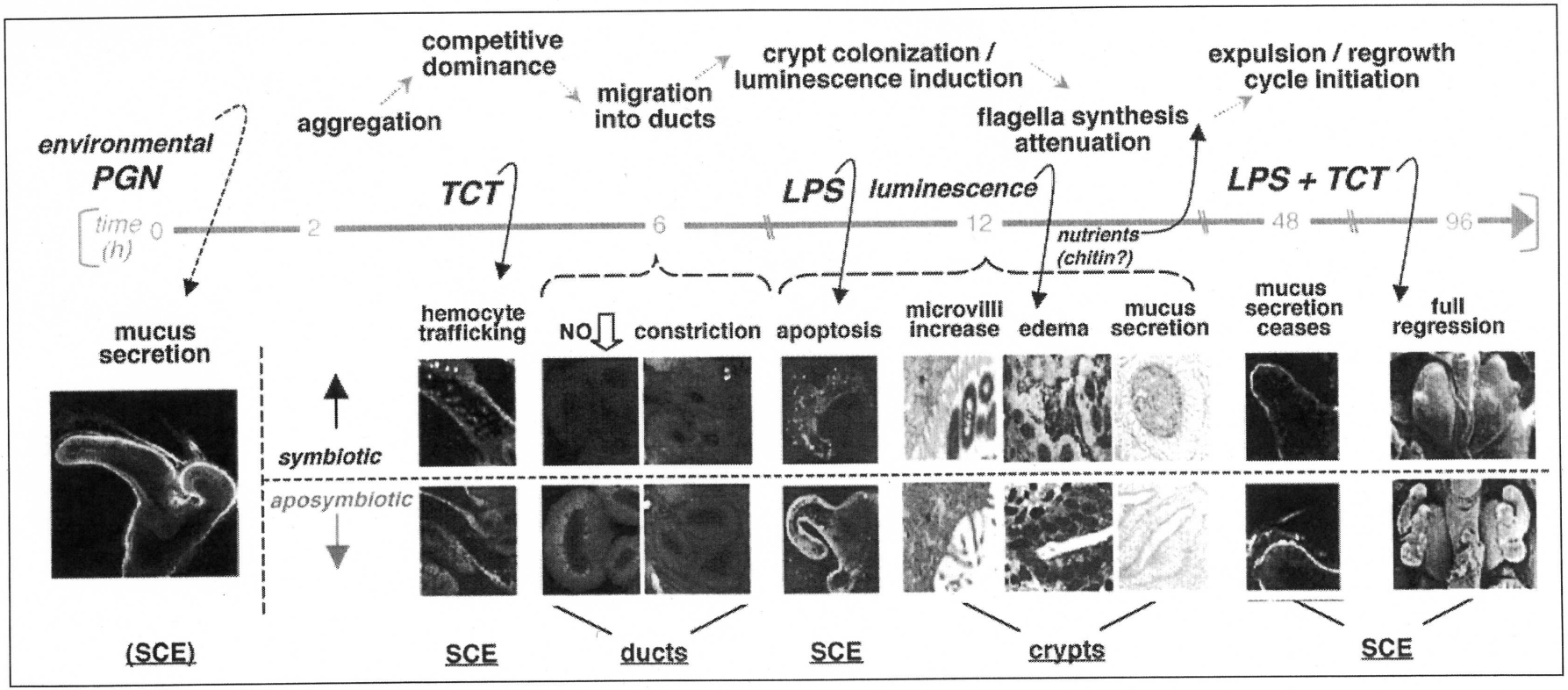

Figure 2. Microbe-associated molecular patterns (MAMPs) as signaling molecules in early infection by *V. fischeri*. The host and symbiont undergo a complex reciprocal dialogue during the first days of colonization that leads to dramatic changes in both partners. In this depiction, events and structures listed above the line illustrate responses of *V. fischeri*, those below indicate the responses of *E. scolopes*. For *E. scolopes*, cell and tissue characteristics in the presence (symbiotic, above the dashed line) or absence (aposymbiotic, below the dashed line) of *V. fischeri* are indicated. Curved arrows indicate direction of signals (italics) communicated between the partners. Host tissues in which the events occur are underlined. LPS, lipopolysaccharide; h, hours following hatching into natural seawater; NO, nitric oxide; PGN, peptidoglycan; SCE, superficial ciliated epithelium; TCT, tracheal cytotoxin, a PGN derivative.

elements are involved in mediating beneficial symbiosis as well.[50-53] *E. scolopes* shares with other animals, including vertebrates and invertebrates,[52,54-57] a series of binding proteins and receptors ('pattern recognition receptors' or 'PRRs'), response pathways and effector molecules. Analysis of the squid light-organ EST database revealed that the light organ expresses during early development three orthologs of the LPS-binding proteins (LBPs), four orthologs of the peptidoglycan recognition proteins (PGRPs) and one ortholog of a Toll-like receptor (TLR).[58] Studies of these molecules in other systems have demonstrated that they can function as receptors themselves, or adaptors that interface the bacterial ligand with its cognate receptor molecule and they can act as monomers, or homo- or heteromultimeric complexes.[59-61] In addition, some of these PRRs, most notably certain PGRPs and LBPs, act as bacteriostatic or bactericidal agents.[57,62,63] The roles of these molecules in mediating specificity of the squid-vibrio symbiosis and in responding to the bacterial MAMPs during development remain to be determined.

Biologists have also identified several conserved response pathways to these receptor-ligand interactions, most notably the NF-kB, JNK and p38 MAP kinase pathway and JAK-STAT pathways.[64,65] In each case, the response induced by the ligand-receptor interaction leads to changes in gene transcription associated with prokaryotic-eukaryotic cell-cell interaction, such as genes that mediate production of antimicrobial agents (e.g., nitric oxide and antimicrobial peptides) or those involved in cytokine production. Thus far, orthologs of proteins in the NF-kB, p38 MAP kinase and JAK-STAT pathways have been found in the EST database of the *E. scolopes*. Expression of these genes during early development suggests that they may be involved in response to interactions with *V. fischeri*, perhaps with *V. fischeri* MAMPs. As with the PRRs, an understanding of the role of these pathways in this symbiosis remains to be elucidated. One interesting avenue will involve how these molecules and pathways are used to manage a beneficial symbiosis and how this differs from the way this animal uses these very same elements to control bacterial pathogenesis.

Luminescence—The Central Feature of the Symbiosis

The application of microbial genetics has revealed a number of *V. fischeri* characters that are required for normal symbiosis. These important aspects of the association have recently been reviewed, so will not be mentioned here.[66] However, one principal feature of the symbiosis, i.e., luminescence, will be covered briefly.

In every symbiosis, the host and symbiont(s) have a 'currency' of exchange that defines the partnership. In the squid-vibrio association, the host provides nutrients for the bacteria and, in exchange, the bacteria produce light that the host uses in its behavior. One might suspect that the bacteria would 'cheat' and not do their part, as luminescence production imposes a metabolic cost to the bacterial cell. However, studies of the association have suggested that the host has mechanisms to ensure that the bacteria are luminous.[67] Mutants in the *luxA* gene, which encodes one of the subunits of the symbiont's luciferase, are incapable of producing luminescence. Such mutants can colonize the light organ initially, but fail to persist, i.e., following the first day, their numbers in the host light organ decline. These mutants also fail to induce the normal swelling of the light-organ crypt epithelial cells that is induced by wild-type *V. fischeri*. Experimental manipulation of the system has indicated that these mutants are defective in obtaining nutrients from the host (E. Ruby, pers. comm.). Although it has not been shown unequivocally, these findings would suggest that the host cell-swelling phenotype is involved in the provision of nutrients to the symbiont population.

How the host cells perceive symbiont luminescence is not understood. However, the nature of the luminescence reaction presents two possibilities. In this reaction, oxygen is consumed and light is produced and all other substrates are recycled.[68] This chemistry suggests that the host perceives either the light itself and/or a change in the oxygen tension in the crypts. An analysis of the light organ EST database revealed a surprising finding—the organ expresses proteins that may perceive light, including the blue-light receptor protein, cryptochrome, as well as many of the components of the visual transduction cascade, including rhodopsin, rhodopsin kinase and arrestin, which are generally eye specific. The expression of these proteins suggests that the light organ tissue has the

biochemical potential to perceive bacteria-produced light. In addition, a large number of proteins associated with the amelioration of oxidative stress are also expressed. Obviously, numerous questions are raised by these observations and resolving the mechanism of light production will require extensive further research on the system. However, these data suggest that an unraveling of how luminescence is controlled in this symbiosis is within reach.

Summary

Studies of the squid-vibrio association have revealed that the partners undergo a very complex reciprocal dialogue that promotes the successful colonization of host tissues. Most notably, experiments with the system have demonstrated that many of the interactions of this beneficial association involve features that have been previously ascribed principally to pathogenesis. Most notably, the bacterial partner presents to the host cells lipopolysaccaride and peptidoglycan derivatives, specific fragments that have been labeled as 'toxins' that damage animal cells and induce inflammation in other systems. However, in the dynamics of the squid-vibrio system, these molecules behave as morphogens. The bacteria use these molecules to communicate to the host partner that symbiosis is established and development can ensue. The developmental program transforms the organ from a colonization morphology to one that associated with the mature, functional symbiosis. Also in common with pathogenesis is the induction in this symbiosis of apoptosis and cellular edema, as well as the involvement of toxic oxygen and nitrogen species. Taken together, these findings demonstrate that many of the molecular responses of animals to their bacterial symbionts are not only ancient, but also that they can be shared by beneficial and pathogenic associations.

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