J Plant Growth Regul (2000) 19:113–130
DOI: 10.1007/s003440000025

JPGR
Journal of Plant Growth Regulation

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THEMATIC ARTICLES

Fundamental Concepts in Symbiotic Interactions: Light and Dark, Day and Night, Squid and Legume

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ABSTRACT

The legume-*Rhizobium* symbiosis and that between *Euprymna scolopes* and *Vibrio fischeri* show some surprising physiological similarities as well as differences. Both interactions rely on exchange of signal molecules, some of which are derived from bacterial cell surface molecules. Although the legume-*Rhizobium* symbiosis is nutritionally based as are many animal-microbe symbioses, it is not obligate because the plant initiates nodule formation only when the soil is deficient in nitrogen. In contrast, the squid-*Vibrio* symbiosis is obligate for the squid but is not nutritionally based. Rather, the bacteria produce light, which enables the animal to evade predators. These similarities and differences are described and discussed in term of the overall question of whether or not these two symbiotic relationships have evolved from commensal or pathogenic/parasitic interactions between prokaryotes and eukaryotes.

Key words: *Euprymna scolopes*; Legume; *Rhizobium*; Sepiolid; Symbiosis; *Vibrio fischeri*

Nor knowest thou what argument
Thy life to thy neighbor's creed has lent.
All are needed by each one;
Nothing is fair or good alone.

—Ralph Waldo Emerson

INTRODUCTION

This review addresses two broad questions: (1) what do a squid and a legume have in common, and (2) have either of the symbioses in which these organisms are involved evolved from a pathogenic interaction or a commensal one? At the outset, these two multicellular organisms would appear to share very few traits. The squid lives in the sea, comes out at night, and produces an eerie luminescence from its light-emitting organ, whereas the plant keeps its roots in the soil hidden in the dark while its aerial parts use light for photosynthesis. Nevertheless, both produce a specialized organ that is inhabited by bacteria (Figures 1A, 2A–C). Host-bacterial interactions enable both the squid and the legume to perform some amazing feats that they could not otherwise do.

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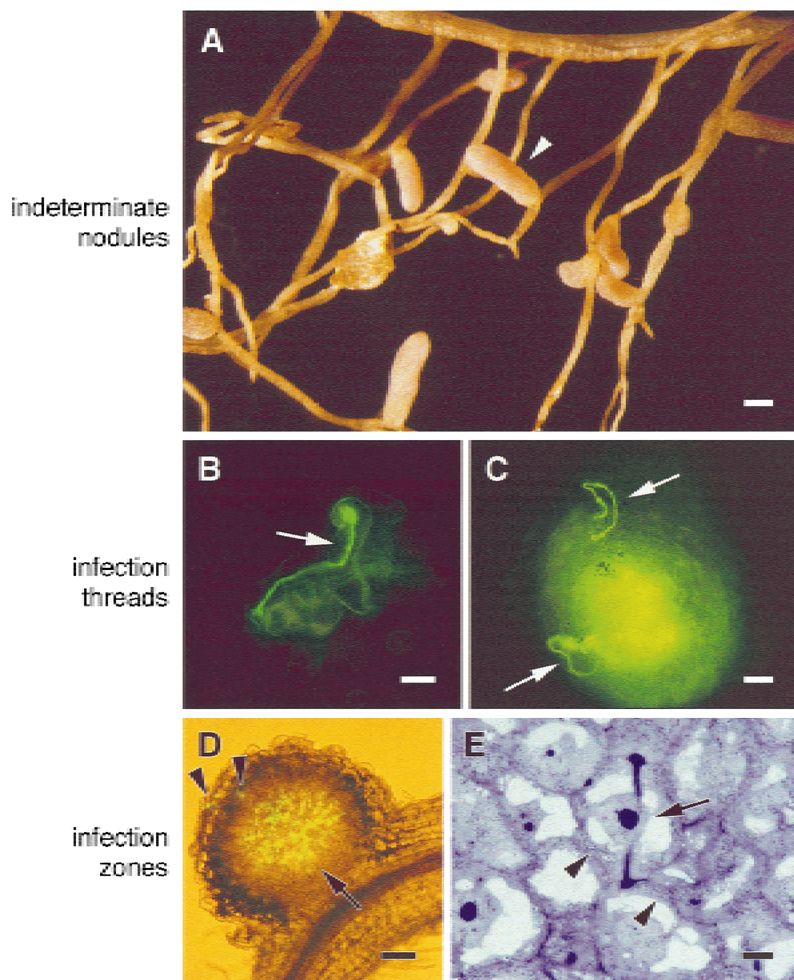

Figure 1. The early stages in indeterminate nodule development. **(A)** Indeterminate nodules from white sweetclover (*Melilotus alba* Desr.). The nodules are pink-colored because of the presence of leghemoglobin. Bar, 1 mm. **(B)** Green-fluorescent protein- (GFP) labeled rhizobia in an infection thread (*arrow*) in an highly deformed root hair, known as a shepherd's crook, of alfalfa (*Medicago sativa* L.). Bar, 20 μm. **(C)**) End-on view of an alfalfa nodule showing two long infection threads (*arrows*) containing GFP-labeled rhizobia. Bar, 20 μm. **(D)** Young nodule containing GFP-labeled rhizobia in the outer edges of the nodule (*arrowheads*) and within the central zone (*arrow*). Bar, 100 μm. **(E)** Infection zone of a mature pea (*Pisum sativum*) nodule. Infection threads (*arrow*) travel from cell to cell disgorging the membrane-bound rhizobia (*arrowheads*) into the nodule cells. Bar, 10 μm.

Early in the development of the field of biology, the biotic world was classified into two main divisions, plants and animals, on the basis of the conspicuous differences in their anatomy, morphology, behavior, and ecology. In the twentieth century, with ever increasingly sophisticated tools of analysis, cellular-level similarities between these two groups became more apparent, until in the late 1970s, plants and animals were grouped together in the Domain Eukarya, which contains all organisms with eukaryotic cells (Woese and others 1990). The other two domains, Archaea and Bacteria (that is, prokaryotes), defined by their lack of a nucleus, separated from the line that was to give rise to eukaryotes probably more than 3 billion years ago.

Biochemical and molecular studies revealed more fundamental diversity among the array of prokaryotes than differences between the plants and animals. More recent molecular analyses of plant and animal cell biology, as well as new information provided by genome sequencing, reveal a surprisingly large number of biochemical pathways/genes controlling signal transduction that are shared by plants and animals (Lam and others 1999; Meyerowitz 1999; O'Neill and Greene 1998; Wei and Deng 1999). Common pathways in these two groups most likely reflect ancient functions, that is, challenges faced by their common ancestor. One such ancient function is the mediation of interactions between eukaryotic cells and environmental prokaryotes. The presence of similar mechanisms underlying these interactions is suggested not only by the ability of certain prokaryotes, for example, *Pseudomonas aeruginosa* and *Vibrio cholerae,* to form relationships with both plant and animal hosts (Epstein 1993; Mahajan-Miklos and others 1999; Rahme and others 1995) but also by the occurrence of similar mechanisms controlling the interactions between plant and animal hosts and their prokaryotic partners (Kopp and Medzhitov 1999; LeVier and others 2000).

De Bary (1879) was the first to use the term

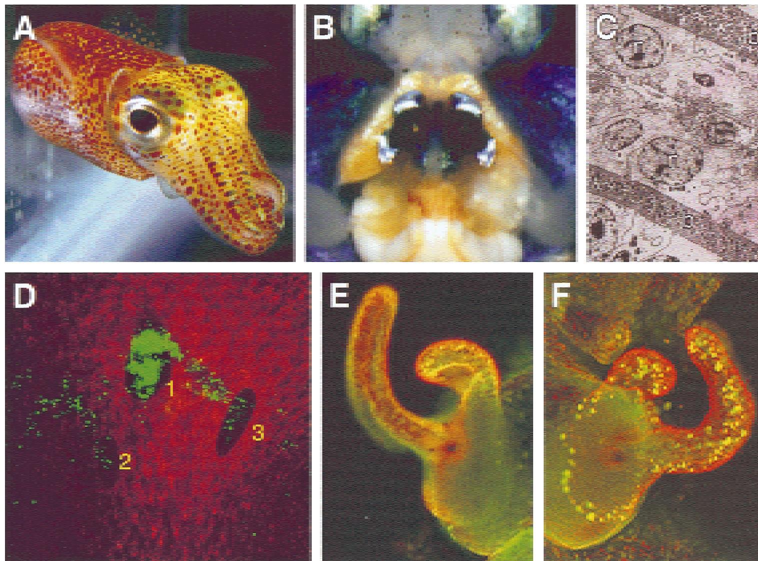

Figure 2. The squid-vibrio symbiosis. **(A)** The living host animal, *Euprymna scolopes.* Bar, 1 cm. **(B)** A ventral dissection of the adult host, revealing the conspicuous, bilobed light organ (*arrow*) in the center of the body cavity. Bar, 1 cm. **(C)** A transmission electron micrograph of the bacteria-containing core of the light organ. The bacteria (*b*) occur extracellularly between sheets of highly polarized epithelial cells of the host; *n* = host nuclei. Bar, 10 µm. **(D)** Initial colonization of the juvenile epithelial crypts. In newly hatched animals, three pores (yellow numbers, 1–3) on the surface of either side of the light organ (stained in this confocal micrograph with CellTracker Red [Molecular Probes, Eugene, OR]) lead to three independent crypts. GFP-labeled *V. fischeri* have become trapped in mucuslike material that has been secreted by the host. At this stage in the process, the bacteria have spent some residence time in the matrix and are now migrating into the three pores. Bar, 20 µm. **(E, F)** Confocal images of the surface of one-half of a light organ of a 12-h aposymbiotic juvenile **(E)** and a 12-h symbiotic juvenile **(F),** both stained with acridine organ. The ciliated field, which consists of a pad of tissue adnate to the light organ and two long appendages, shows no punctate nuclei, which are characteristic of the condensed chromatin of apoptotic cells, on the light organ of the aposymbiotic juvenile, but numerous punctate nuclei on the light organ of the symbiotic animal. Bar, 100 µm.

"symbiosis," which he defined as the "living together of differently named organisms" ("des Zusammenlebens ungleichnamiger Organismen"). In current usage, symbiosis often implies mutualism—a beneficial arrangement in which, in an ideal situation, each partner gives and takes equally, but De Bary used this term to describe both symbiotic and parasitic interactions, in which one partner takes more than gives. Pathogenesis is microbial parasitism that results in a disease or infection. Most of these complex interactions are also defined on the basis of nutrition, but the benefits (or stakes) can also be physical ones. In any case, how modern-day symbiotic interactions between prokaryotes and their plant and animal partners evolved is unclear. However, one hypothesis is that parasitism or pathogenesis is the default, and the host is manipulated by its parasite (see Corsaro and others 1999; Lederberg 2000; Steinert and others 2000). One of the many questions that a study of diverse eukaryotic-prokaryotic partnerships might address is whether the first bi-domain associations were mutualistic, parasitic, or something else.

This review explores some of the similarities and differences between a well-studied plant-bacterial symbiosis, the relationship between legumes and nitrogen-fixing rhizobia, and a more recently developed model of animal-bacterial symbiosis, the association of the squid *Euprymna scolopes* (Figure 2A) and its luminous prokaryotic partner, *Vibrio fischeri.* A comparison of these associations offers the opportunity to consider which features are shared by broadly divergent host-symbiont interactions and which characteristics may be particular to interactions with either plants or animals, but not both. In addition, because both of these symbioses are considered beneficial or mutualistic, analyses of their similarities and differences should provide insight into traits that are unique to beneficial interactions. Similarly, if these shared responses are also present in parasitic/pathogenic associations, as well as across these broad phylogenetic boundaries, they may represent a class of general responses that mediate plant and animal interactions with microbes no matter what the outcome of the interaction.

The General Nature of the Symbioses

Both the legume-rhizobia (LR) and the squid-vibrio (SV) symbioses have been reviewed individually recently (Crespi and Gálvez 2000; McFall-Ngai 1999; Stougaard 2000; Visick and McFall-Ngai 2000). Thus, detailed descriptions in this review will be restricted to those aspects that are useful in the comparison of the associations.

Symbioses are often classified on the basis of their broad characteristics, for example, how they are

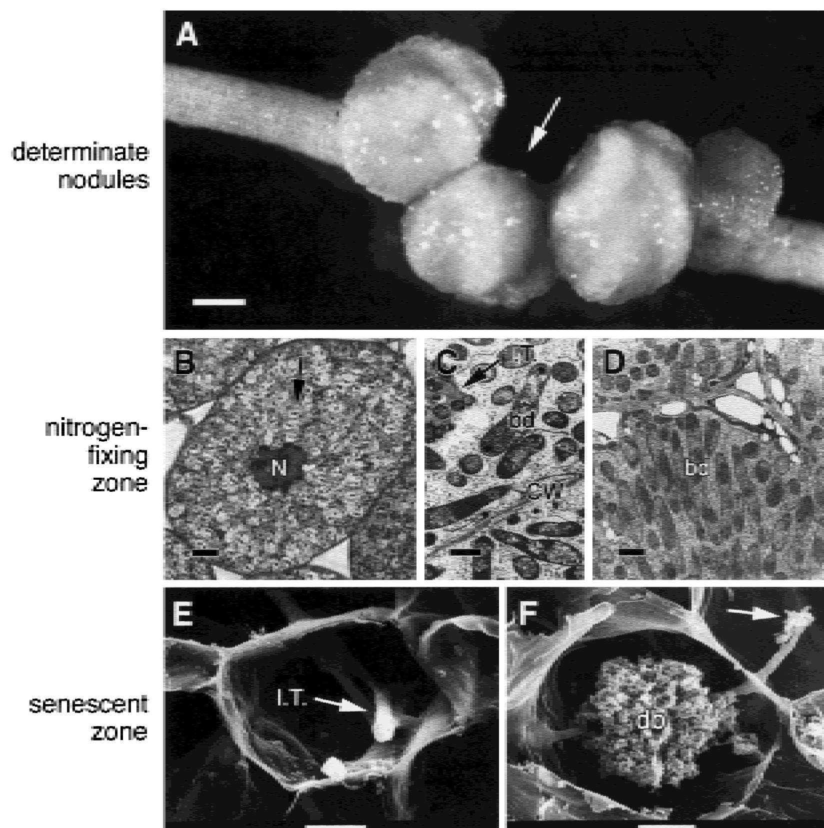

Figure 3. The later stages of indeterminate and determinate nodule development. **(A)** Determinate nodules of *Lotus corniculatus*. Bar, 1 mm. **(B)** Light micrograph from a section through an infected nodule cell of a soybean (*Glycine max*). Bar, 10 μm. **(C)** Transmission electron micrograph (TEM) of the transition from bacteria within infection thread (*I.T., arrowheads*) to bacteroids (*arrow*) in an alfalfa nodule. Two host cells separated by a cell wall (*CW*) are shown. On release from the infection thread, the rhizobia (enclosed singly by a peribacteroid membrane) elongate and differentiate. Bar, 1 μm. **(D)** TEM of nitrogen-fixing bacteroids (*bd*) in an alfalfa nodule. Bar, 1 μm. **(E)** Scanning electron micrograph (SEM) of an infection thread traversing a senescent nodule cell of alfalfa. Bar, 10 μm. **(F)** SEM through a similarly senesced cell, but here the bacteria have degenerated around the thread. Vegetative rhizobia have been released into the host cell (*arrow*). Bar, 10 μm.

maintained between generations, whether they are obligate or facultative under field conditions, and the types of "products" that are exchanged between the partners (Douglas 1994). Both the LR and SV symbioses are horizontally transmitted between generations; that is, the symbiont is not passed in or on the host's germ cells, but rather with each host generation, the symbiont must be acquired anew from the environment. In contrast with the facultative LR associations, which occur only under conditions of nitrogen limitation in the soil, the SV symbioses appear to be obligate for the squid; squid hosts have never been found without their symbionts. This difference in the two associations is reflected in the nature of the exchange between the partners. In the LR symbiosis, the bacterial cells are believed to undergo a terminal differentiation, at least in the case of those bacteroids living within the cells of indeterminate nodule-forming hosts (Figure 3C, D). The bacteroids fix nitrogen and transport nitrogenous compounds to the legume (Hirsch 1992). Thus, the benefit of this symbiosis to the host is nutritional, and whether a symbiosis is formed and whether it persists depends on environmental conditions. In contrast, in the SV symbiosis, the extracellular bacterial partners produce light, which the host uses to camouflage itself, presumably as an essential antipredatory strategy (McFall-Ngai 1990). However, under laboratory conditions, the squid host can be antibiotically cured of its symbionts with no ill physiologic effects (Doino and McFall-Ngai 1995). In other words, no evidence exists that the symbionts provide essential nutrients to the host. In addition, although the bacterial cells differentiate in the SV symbiosis, it is not a terminal differentiation. *V. fischeri* cells can, and do each day (see below), revert to their free-living state (Graf and Ruby 1998; Nyholm and McFall-Ngai 1998). Another obvious difference between the two associations is in the type of environment in which they are found. The LR symbiosis is terrestrial, whereas the SV relationship is aquatic. These important characteristics of the two symbioses define the essential nature of the two associations and affect the patterns of their establishment, development, and stable maintenance over the life history of the host.

Initiation of the Interaction

In horizontally transmitted symbioses, mechanisms must exist to bring the symbiont into the vicinity of susceptible host tissues. This process has been extensively studied in the LR symbiosis, and a wide vari-

ety of genes and chemical signals produced by either the host or symbiont have been identified as crucial players in mediating this process. In contrast, only the broad outlines of the process by which the light organ is induced have been defined in the SV symbiosis, but the little that is known suggests that some intriguing similarities exist between this association and the LR symbiosis.

Attraction

The LR symbiosis takes place in the soil, which is composed of a suspension of particles made up of organic and inorganic material dispersed in water. Compared with the relatively chaotic, aquatic environment, the soil appears relatively stable. However, soil can be inundated with torrential rains, leading to erosion and disruption of soil layers. Rhizobia may also be widely dispersed and few in number, especially in soils where legumes do not routinely grow. Some estimates have suggested that there may be fewer than 10^2 rhizobial cells/g of soil (Singleton and others 1992). Therefore, to maximize the possibility of interaction, legume seeds and roots secrete flavonoids and related molecules that attract the rhizobia. These chemoattracting molecules, which exhibit some bacterial strain specificity, also serve to induce rhizobial *nod* genes so that the bacteria synthesize the primary morphogenetic signal (Nod factor) for inducing the host's response (see reviews by Crespi and Gálvez 2000; Long 1996; Schultze and Kondorosi 1998). Nod factors are variable-length *N*-acetylglucosamine oligomers with either a C-16 or C-18 acyl tail at the nonreducing end and various other substitutions at the reducing end (Figure 4A). Nod factors appear to be the main rhizobial inducer molecules for nodulation because the purified molecules elicit, in a host-specific way, many of the plant responses observed in the early stages of nodule formation (Crespi and Gálvez 2000). Eventually, the rhizobia enter a deformed root hair by way of an infection thread (Figure 1B), but cell-cell contact is required for the thread to form.

Recent studies of the SV association have revealed striking similarities to the LR symbiosis in these early events. On hatching, squid tissues that are destined to become symbiotic are exposed to microbes in the ambient seawater, which bathes the internal squid tissues through the normal ventilatory movements of the host. The interaction of the host with environmental gram-negative bacteria induces the secretion of mucuslike material in the vicinity of susceptible host tissues (Nyholm and others 2000). Juvenile squid have unique, complex ciliated

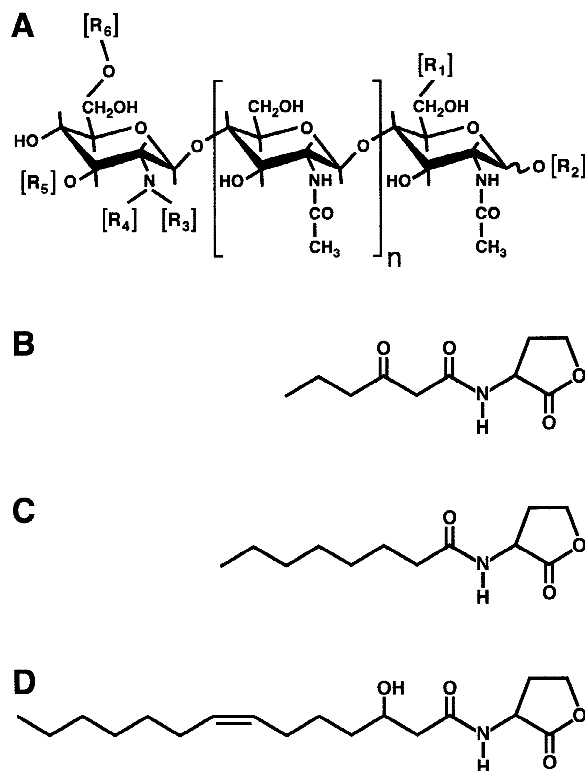

Figure 4. Molecules involved in microbe-symbiont communication. **(A)** Generic Nod factor. *n* refers to the number of glucosamine residues in the backbone. R_1 can be H, sulfate, fucose, methylfucose, sulfomethylfucose, acetylmethylfucose, or D-arabinose. R_2 is either H or glycerol, whereas R_3 is either H or CH_3. R_4 is an acyl group of usually 16 or 18 carbons. R_5 can be either H or a carbamoyl group, and R_6 can be H or an acetyl or carbamoyl group. **(B)** VA1, *N*-(3-oxohexanoyl)-L-homoserine lactone autoinducer produced by *Vibrio fischeri.* **(C)** VA-2, a second *V. fischeri* autoinducer. **(D)** The autoinducer from *Rhizobium leguminosarum* bv. *viciae*.-

fields of epithelia (McFall-Ngai and Ruby 1991) that maintain the secreted mass in place, preventing it from being washed away by the ventilatory currents of the host (Figure 5A). Bacteria aggregate in the secreted matrix of this mucuslike material and, by some yet undetermined mechanism, the population of *V. fischeri* cells preferentially accumulates in this aggregate. After a residence time of several hours within the matrix, the bacterial cells migrate to and enter pores on the surface of the light organ (Figure 2D). These pores lead, by way of long ciliated ducts, to epithelia-lined crypt spaces that become filled by the growing population of *V. fischeri* cells. Although other types of bacterial cells will aggregate, only *V. fischeri* cells are capable of successfully completing this migration to enter the crypts where they proliferate. The bacterial symbionts remain extracellular,

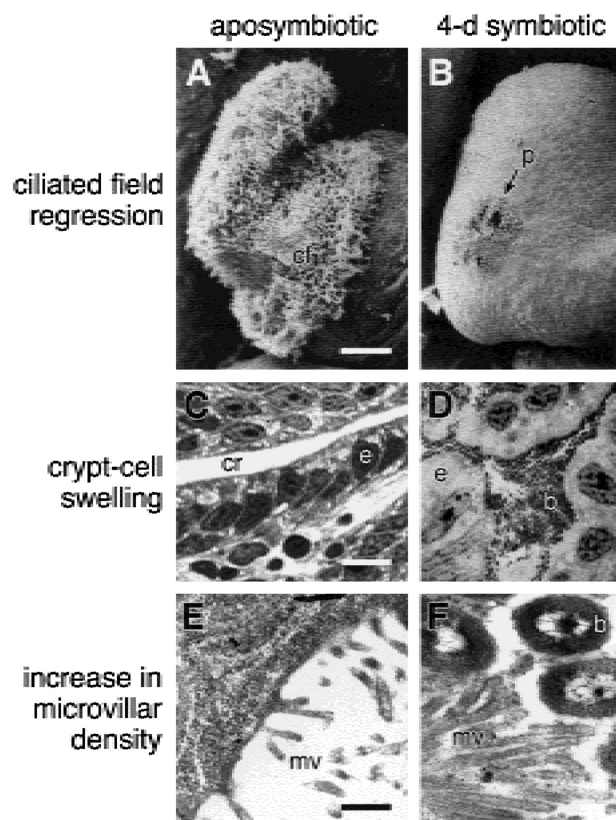

Figure 5. Comparison of developmental changes in the juvenile light organ over the first 4 days after hatching in animals not exposed (*left three panels*) and exposed (*right three panels*) to *V. fischeri*. *b,* bacteria; *cf,* ciliated field; *cr,* crypt; *e,* epithelial cell; *mv,* microvilli; *p,*pores. Bars **A, B;** 50 µm. Bars **C, D;** 20 µm. Bars **E, F;** 0.5 µm.

associated with the apical surfaces of the host crypt cells, throughout the life history of the host (Figure 2C). Studies with *V. fischeri* mutants that are defective in motility (Graf and others 1994) indicate that, although they aggregate, they do not leave the matrix to colonize host tissues. In addition, the bacteria, when they do eventually move to colonize, show a strong vector toward the site of entry, suggesting that chemotaxis is also essential (Figure 2D).

The principal differences between the LR and the SV symbioses in these early events are in the order in which they occur. In the relatively structured environment of the soil, more extensive chemical gradients can be established. These gradients appear to provide the mechanism by which the rhizobial symbionts are initially recruited to the root from the general rhizosphere population of bacteria; that is, their enrichment in the vicinity of the root hair is a direct result of chemotaxis to the root. In the fluid environment of the seawater, which is dominated by high shear stress and turbulent flow, stable chemical gradients are less apt to form. Thus, it is likely that only after the bacteria accumulate in the mucuslike material that is adjacent to the susceptible tissues does chemotaxis of the bacteria toward host tissues play a significant role in colonization. The molecules that attract the two symbionts are likely to be different. For rhizobia, plant-secreted secondary metabolites (flavonoids, anthocyanins and related molecules [Paiva 2000]) are the major attractants, whereas for the vibrios no specific chemical has been identified yet.

Autoinduction

Many bacterial species that occur in associations with eukaryotic tissues, whether the relationship is pathogenic or beneficial, exhibit a group behavior called "quorum-sensing" (Fuqua and others 1996). In this behavior, the bacteria constitutively produce low levels of one or more specific pheromone-like molecules, or autoinducers, that accumulate in the surrounding environment only when that species of bacterium achieves a high population density in a confined area (Visick and Ruby 1999). In many gram-negative bacteria, these autoinducers are a family of chemically distinct, but homologous, acyl-homoserine lactones (HSL; Figure 4B–D). Having reached a critical ambient concentration, the autoinducer diffuses back into the bacteria, positively up-regulating its own production, as well as the transcription of a series of other genes organized as a regulon. Typically, the gene expression up-regulated by autoinducers directs the synthesis of products that are characteristic of the symbiotic state.

The phenomenon of autoinduction was first described in *V. fischeri,* which was found to produce luminescence in culture only after achieving a high population density (Nealson 1999; Nealson and Markovitz 1970; Nealson and others 1970). The *luxI* gene of the luminescence (or *lux*) regulon encodes the gene that directs synthesis of a 3-oxo-hexanoyl HSL (Figure 4B) in *V. fischeri* (Eberhard and others 1981). The product of the *luxR* gene is a transcriptional activator that, when bound to autoinducer, directs the induction of the *luxCDABE* genes, which encode the structural proteins required to drive the emission of bioluminescence (Engebrecht and others 1983). Recently, a second autoinducer synthase gene (*ainS*) and an associated regulatory gene (*ainR*) have been identified in *V. fischeri* (Gilson and others 1995). The relative roles of these two autoinducers (Figure 4B, C) in the control of bacterial luminescence in the various niches occupied by *V. fischeri* have not yet been fully defined (Visick and others 2000). Homologues of *luxI* and *luxR* have been

found in at least 15 other bacterial genera, including *Rhizobium, Pseudomonas,* and *Agrobacterium* (Dunny and Winans 1999).

Studies of *V. fischeri* in symbioses, such as that in *Euprymna scolopes,* have revealed that 3-oxo-hexanoyl HSL accumulates to inducing levels in the confines of the light organ crypts (Boettcher and Ruby 1995), producing a brightly luminescent population of symbionts (Boettcher and Ruby 1990). When and where during the initiation of the SV association the initial autoinduction takes place remains unresolved. Perhaps the several-hour residence time of *V. fischeri* in the aggregates outside of the light organ is a period when the bacteria induce specific genes, such as those associated with autoinduction, that prepare the bacteria for interaction with host tissues.

Rhizobia also undergo autoinduction or quorum-sensing. The *rhiABC* (*rhi* for rhizosphere-expressed) genes of *R. leguminosarum* bv. *viciae* are expressed in the rhizosphere or in stationary-phase laboratory cultures. RhiA is the most prominent single protein of the cell and is not expressed in bacteroids, the nitrogen-fixing form of rhizobia (Dibb and others 1984). The *rhiABC* operon is regulated by RhiR and is induced by *N*-acylated homoserine lactones (AHL; Figure 4D) (Gray and others 1996). The *rhiI* gene is also regulated by *rhiR* in a cell-density–dependent manner, and like *luxI, rhiI* appears to be involved in AHL synthesis (Rodelas and others 1999). It is not known how important autoinduction is for nodulation. In *R. leguminosarum* bv. *viciae,* the *rhi* genes are located within the *nod/nol/noe* operons, and repression of *rhi* expression by *nod/nol/noe*-induced compounds suggests an interaction between these two regulons (Economou and others 1989). Defects in nodule development are detected in certain *nod rhi* double mutants (Cubo and others 1992). As in the SV symbiosis, the aggregation of rhizobia in the rhizosphere before root hair penetration may be required for the induction of genes for later stages of the symbiosis. In addition, in *R. leguminosarum* bv. *phaseoli,* more cells survive stationary phase after carbon or nitrogen starvation if grown at a high rather than a low density, and survival of low-density cells can be improved by adding AHL (Thorne and Williams 1999).

Cell Surfaces

Rhizobia are typical gram-negative bacteria with a cytoplasmic and outer membrane enclosing a periplasmic space. They secrete various types of extracellular material, much of which is composed of polysaccharides—lipopolysaccharides (LPS), capsular polysaccharides (CPS), cyclic β-glucans, and acidic exopolysaccharides (EPS); the latter are secreted into the culture medium. Mutants that are defective in the production of these polysaccharides are often blocked in various stages of nodule development, particularly nodule invasion by means of infection threads.

The succinoglycan biosynthetic pathway for exopolysaccharide production in *Sinorhizobium meliloti* is perhaps one of the best understood for the rhizobial cell surface molecules. This polysaccharide is an octasaccharide polymer composed of one galactose and seven glucose residues, with acetyl, succinyl, and pyruvyl modifications (Aman and others 1981; Reuber and Walker 1993). *S. meliloti* mutants that do not produce EPS or synthesize defective EPS form small, bacteria-free nodules on alfalfa with infection threads that abort within the root hairs (Cheng and Walker 1998; Finan and others 1985; Leigh and others 1987). One hypothesis explaining the function of EPS is that it serves as a suppressor of plant defense reactions, thereby enabling rhizobia to enter the host cell (Becker and others 2000). Another function for EPS, especially low molecular weight EPS II, is that it serves as a signal molecule (González and others 1996). Other experiments in which EPS mutants were inoculated onto transgenic legumes carrying a nonhost lectin gene demonstrate that rhizobial exopolysaccharide may interact with lectin in the attachment stages of nodule formation (van Rhijn and others 1998; van Rhijn and others personal communication. The specific binding of a legume lectin to a compatible *Rhizobium* allows the two symbionts to recognize each other (see Hirsch 1999). This binding, along with other proteins involved in rhizobial attachment, enables a consortium of bacteria to become established at the tip of a susceptible root hair. The resulting increase in concentration of compatible Nod factor brings about root hair deformation and, at higher concentrations, infection thread formation and nodule development on a heterologous host (van Rhijn and others 1998; van Rhijn and others submitted). Thus, EPS appears to play a critical role in the earliest stages of nodule development.

A symbiotically active form of a strain-specific K antigen, a capsular polysaccharide containing a Kdo (3-deoxy-D-manno-octulosonic acid derivative) enables *S. meliloti* AK631 to nodulate alfalfa even though it synthesizes neither succinoglycan or EPS II (Putnoky and others 1990). Mutagenizing genes involved in K antigen synthesis eliminated AK631's ability to induce nitrogen-fixing nodules (Campbell and others 1998). Several of these mutants were also found to be defective in LPS.

LPS is composed of three domains: lipid A, core oligosaccharide, and the O-antigen (OPS) (see Kannenberg and others 1998; Noel and Duelli 2000). LPS molecules are anchored in the bacterial outer membrane with polysaccharide moieties extending into the environment, making them potential candidates for cell-cell interactions. LPS is likely to be involved in infection thread development and bacterial release into the host cell on the basis of studies with mutants and monoclonal antibodies (MABs) made to LPS. However, so far, many of the mutants that have been studied and that induce nodules with symbiotic defects are OPS^{-}, that is, mutants with small amounts of LPS or with truncated OPS (Noel and Duelli 2000). Similarly, the MABs used so far only detect structural changes in the O-antigen portion of the LPS and not in the core or lipid A (Kannenberg and others 1998). Moreover, the symbiotic defects observed in response to OPS mutants vary depending on whether the host legume produces determinate or indeterminate nodules. Determinate nodules are those that cease cell division early and increase in size by cell expansion (Figure 3A), whereas indeterminate nodules have a distal meristem that continually adds new cells (Figure 1A) (Crespi and Gálvez 2000; Hirsch 1992).

For determinate-nodule–producing hosts, the defects are much more serious. A non-nodulation phenotype depending on the soybean variety has been described for *Bradyrhizobium elkanii* with a mutated OPS, but on some varieties callus developed (Stacey and others 1991). *R. etli* mutants induce nodules on bean, but the nodules are arrested in their development and appear more rootlike in that they have a central vascular bundle rather than peripheral bundles (Noel and others 1986). For indeterminate-nodule–forming plants, OPS mutants elicit the formation of nodules with abnormal infection thread formation and bacterial release (de Maagd and others 1989; Perotto and others 1994). The reason for these differences is unknown but may relate to the origin of cell divisions for the nodule primordia: outer cortex for determinate nodule-forming hosts and inner cortex for indeterminate nodule-forming plants. Also, the infection thread path for indeterminate nodules is significantly longer than in determinate nodules. Infection threads are evident in the young developing nodule (Figure 1C, D), in the infection zone (Figure 1E) of the mature nitrogen-fixing nodule, and also in the proximal regions of senescing nodules (Figure 3E, F).

Mutants in the core nonrepeating oligosaccharide have been described for *R. leguminosarum* bv. *viciae* (Kadrmas and others 1998), and it is likely that the *S. meliloti* mutant described by Niehaus and others (1998) represents an LPS core mutation. The *R. leguminosarum* bv. *viciae* genes *lpcA-C* encode novel glycosyltransferases—a galactosyltransferase is encoded by *lpcA,* a Kdo-transferase by *lpcB,* and a mannosyltransferase by *lpcC* (Kadrmas and others 1998). *R. leguminosarum* bv. *viciae lpcA* mutants infect root nodules by way of enlarged infection threads, but the released rhizobia fail to differentiate into bacteroids (Priefer 1989). A similar phenotype was observed for *lpcC* mutants when they were inoculated onto pea roots (Kadrmas and others 1998).

The cell surfaces of *V. fischeri* are less well described than those of rhizobia. *V. fischeri* does not produce a polysaccharide capsule in culture (P. Fidopiastis, personal communication), and whether a capsule is present when they are in symbiosis with the squid host is yet unresolved. However, host-symbiont cell interactions have been implicated at all stages of the SV symbiosis. Adhesion of bacterial cells to host ligands appears to be involved in the very first interactions with the mucus aggregates (Nyholm and others 2000), in the early adhesions of cells to the crypt cell surfaces (McFall-Ngai and others 1998), and in the retention of cells in the crypts during the host's diel venting of symbionts into the environment (Nyholm and McFall-Ngai 1998).

Although the precise mechanisms that govern this range of interactions have not been fully defined, a number of specific cell surface molecules have been identified as critical elements in the interactions between the light organ and the bacterial symbionts. Several lines of evidence suggest that mannose adhesin-glycan interactions are essential in the establishment of the association (McFall-Ngai and others 1998). *V. fischeri* cells hemagglutinate guinea pig red blood cells, a behavior that is indicative of mannose-recognizing adhesins on their cell surface. These glycan-binding molecules can be either associated or not associated with pili, proteinaceous appendages extending from the outer surface of the bacterial cell. In addition, mannose glycans are the only abundant sugars on the apical surfaces of host crypt cells, and when analogs of mannose are introduced into the seawater during the initiation of the symbiosis, host tissues do not become colonized by the symbiont. Other sugars and their analogs are not capable of inhibiting host colonization.

Another *V. fischeri* surface ligand, OmpV (Omp for outer membrane protein), is also a candidate molecule in host-symbiont cell interactions (Aeckersberg and others 1998). When presented alone to the host, *V. fischeri* cells mutant in the *ompV* gene colonize host tissues normally. However, when introduced to the host at a ratio of 50:50 with the parent strain, these mutants are at a disadvantage, being

significantly outcompeted by the parent strain in colonizing the organ crypts.

The LPS of *V. fischeri* is another likely candidate for mediating the interactions of the partners in the SV symbiosis. Bacterial LPS causes a variety of responses in animal cells, perhaps the best described being the inflammatory response in vertebrates. Thus far, two possible aspects of the SV symbiosis may be influenced by bacterial LPS: the induction of mucus aggregates during the first phases of the symbiosis, and induction of cell death associated with *V. fischeri*–triggered development of the host. As mentioned earlier, in response to gram-negative environmental bacteria, the host produces a mucuslike secretion in which the symbionts become entrapped. In experiments describing this phenomenon, when presented to the host, gram-negative bacterial cells, living or dead, induce host cell mucus production. This mucus production does not occur in response to the exposure of the host to gram-positive bacteria. One of the most conspicuous characteristics that align all gram-negative bacteria is the presence in their outer membranes of LPS, a molecule whose biochemical activity is independent of the viability of the bacterial cell. In addition, bacterial LPS is a well-known inducer of mucus production by animal cells (Jeffery and Li 1997). These data provide only circumstantial evidence that LPS is involved in the process of host cell mucus production. Definitive proof of the involvement of this molecule awaits experiments with bacterial mutants defective in the synthesis of normal LPS.

LPS may also trigger the development of host tissues. During the symbiont-induced morphogenesis of the squid light organ (see later), the bacteria induce cell death and the eventual loss of the remote, superficial ciliated epithelia (Figure 5B) (Foster and McFall-Ngai 1998); McFall-Ngai and Ruby 1991; Montgomery and McFall-Ngai 1994) that are involved in the suspension of the mucus aggregates (Figure 2D). Early experiments describing this phenomenon showed that *V. fischeri* must enter the light organ crypts to trigger the cell death program of this epithelial field (Doino and McFall-Ngai 1995); exposure of the host to large numbers of environmental bacteria does not result in apoptosis or loss of the field. Because LPS is a well-known inducer of animal cell apoptosis (Aliprantis and others 1999; Guichon and Zychlinsky 1996; Norimatsu and others 1995), *V. fischeri* LPS was tested as a possible inducer of host cell death (Foster and others 1999). Purified *V. fischeri* LPS, as well as lipid A, the LPS constituent that is responsible for LPS endotoxin activity, instigated the programmed cell death in a similar spatial pattern and time frame to that observed for living, intact *V. fischeri* cells. The lipid A component of the LPS is conserved throughout gram-negative bacteria, so the LPS derived from any gram-negative species was capable of inducing cell death in the host epithelium. Using fluorescently labeled LPS and confocal microscopy, the LPS was found to interact with two sets of host cells, the epithelia lining the crypts and a population of host macrophages that samples the crypt spaces. Thus, although all gram-negative bacteria have lipid A, only *V. fischeri* is capable of entering the light organ and interacting with cells in the crypts, thereby transducing the signal to the superficial epithelium.

Is There an Oxidative Burst?

One of the key early responses in plant-pathogen interactions is an oxidative burst, which results in the production of reactive oxygen species (ROS): (1) superoxide anion (O_2^-), produced from oxygen by means of a membrane-bound NADPH oxidase that resembles the mammalian neutrophil enzyme and (2) hydrogen peroxide (H_2O_2), produced from superoxide by superoxide dismutase (SOD) (Keller and others 1998; Lamb and Dixon 1997; Xing and others 1997). In combination with nitric oxide (NO), H_2O_2 leads to a hypersensitive response (HR) and death of the plant cell (see Durner and Klessig 1999). ROS species are also believed to function directly as antimicrobial agents, and H_2O_2 is a likely substrate for oxidative cross-linking of cell wall proteins by wall-bound peroxidase (Bradley and others 1992; Lamb and Dixon 1997). NO is also a signaling molecule in both mammalian systems and in plant-pathogen interactions. The fact that ROS and NO lead to similar effects in animals and plants implies a common ancestry in an innate immune system. Do such responses occur in symbiotic associations? If so, this might imply a close relationship between pathogenic and symbiotic interactions.

Cook and colleagues observed that expression of a rhizobial-induced peroxidase (*rip1*) is tightly correlated with the early stages of nodule development. This legume gene is expressed first in the differentiating root epidermis and then in the nodule primordium (Cook and others 1995; Peng and others 1996). There appear to be putative H_2O_2-inducible *cis* elements in the *rip1* promoter. Nod factor treatment increases the expression of *rip1,* and the question is whether an oxidative burst occurs in the nitrogen-fixing symbiotic interaction. Experiments show that treatment of roots with H_2O_2 was sufficient to induce *rip1* gene expression, and pretreatment with diphenylene iodonium (DPI), an inhibitor of heme-containing oxidases such as NADPH

oxidase, blocked *rip1* induction by Nod factor (D. Cook, personal communications). Other experiments suggest that superoxide is produced after Nod factor treatment, and its production, along with *rip1* induction in *Medicago* sp., occurred only in response to sulfated Nod factor.

One of the first descriptions of nitric oxide synthase (NOS) activity in plants was in roots and nodules of *Lupinus albus* (Cueto and others 1996). Three sites of NADPH-diaphorase staining, which is often used to localize NOS activity, were detected in the nodules: in the vascular bundles, in the periphery of the infected zone, and in the infected zone of the nodule. However, other enzymes also have NADPH-diaphorase activity, and the staining might overestimate the amount of NOS. The biological significance of NOS may lie in the fact that NO inhibits nitrogenase and consequently nitrogen fixation (Meyer 1981). For example, NO binding to leghemoglobin or the oxygen-regulated FixL may inhibit the function of these two heme proteins (Mathieu and others 1998). Cueto and others (1996) speculated that rhizobial LPS may induce NOS, but so far no experiments have been reported. In any case it is not certain how NO is involved in symbiotic interactions. No evidence of programmed cell death exists in response to a compatible rhizobial strain, so it seems unlikely that ROS and NO are involved to a large scale.

Respiratory burst activity has not yet been measured in squid cells in response to interactions with *V. fischeri,* but several lines of evidence suggest that the oxidative environment is critical in the dynamics of the symbiosis (Ruby and McFall-Ngai 1999). The light organ has high levels of an mRNA species that encode a protein with significant sequence similarity to mammalian myeloperoxidase (MPO) (Tomarev and others 1993), a protein abundant in cells that mediate the innate immune response in vertebrates (Klebanoff 1991). MPO converts H_2O_2, generated as a consequence of host cell respiratory burst, and halide ions to hypohalous acid, a potent antimicrobial agent that acts on phagocytosed potential pathogens. The squid peroxidase (SPO) has biochemical and antigenic properties similar to that of mammalian MPO. SPO occurs in regions of the organ that interact directly with the symbionts, that is, the apical surfaces of the crypt cells, the crypt spaces, and the macrophage-like cells that sample the crypt spaces (Weis and others 1996).

Bacteria typically are unable to withstand the activity of halide peroxidases but are known to rely on two mechanisms by which to circumvent this antimicrobial activity: by inhibiting respiratory burst activity or by competing with the peroxidase for the substrate H_2O_2 resulting from the respiratory burst, Dukan and Touati 1996; Tartaglia and others 1989). Studies of *V. fischeri* suggest that both strategies are used. The occurrence of a respiratory burst in host defense depends on the availability of molecular oxygen. The luciferase (the mixed-function oxidase that catalyzes the production of light) of *V. fischeri* has an unusually high affinity for oxygen (Ruby and McFall-Ngai 1999), and analyses of luciferase activity in the symbiosis have indicated that it drastically lowers the free oxygen in the crypt spaces (Boettcher and others 1996). Lowered oxygen would impair the activity of any enzymes involved in the initial respiratory burst that gives rise to the H_2O_2. Although direct measurements of oxygen tension in the symbiotic light organ have not been made, mutants of *V. fischeri* that are defective in light production show a defect in their ability to persist in the light organ (Visick and others 2000), a phenotype that may be linked to a change in their oxygen use. *V. fischeri* may also effectively compete for the H_2O_2 resulting from any respiratory burst activity that has occurred. Visick and Ruby (1998) described a periplasmic, group III catalase in *V. fischeri* that is induced by oxidative stress.

Host Range

The diversity of the squids with light organ symbioses is relatively low compared with the LR associations, which are highly host specific. Bacterial light organs are found in a couple of dozen species in eight genera of sepiolid squids, which occur as a family in both nearshore and deeper water environments in the tropics to polar latitudes (McFall-Ngai 1999). Thus far, members of two host groups have been studied, species of the IndoPacific genus *Euprymna* and the Mediterranean/Atlantic genus *Sepiola.* Strains of only two bacterial species, *V. fischeri* and *V. logei,* have been isolated from these host light organs (Fidopiastis and others 1998). All *Euprymna* spp. analyzed thus far harbor *V. fischeri* in their symbiotic tissues, whereas the *Sepiola* spp. have *V. fischeri* or *V. logei,* either as a monoculture of one of these species or as a mixed culture of these two vibrio species within a single light organ.

The only data presently available for host range of the vibrios in the squid symbioses has been provided by (1) characterization of the phylogenies of several hosts and their symbionts and (2) experiments that have explored the recognition of symbionts from various host species by the Hawaiian host *Euprymna scolopes* (Nishiguchi and others 1998). The molecular phylogenies of *V. fischeri* from various *Euprymna* and

Sepiola hosts provided strong evidence of strain differences among the microbial symbionts. Furthermore, congruent cladograms of the hosts and symbionts resulted when the molecular phylogenies of the partners were aligned, a finding that suggested that coevolution of the partners has accompanied the radiation of the host. This hypothesis was supported by experimental manipulation of colonization in *E. scolopes.* This series of studies showed that *E. scolopes* will be colonized by any bacterial strain that has been a symbiont in a sepiolid squid when that strain alone is presented to the host. However, in experiments in which *E. scolopes* is exposed to a 50:50 mix of native and non-native *V. fischeri* strains, both strains enter the light organ in equal numbers, but the non-native strain is gradually eliminated over the first 2 to 3 days after the onset of colonization. And, when *E. scolopes* is exposed to two non-native vibrio strains, the symbiont from the host that is most closely related to *E. scolopes* will persist. A matrix of experiments in which all possible pairs of *V. fischeri* strains were presented to *E. scolopes* showed that the ability to colonize the organ competitively directly mirrors the phylogenetic trees of the partners. These data suggest that subtle, incremental changes have occurred over evolutionary time that have resulted in the ability of the host and/or symbiont to discriminate, during the very early period of the association, between an appropriate and inappropriate partner. Because these experiments have suggested such a tight coupling between the phylogenies of the partners and their symbiotic competence, the squid-vibrio system offers the unique opportunity to determine, at least in one group of organisms, the mechanisms by which specificity determinants evolve.

Phylogenetic studies based on the chloroplast gene *rbcL* indicate that there are likely to be multiple origins of nodulation in dicotyledonous plants (Soltis and others 1995). In addition to the legumes, diverse members of the Rosid I (dicot subclass Rosidae of the Rosaceae) line are nodulated, but by the gram-positive actinomycete *Frankia* instead of *Rhizobium* (see Wall 2000). A more complete study of actinorhizal plants and their nonactinorhizal relatives suggests that the interactions with *Frankia* evolved at least four times and perhaps as many as six times during angiosperm evolution (Swenson 1996). Within the legumes, there is some question as to whether there is a single or multiple independent origins of nodulation (Doyle 1998). The legume family is divided into three subfamilies: Caesalpinoideae, Mimosoideae, and Papilionodeae. Caesalpoinoid legumes are the most basal and less commonly nodulated (23% of approximately 2000 species) than the other two subfamilies (90% of approximately 3000 mimosoid species and 97% of approximately 13,000 papilionoid species). Complicating an understanding of the evolution of symbiotic nitrogen fixation is the information obtained from molecular phylogenetic studies of rhizobia. Here, data inferred from 16S RNA gene sequences indicate that there is considerable genetic diversity in the bacteria that nodulate legumes (Young 1996). However, in contrast to the SV symbiosis, there appear to be no congruent trees on the basis of 16S RNA analysis for members of the *Rhizobium* complex (*Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium,* and *Sinorhizobium*) and those for specific legumes based on *rbcL.* One explanation is that clusters of symbiotic genes were horizontally transferred from one unrelated bacterium to another (Sullivan and others 1995), similar to the situation for certain pathogenesis genes (see Bird and Kolhai 2000). Thus, the evolutionary pressures resulting in the specificity observed in the LR symbiosis remain independent from molecular phylogeny.

Although some rhizobia such as strain NGR234 nodulate numerous legumes, as well as the nonlegume *Parasponia*, most partnerships in the LR symbiosis, especially for the most-studied members of the family, the papilionoid legumes, are highly host-specific. For example, *Sinorhizobium meliloti* nodulates alfalfa, sweetclover, and fenugreek, but not soybean, and *Bradyrhizobium japonicum* nodulates soybean, but not alfalfa, sweetclover, or fenugreek. In part, specificity in LR associations is mediated by rhizobial Nod factor. For example, the *S. meliloti* Nod factor has a C-16 acyl tail at the nonreducing end and a sulfate at the reducing end, whereas the *R. leguminosarum* bv. *viciae* Nod factor has a C-18 fatty acyl residue and no sulfate. A mutation in *nodH* leads to the production of non-sulfated *S. meliloti* Nod factors and a change in the symbiotic host range from alfalfa to *R. leguminosarum* bv. *viciae* hosts such as pea or vetch (Roche and others 1991). Another example of specificity is observed in the *sym2* mutant of pea (*Pisum sativum*), which is nodulated by only some strains of *R. leguminosarum* bv. *viciae* (strain TOM). Strain TOM has a gene *nodX,* which encodes an *O*-acetyl transferase that puts an *O*-acetyl group on the C-6 of the reducing *N*-acetylglucosamine residue (Firman and others 1993). This modified Nod factor is required to overcome the nodulation resistance of peas carrying *sym2.*

Host specificity in the LR symbiosis can also be influenced by other factors such as lectins (Hirsch 1999). Introduction of a soybean lectin (SBL) gene into *Lotus corniculatus* yielded transgenic plants that were nodulated by *B. japonicum,* the soybean sym-

biont (van Rhijn and others 1998). However, for this response to occur, there must be recognition by the plant of the rhizobial Nod factor. An example is demonstrated by transgenic alfalfa lines carrying either SBL or PSL (pea lectin) genes, which do not nodulate unless the heterologous rhizobial cells used as inoculum express *S. meliloti* genes for producing a sulfated Nod factor (van Rhijn and others personal communication). It is possible that lectin function is analogous to that of the mannose adhesin-glycans on the surfaces of the squid host crypt cells in that the lectin aids colonization of the host by the bacteria. In any case, there appears to be multiple points of specificity in the LR interaction. The fact that nodule development can be arrested at several developmental stages by mutations in either bacterial or plant genes indicates that considerable "hand-shaking" goes on between the two symbiotic partners (see Crespi and Gálvez 2000). The nature of this "hand-shaking" is still not very well understood. It is likely that many of the signals exchanged are specific to each symbiotic partner, whereas others may be shared among the broad spectrum of the LR associations.

Developmental Similarities and Differences

The Later Stages

The progression of the developmental program of the symbiosis is the best understood aspect of both the LR and SV associations. Several very basic similarities and differences can be noted. In both symbioses, bacteria eventually reside deep in host tissues. However, the obligate nature of the SV symbiosis has developmental consequences for the squid. During embryogenesis of the squid host, a nascent organ is formed, the sole function of which is to house the symbiosis (Montgomery and McFall-Ngai 1993). Similarly, the legume nodule develops from root cortical cells, which de-differentiate, undergo cell divisions, and initiate a new organ, its sole purpose to house the nitrogen-fixing rhizobia (Hirsch 1992). However, in the presence of adequate N, nodule development does not proceed, or the already developed nodules senesce, whereas in the case of the SV symbiosis, the light organ is retained until the squid dies, perhaps because nutrition is not a factor in its development.

In both symbioses, interactions of the host with the symbiont induce developmental changes in the cells directly in contact with the symbiont, as well as in remote tissues. In the LR symbiosis, attachment to the root hair cells induces deformation of the root hair and the eventual formation of the infection thread (Figure 1B, C), the path through which rhizobia wend their way to the recently divided cortical cells, which form the nodule primordium (Figure 1D). However, cortical cell division can begin in some legume roots without direct contact with the symbionts, implying the presence of a diffusible or transmittable signal either directly or indirectly related to Nod factor.

In the SV symbiosis, *V. fischeri* induces the crypt cells, with which it directly interacts, to swell and to increase the density of their brush border microvilli (see Figure 2B–E) (Montgomery and McFall-Ngai 1994; Lamarcq and McFall-Ngai 1998). Whereas no mutants have been identified that are defective in inducing an increase in microvillar density of the crypt brush border, mutants defective in light production fail to induce host cell swelling (Visick and others 2000). This phenotype may be due to the inability of these cells to lower oxygen levels, as previously mentioned; hypoxia is a well-documented inducer of animal cell swelling (Hierholzer and others 1997; Mairbaurl and others 1997). Animal cell swelling is also known to cause an increase in membrane transport (Okada and others 1994), which, in this case, may be critical in satisfying the nutritional demands of the symbionts. Thus, the observed lack of persistence of mutants in light production may be due to their inability to access host cell nutrients. Remotely, the microbial symbionts induce the complete loss of the superficial, ciliated epithelium over the first several days after the initial colonization of the crypts (McFall-Ngai and Ruby 1991; Montgomery and McFall-Ngai 1994). Some or all of this process is mediated through bacteria-induced apoptosis (Foster and McFall-Ngai 1998; Montgomery and McFall-Ngai 1994; Figure 2D, E).

Legume nodules originate in the root cortex, and mitotic divisions in the pericycle are either concurrent or soon follow (see Crespi and Gálvez 2000). As these cell divisions are taking place, the infection thread continues its course through the infected root hair and adjacent cortical cells toward the nascent nodule primordium. The cytoskeletal architecture of the cortical cells is altered to form a cytoplasmic bridge before the entry of the infection thread (van Brussel and others 1992). Once it expends into the nodule primordium, the infection thread disgorges its rhizobial contents by an endocytotic-like process (Figure 1E). The rhizobial cells become surrounded by host-derived membrane as they enter the host cell cytoplasm (Figure 3C). Eventually, the host cell becomes packed with numerous membrane-encircled bacteria (the combination is oftentimes called a symbiosome) and along with their host cell

differentiate (Figure 3B). The result is an expanded host cell that is filled with nitrogen-fixing bacteroids (Figure 3B, D). Nevertheless, a significant number of cells in the nodule remain uninfected. These include the cells of the nodule cortex that border the central zone, the endodermis, and the cells of the vasculature, as well as a number of uninfected central cells, which are smaller in size than their infected counterparts.

Once they are within the host cells, the rhizobia remain there until the nodule senesces. This persistence contrasts to the SV symbiosis, in which 90 to 95% of the symbiotic population of vibrios is expelled from their host on a daily basis (Graf and Ruby 1998; Lee and Ruby 1994). In the well-studied indeterminate nodule-forming symbiosis, rhizobia sequestered within infection threads are untouched by the lysis of the nodule cell, and eventually are released into the soil when the nodule decays (Figure 3E, F).

Are Legumes and Squids Hard-Wired to Interact with Their Symbionts?

Some evidence suggests in the LR symbiosis that some legumes are programmed to form a nodule even without bacterial involvement. Alfalfa Nar (*n*odulation in the *a*bsence of *r*hizobia) mutants develop nodules spontaneously (Truchet and others 1989). This mutation is dominant (Caetano-Anollés and others 1992) and results in the development of elongated nodules that not only express early nodulin genes but also develop a discrete nodule meristem and peripheral vascular bundle (see Crespi and Gálvez 2000). However, instead of bacteroids, the nodule cells are filled with amyloplasts, starch-filled plastids. The existence of the Nar mutant indicates that some legumes have the capacity to form nodules without a rhizobial stimulus. It further implies that such plants are hard-wired in terms of gene expression and pattern of cell divisions and cellular differentiation. However, this phenotype has not been described for legumes other than alfalfa, indicating that it is not a common occurrence. Most legumes do not exhibit this much control over their own destiny; indeed, many do not even undergo cell division in response to added Nod factor.

In both the LR and SV symbiosis, during development of would-be symbiotic tissue, specific receptors must be expressed in cells that mediate the initial phases of the symbiosis, that is, on the root hair membranes that interface with the soil and along the brush border of the crypt cells. In the LR symbiosis, these early interactions generally trigger much of nodule development, whereas in the SV symbiosis, the bacteria only participate in the early remodeling of the nascent organ that has already formed during embryogenesis. The late developmental events of light organ development, specifically, the elaboration of accessory tissues that are involved in the function of modulating bacterial light production, are "hard wired," that is, they do not require interaction with *V. fischeri* (Montgomery and McFall-Ngai 1998). Thus, unlike the LR symbiosis in which development involves a more elaborate, reciprocal dialogue, bacteria-induced morphogenesis of squid host tissues is restricted to a discrete period of only a few hours after onset of the symbiosis.

Conclusions

We have concentrated in this review on the initial stages involved in the establishment of the SV and LR symbioses. The later stages of their interaction show much greater divergence than the earlier ones, most likely as a result of the disparate functions and habitats of these two symbioses, as well as other factors. For example, development in multicellular plants and animals is significantly different—the lack of cell migration in plants and the differences in the types of hormones produced in plants compared with animals. However, it appears that the stages that occur early in each symbiosis, those that take place at the molecular and cellular levels, are relatively conserved. Both symbioses are initiated after signals emanating from the host are perceived; in both cases, the symbionts are attracted to and are attached to their respective host. For the LR and SV symbioses, cell surface molecules are critical for cell-cell contact. As a consequence of this initial encounter, the concentrations of reactive oxygen species are changed in the two interactions. Moreover, each symbiosis uses quorum sensing to induce bacterial genes encoding products that will have a further influence on the host. In the legume, building a consortium of bacteria induces a wide range of cytologic changes that ultimately result in the fixation of N_2, whereas for the squid, quorum sensing triggers the expression of the *lux* operon and the production of light. In both symbioses, a specialized structure is built from host tissues for bacterial habitation.

Evolution of the LR and SV Symbiotic Interactions

As mentioned in the Introduction, some have likened symbiosis to an "arms race," in which each participant thrusts or parries. This concept has led to the hypothesis that symbiotic associations are de-

rived from parasitic associations—"Domesticating the host is the better long-term strategy for pathogens" (Lederberg 2000). However, rather than the host being domesticated, we believe it is more likely that the prokaryote was "tamed." Prokaryotes evolve much faster than eukaryotes, and it is reasonable that the host would select those bacteria that were more likely to do good rather than harm. This positive selection might have led to the evolution of the complex lock and key system of recognition in the LR symbiosis for discriminating against harmful bacteria or free-loaders.

In contrast to the master-slave relationship exemplified by the eukaryotic cell and its chloroplast or mitochondrion or by the VA mycorrhizal association, in which the fungus is completely dependent on its host for survival (see Barker and Tagu 2000), the partners of the LR and SV symbioses can live independently of one another, although in nature the squid is not found without its symbiont. Although the LR symbiosis is nutrition-based, as are most animal symbioses, it only occurs in the absence of N in the soil. If N is present, the symbiosis is not initiated. Thus, it seems highly unlikely that the ancestral interaction between plant and rhizobia was parasitic. Unfortunately, the fossil record for the LR symbiosis is nonexistent, but it is doubtful that this symbiosis would have been present before the evolution of the angiosperms, that is, some 250 million years ago. Data suggest that the ancestral interaction between legumes and rhizobia may have been commensal or at least saprophytic. (1) For example, commensal *Mesorhizobium loti* become symbiotically competent under field and laboratory conditions on transfer of a large region of DNA, a so-called symbiosis island that contains both *nod* and *nif* genes, from other rhizobia (Sullivan and Ronson 1998; Sullivan and others 1995). The process is no doubt analogous to the transmission of pathogenicity islands seen in mammalian and plant pathogens, but the end result is not homologous. (2) A suggestion that LR association was evolutionarily less intimate comes from a study of basal legumes such as *Gleditsia* (tribe Caesalpinieae), which are reported to have rhizobia within their roots. These rhizobia, which presumably fix nitrogen, resemble bacteroids, but do not induce nodule formation (Bryan and others 1996). (3) Various nonlegumes such as sugar cane and kallar grass also associate with nitrogen-fixing bacteria; these do not enter the host cells, but rather proliferate in the apoplast or live as epiphytes (see Reinhold-Hurek and Hurek 1998). These bacteria cannot be considered parasitic.

In contrast, animal symbioses appear to be almost totally nutrition-based, so it is possible that parasitism could have been the ancestral mode whereby an invader evolved into a symbiotic form. However, the SV symbiosis is a notable exception to this nutritional mode of symbiosis in that the outcome results primarily in light production and not in nutritional enhancement. Moreover, each day, the vibrios are expelled from the squid light organ, and the repopulation of the organ is the result of growth of those cells that have remained in the light organ after expulsion (Nyholm and McFall-Ngai 1998). Ruby and Morin (1979) proposed that the luminescent symbioses between marine fish and *Vibrio* or *Photobacterium* evolved from a transient relationship of the bacteria with the skin or gut of the host. This seems likely, and thus this symbiosis also appears not to be derived from parasitism.

This discussion illustrates that the "one-size-fits-all" model of the evolution of symbiosis from parasitism does not hold for the SV and LR associations. Each host-microbe interaction must be studied carefully for its differences and similarities. Our current bias toward studying human disease and infections and the concomitant emphasis on host-pathogen interactions have perpetuated the concept that eukaryotes and prokaryotes are "at war." However, it may be more useful to recognize that evolution works on basic mechanisms that are already established in the cell. For example, *bacA*, a rhizobial gene that encodes a putative cytoplasmic membrane transporter with seven transmembrane domains, has been shown to be conserved in the mammalian pathogen *Brucella abortus* (LeVier and others 2000). Rhizobial *bacA* mutants lyse on release from the infection threads, whereas mutating the *bacA* homolog in brucellae decreased their survival in macrophages. Does this result demonstrate that rhizobia are evolved from pathogenic bacteria? We think not. Rather what it shows is the remarkable ability of organisms to manipulate their molecular and cellular machinery to adapt to new environments that require either cooperation (symbiosis) or competition (pathogenesis). What we need to do now is learn more about the basic operating principles that enable these symbioses to occur.

ACKNOWLEDGMENTS

We are grateful to members of our respective laboratories, many of whom are responsible for the research described herein, for their comments on the manuscript. We thank Yimei Lin for Figure 1A, Nancy A. Fujishige for Figure 2A, and Pieternel van Rhijn for Figure 3A. The late John G. Torrey provided Figure 3B. We also thank Margaret Kowalczyk for assembling the final figures. We also thank Drs.

Stefan J. Kirchanski and Edward G. Ruby for their helpful comments. The research in AMH's laboratory is funded by USDA Cooperative Agreement 96-35305-3583, NIH AT00151, and UCBioSTAR S98-86. The research in MMN's laboratory is funded by NSF IBN 9904601 and NIH RO-1-RR12294.

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