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Plant-Microbe Symbioses: A Continuum from Commensalism to Parasitism

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Abstract

Photosynthetic organisms establish symbioses with a wide range of microorganisms. This review examines the diversity of symbiotic interactions, and proposes that there is a continuum from commensalism to mutualism to pathogenesis/parasitism in plant-microbe associations. The advantage of considering commensalism, mutualism, and pathogenesis/parasitism as a continuum rather than as discrete relationships between hosts and microbes, as they have been considered in the past, is that it will motivate us to focus more on common molecular mechanisms.

Keywords: ? ?

1. Introduction

Plants establish mutualistic, often described as symbiotic, interactions with myriad organisms, both prokaryotic and eukaryotic. Some of the most prominent photosynthetic mutualisms are illustrated in Fig. 1. Although technically not a plant symbiosis, lichens are photosynthetic and represent an excellent example of a beneficial interaction (Fig. 1A).

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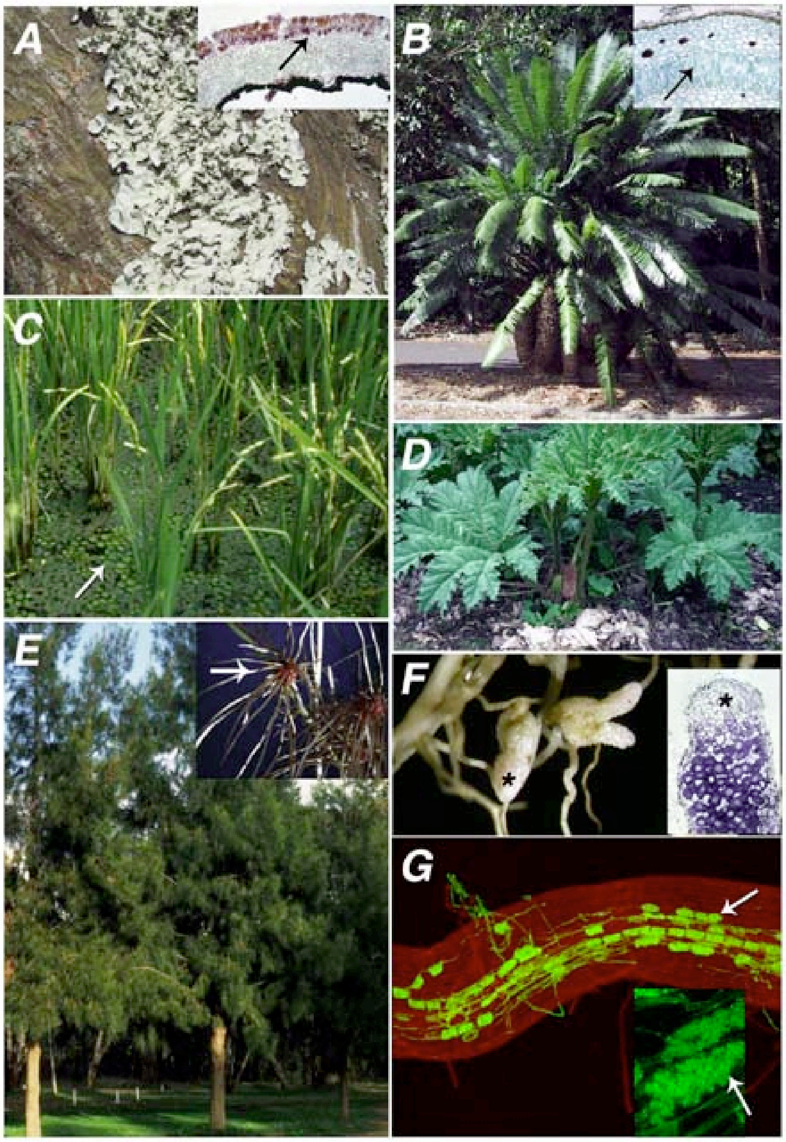

Figure 1. See legend on next page.

Lichens are composites of fungi (mostly ascomycetes) and a photosynthetic partner, either cyanobacteria or green algae, or both. Cyanobacteria also associate with the roots of cycads (Fig. 1B) and are found within the leaf of the highly reduced water fern *Azolla* (Fig. 1C). In the latter symbioses, the cyanobacteria provide nitrogen rather than carbon (see Rai et al., 2000 for a review). In many parts of Asia, *Azolla* ferns are planted in rice fields as a bio-fertilizer. Cyanobacteria also establish a symbiotic association with one angiosperm, namely *Gunnera* (Fig. 1D). The symbiosis develops in specialized glands in nonphotosynthetic stem tissues. In addition to these associations, cyanobacteria form symbioses with diatoms, hornworts, liverworts, and the soil fungus *Geosiphon pyriforme* (Rai et al., 2000).

Non-cyanobacterial, nitrogen-fixing bacteria that develop *de novo* organs on plant roots have been intensively studied because of their ecological and agronomic importance. Fig. 1E illustrates *Casuarina* trees, the roots of which establish a symbiosis with *Frankia*, a member of the Actinomycetales. *Casuarina* nodules are highly modified lateral roots, which develop from the pericycle after some initial cell divisions in the root cortex (Hirsch and LaRue, 1997). In *Casuarina*, the nodules terminate in ageotropic, stunted roots (insert, Fig. 1E), but in *Alnus*, a very large cluster of nodule lobes develops.

Undoubtedly, the best known of the nitrogen-fixing root symbioses is that between members of the Rhizobiaceae and the majority of members of the plant family Fabaceae. Cell divisions initiating the nodule occur in inner cortical cells for indeterminate nodules such as alfalfa and pea, and in the outer cortex for determinate nodules such as soybean (Hirsch, 1992). Legume root nodules of the indeterminate type are depicted in Fig. 1F.

Figure 1. Representatives of the different plant-microbial symbioses described in the text. Unless otherwise noted, all photographs are by the author. (A) Crustose lichen. Insert, transverse section through the thallus of *Parmalia* sp. The arrow points to one of several round algal cells that serve as the photobiont whereas the lower part of the thallus is composed of fungal hyphae. (B) Habit of Cycas. Insert, transverse section of a part of a large coralloid root of Cycas. The arrow points to the layer of cells that contain the cyanobacteria; the symbiont-containing cells are arranged in a ring when viewed in cross-section. (C) *Azolla* (arrow) used as a biofertilizer in a rice field in Indonesia. (D) A species of *Gunnera* growing in New Zealand. (E) Trees of *Casuarina*. Insert, *Casuarina* nodules with their ageotropic nodule roots (arrow). Photo, J.G. Torrey. (F) Nitrogen-fixing nodules of *Melilotus alba*. The asterisk indicates the nodule meristem. Photo, Y. Lin. Insert, longitudinal section through the distal portion of an indeterminate nodule; the asterisk indicates the nodule meristem. (G) Confocal microscope view of a *Melilotus alba* root colonized by *Glomus intraradices*. The arrow points to an arbuscule. Insert, enlargement of an arbuscule showing the convoluted surface (arrow). Photos, M.R. Lum.

More extensive discussion of the diversity in legume nodulation can be found in Sprent (2001).

Mycorrhizas are examples of a mutualism that occurs between plant roots and fungi; the fungus is involved in phosphate acquisition and the plant supplies carbon. The fungi are taxonomically diverse in the case of ectomycorrhiza, but most are ascomycetes. The arbuscular mycorrhizal (AM) association is limited to the Glomales (zygomycetes), which have recently been re-classified as Glomeromycota (Schüssler et al., 2001). Fossilized fungal spores resembling *Glomus* sp. have been dated from the mid-Ordovician, almost 495 million years ago (Redecker et al., 2000). The development of arbuscules or "little trees" within the root cells distinguishes the AM symbiosis (Fig. 1G). The convoluted membrane that lines the arbuscule increases the area of the interface between host and fungus. A number of significant developmental and genetic similarities have been observed in the legume-rhizobia and AM-fungal mycorrhizal symbioses (see review by Lum and Hirsch, 2003). Examination of these similarities has led to the elucidation of signal transduction pathways in both symbioses (see review by Kistner and Parniske, 2002).

Although "symbiosis" in recent times has been used mostly to describe mutualistic associations rather than parasitic ones, there are many similarities in the ways symbionts and pathogens associate with their eukaryotic hosts. The word "symbiosis" was coined by DeBary (1879) to describe "unlike organisms living together", and his original definition included both mutualists and parasites. This initial definition of symbiosis is being re-examined in animal biology, and indeed has been broadened to encompass commensal ("dining at another's table") associations. Hentschel et al. (2000) described a continuum from commensal (one organism benefiting with little or no detriment to the other's fitness) to mutualistic (two organisms benefiting one another) and finally to pathogenic/parasitic (one partner benefits at the expense of the other). Such a continuum can exist even for one organism, e.g., *Helicobacter pylori*, which can be commensal or pathogenic depending on the host it colonizes (Hooper and Gordon, 2001).

This same idea of continuum can be applied to photosynthetic symbioses, but they are rarely discussed in this light. Moreover, symbioses involving plants are often considered in isolation, not only from animal symbioses, but also from each other. [To be comprehensive, I am including lichens in the general category of plant symbioses.] Like animal symbioses, the symbionts may be obligate or facultative, consortial or monospecific; they may be transmitted horizontally (environmentally) or vertically (through the germ line) (McFall-Ngai, 2001). These various modes of organism-to-organism association will be discussed in this review. I will concentrate mostly on the interactions made up of a microbial partner and a eukaryotic host, and where possible, a brief comparison with animal symbioses will be made. In addition, I will discuss the concepts of recognition and specificity, particularly the relevance of each for the establishment and functioning of the symbiosis. The idea of symbiosis as a driving force in development, particularly in reference to its role in eliciting new organ formation in plants, will also be briefly discussed.

2. Consortial versus Monospecific: Many Suitors versus One

Symbioses involving plants may involve consortia of associating organisms, including both bacteria and fungi, or may be monospecific as described for animal symbioses, e.g., for the *Euprymna scolopes*-*Vibrio fischeri* interaction that results in the formation of a light organ in the squid (McFall-Ngai, 2001). For plants, examples of consortia are the rhizosphere and the phyllosphere whereas the *Rhizobium*-legume symbiosis is an archetypal example of a monospecific association. There are also instances where the symbioses are tri- or quadripartite, such as lichens, which can be comprised of cyanobacterial and green algal photobionts and a fungus, as well as some nitrogen-fixing root symbioses, which also incorporate mycorrhizal fungi. Moreover, many AM fungi contain their own intracellular inhabitants, bacteria closely related to *Burkholderia* (see Bonfante, 2003).

The rhizosphere

The now classic studies of Torsvik et al. (1990) provided the first estimate of more than 4000 culture-independent prokaryotic genomes in a deciduous forest soil. This corresponds to ca. 3.8×10^6 bacteria clones per gram of dry soil, an estimate that is significantly higher than that obtained by culturing organisms from bulk soil (see literature cited in Kent and Triplett, 2002). However, the rhizosphere – the up to 1500-μm thick interface between the root and the soil – should support even more microbial diversity than bulk soil because of the presence of plant root exudates. Early studies demonstrated that numerous microorganisms live in the rhizosphere, especially within 50 μm of the root surface. Roots release approximately 50 to 100 milligrams of exudate per gram of root, which is enough to support 2×10^{10} cells (36 milligrams) of bacteria (Foster et al., 1983). In addition to bacteria, many other organisms – viruses, fungi, protozoans, algae, mites, and so on – live in the rhizosphere. These organisms show a diversity of life styles, living as commensals, mutualists, parasites, or saprobes.

One of the important conclusions reached from surveys of the rhizosphere is that the types of plant species or plant communities present have a tremendous effect on microbial diversity. For example, studies where a member of the Rhizobiaceae was included in the soil microbiota show the influence of a legume on the microbial population. From field plots, which were earlier inoculated with luciferase gene-marked *Sinorhizobium meliloti* and where alfalfa and *Chenopodium album* were both growing, a larger number of bacteria was cultured from the rhizospheres of the plants from the inoculated soils compared to the uninoculated ones. Interestingly, the alfalfa rhizosphere had two to three orders of magnitude more *S. meliloti* bacteria than did the *C. album* rhizosphere, indicating that the legume host was more efficiently colonized than the non-legume (Schwieger and Tebbe, 2000). Moreover, even in uninoculated fields, other rhizobial species in addition to luciferase-marked *S. meliloti* were detected in the alfalfa rhizosphere using culture-independent methodology (Schwieger and Tebbe, 2000). Alfalfa and other plant roots secrete compounds that are reported to chemoattract rhizobia (Dharmatilake and Bauer, 1992), and thus the enrichment of these species in the rhizosphere may be a consequence of the exudates produced by the host.

Nevertheless, no unifying conclusions can be reached about the numbers or diversity of bacteria in the rhizosphere because many variables, such as the state of plant health, production of bacterial antibiotics, plant nutrient status, etc., come into play (Kent and Triplett, 2002). More studies need to be done. In any case, from those studies that have been reported, bacterial survival appears to be increased in the rhizosphere, and perhaps even in bulk soil, as a result of commensalism.

In a sense, the rhizosphere is analogous to the oral cavity, which has an extremely diverse microbial population, most of which are commensal or opportunistic pathogens. In human subgingival plaque, 500 clusters of rDNA sequence or phyllotypes of bacteria were uncovered (Paster et al., 2001). This number is comparable to that obtained by cultivation methods; about 50% of oral bacteria have been cultured. When the microbial communities were analyzed in tongue dorsa, the number of phyllotypes detected was 600 (Kazor et al., 2003) whereas only 30% of the viable microbes had been detected earlier by culture methods (Kazor et al., 2001). However, in the rhizosphere, only 1% of the population of bacteria in soil is estimated to have been cultured (Torsvik and Øvreås, 2002). If studies using both non-culture and culture-based methods could be applied as intensively to the rhizosphere as they have been to the oral cavity, our understanding of the diversity in the plant-root interface would be significantly enhanced.

The phyllosphere

In the phyllosphere, more than 75 different bacterial species (37 named and 12 unnamed genera) were cultured from leaves of field-grown sugar beet from differently aged leaves over a complete growing season (Thompson et al., 1993), this in spite of the fact that the leaf surface is a hostile environment. Except for a few microenvironments, the leaf surface is desiccated and lacking in nutrients. Carbon-containing nutrients on leaf surfaces serve as major determinants of colonization by phyllospheric bacteria (see Lindow and Brandl, 2003).

Yang et al. (2001) used a PCR-based approach to analyze uncultivable bacteria from the phyllosphere and found 17 unique rDNA sequences, only four of which had been previously identified. These data suggest that the phyllosphere may be much more complex than originally envisioned. Both culture and PCR-based methods are needed for an assessment of the true complexity of this environment.

3. Courting the Monospecific Symbionts

Horizontal or environmental transmission is the usual way that symbionts are acquired in plant-microbe interactions. Exceptions are *Azolla*, where the symbiont is transmitted in the megaspore (but the symbiont can also be horizontally transmitted), and lichens. In lichens, vegetative units of reproduction, known as soredia (globular clusters of algal cells enveloped by hyphae; Armaleo, 1991), can be dispersed into the environment to propagate new individuals.

In monospecific symbioses that are horizontally transmitted, one of the major stumbling blocks to colonization and subsequent infection is getting the two partners together. This is especially difficult in situations where the microbial partner may be very dilute as is *V. fischeri* in the ocean, and *Nostoc* or rhizobial species in the soil. One of the first steps is thus a concentrating or harvesting mechanism. In the *V. fischeri-Euprymna* symbiosis, this is accomplished by entrapping the luminescent bacteria in a mucus mass that is produced by the squid (Nyholm et al., 2000).

Similarly, in the *Nostoc-Gunnera* nitrogen-fixing association, hormogonia, motile chains of cyanobacterial cells, are captured by mucilage exuded by the plant through gland openings (Johansson and Bergman, 1992). In the legume-rhizobia symbiosis, plant lectins secreted into the rhizosphere aggregate rhizobia near the susceptible root hairs so that a threshold level of competent bacteria can initiate root hair curling and nodule development (Kijne et al., 1992; Hirsch, 1999). These harvesting steps are part of the initial recognition process, and the prokaryotic ligands that interact with these host-derived molecules are likely to be associated with the cell-surface of the microbe (see Hirsch and McFall-Ngai, 2000). Interestingly, these recognition events appear to be density-dependent, strongly suggesting that quorum sensing takes place in the initial stages of the association between the two organisms.

It is not known whether concentrating mechanisms exist for other plant-microbe symbioses, e.g., for arbuscular mycorrhizae or for lichens. Although there is some evidence for chemoattraction of the fungus to the host and for the production of a branching factor secreted by the host, the chemical identities of neither has been elucidated (see literature cited in Hirsch and Kapulnik, 1998), although research is underway. Understanding the initial stages of the lichen symbiosis is challenging because of the slow growth of the lichenized fungus and the concomitant difficulty of re-synthesizing most lichens from their component parts.

4. Who's that Knocking on the Door(s)?

Recognition and specificity are frequently used interchangeably to describe symbiotic interactions, but generally recognition refers to the state of being perceived whereas specificity relates to an interaction with a particular species, e.g., a disease or syndrome caused by a specific organism. A further description of the *Rhizobium*-legume symbiosis will clarify some of the differences.

After the rhizobia are attracted to the legume roots, flavonoids secreted by the plant induce the *nod* genes in the rhizobia (Long, 1996). Host specificity is exhibited even at this stage as certain flavonoids induce *nod* genes in one *Rhizobium* species, but not in another. Upon induction of the *nod* genes, the rhizobia synthesize Nod factor, a lipochitooligosaccharide molecule that serves as a primary morphogen for inducing the host's nodulation pathway. Different Nod factors exhibit a number of substitutions at both the reducing and non-reducing ends of the molecule that help mediate this host specificity (Perret et al., 2000). For example, if a sulfate group is missing on the reducing end of the Nod factor molecule because of a mutation in the *nodH* gene of *Sinorhizobium meliloti*, the bacteria no longer induce nodule formation on their host alfalfa.

Perception of Nod factor is specific with a particular legume responding to its cognate Nod factor signal. Signal perception and transduction is proposed to occur by means of a series of proteins with leucine-rich repeat (LRR) extracellular domains linked by a transmemembrane domain to a cytoplasmically located kinase domain. LRR domains are known to interact with extracellular ligands, particularly other proteins or small peptides. Legumes that are capable of establishing a symbiosis with both AM fungi (Myc$^+$) and rhizobia (Nod$^+$) have been excellent candidates for searching for genes encoding proteins involved in these early stages. A gene, which when mutated gives rise to both a Myc$^-$ and Nod$^-$ phenotype, indicating that the mutation co-segregates, has been found to encode an LRR-RLK (Receptor-Like

Kinase). The protein has been designated NORK (Nodule Receptor Kinase) for alfalfa and other indeterminate legumes and SymRK (Symbiotic Receptor Kinase) for *Lotus japonicus* (Endre et al., 2002; Stracke et al., 2002). More recently, additional receptor proteins that are putative Nod factor binding proteins have been identified. These are serine/threonine RLKs with extracellular LysM domains (Limpens et al., 2003; Madsen et al., 2003, Radutoiu et al., 2003). LysM domains are characteristic of proteins that bind peptidoglycan and chitin. Upon binding to Nod factor, these proteins could potentially interact with NORK/SymRK via protein-protein interactions (Fig. 2). However, there is no evidence so far, for either the direct or indirect interaction of a putative Myc factor with the NORK/SymRK proteins or of the Nod factor with the LysM motif-containing proteins.

LRR-RLK proteins are also implicated in plant-pathogen recognition and specificity. They are the products of the so-called R or resistance genes, which are important in cultivar-dependent host resistance. One of the tenets of the gene-for-gene hypothesis in plant pathology (Flor, 1971) is that the R proteins interact with products of the pathogen *avr* genes, although the evidence for a direct interaction between the two *in vivo* has not yet been obtained (Cohn and Martin, 2003). When recognition occurs, the result is a hypersensitive response (HR). The HR is characterized by localized plant cell death, thereby bringing about disease resistance by limiting the pathogen's advance. If there is no recognition, disease ensues.

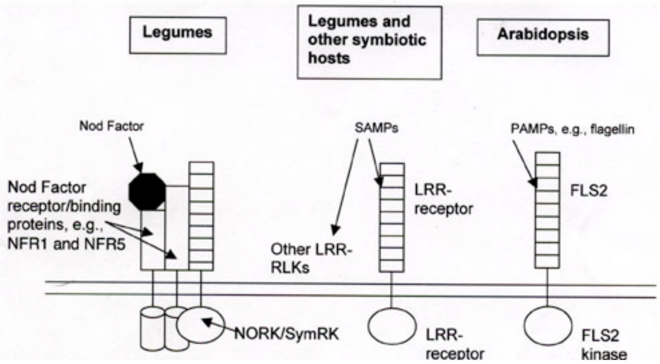

Figure 2. Interaction of ligands with leucine-rich receptors. In legumes, substituted Nod factors are believed to bind to a Nod factor-binding protein/receptor designated NFR1 and NFR5 in *Lotus japonicus*. Upon binding Nod factor, one or both undergo a conformation change and are then proposed to bind to NORK/SymRK, which triggers the downstream signaling transduction cascade leading to root nodule development. Other microbe-produced factors, i.e., SAMPs, are proposed to interact with LRR-RLKs in both legumes and other symbiotic hosts, in a manner similar to PAMPs, such as flagellin, which interacts with FLS2 and triggers non-cultivar host defense in *Arabidopsis*.

Following recognition of the appropriate Nod factor, infection threads that carry the dividing rhizobia are established in the responding root hairs. In addition to the correct Nod factor, cell surface molecules such as exopolysaccharide (EPS) and lipopolysaccharide (LPS) are required for proper infection thread development, which is necessary for the rhizobia to enter into the host cells so that they can differentiate into nitrogen-fixing bacteroids.

However, the details of infection thread development and subsequent endocytosis of the rhizobia into the nodule cells are still incompletely understood, but research is in progress. Mutagenesis studies indicate that a number of genes are critical for proper nodule development. Moreover, many of the stages resulting in further nodule development appear to require host specificity (Limpens et al., 2003). In conclusion, the very narrow host range of some rhizobia and the extensive transformation that takes place in the host to establish the root nodule argue for several levels of specificity, i.e. the presence of many legume "doors" and associated *Rhizobium*-synthesized "keys" to open them.

Lectins apparently participate in the initial stages of the cyanobacterial symbioses (Rai et al., 2000) and are likely to mediate the early recognition events as they do in the *Rhizobium*-legume interaction. Beyond this initial recognition, however, very little additional differentiation occurs in the various cyanobacteria-mediated symbioses. For example, cyanobacteria do not initiate coralloid roots in cycads *de novo*. Studies in the cycad *Macrozamia* show that precoralloid roots are established, but they do not become geotropic or exhibit extensive lenticel formation until they are colonized by the cyanobiont (Ahern and Staff, 1994). Colonization also leads to the expansion of the cortical cells that house the cyanobacteria (insert, Fig. 1B). In the *Azolla* leaf cavities, minimal developmental changes occur upon cyanobiont colonization whereas in *Gunnera*, infection with cyanobacteria leads to a stimulation of mitosis in certain host cells (Rai et al., 2000). Nonetheless, there is very little species specificity exhibited in the cyanobacterial symbioses, except for *Azolla*, which is vertically transmittted (Rai et al., 2002). Some coralloid cycad roots can be inhabited by more than one *Nostoc* strain, and this is also the case for *Gunnera*, indicating a low specificity (Lindblad et al., 1989; Nilsson et al., 2000). Moreover, the lack of extensive morphological and physiological differentiation would seem to obviate the need for frequent questioning of "who's that knocking at the door" and repeated recognition events.

5. PAMPs and SAMPs – Recognition of Non-Self

The R proteins and other plant LRR-RLKs are functionally related to

Drosophila Toll-like receptor (TLR) proteins, which are involved in innate immunity. TLRs and other pattern recognition proteins (PRRs) detect non-self determinants, also known as PAMPs (Pathogen-Associated Molecular Patterns), which are usually derived from infectious microbes (Janeway, 1989). Bacterial flagellin, glycans, and LPS are examples of molecules that elicit either plant or animal defense by interacting with a broad range of RLKs that act as PRRs (see reviews by Gómez-Gómez and Boller, 2002; Parker, 2003). Flagellin and other PAMPs are not products of the *avr* genes, which are cultivar-specific. Rather, they are general-purpose "elicitors" that trigger plant defense in a non-cultivar-dependent way (Parker, 2003). A PRR, an LRR-RLK designated FLS2, has been identified in *Arabidopsis* for flagellin perception in non-cultivar-dependent recognition (Gómez-Gómez and Boller, 2000) (Fig. 2). It is likely that other PRRs for PAMPs in addition to flagellin will be discovered in plants. Activation of the PRRs by PAMPs triggers innate immunity in animals and a similar type of response in plants. A MAP kinase signaling cascade that is downstream of flagellin recognition has recently been identified in *Arabidopsis* (Asai et al., 2002). This cascade shows similarities to the signal transduction pathways in insects and mammals (Gómez-Gómez and Boller, 2002).

In non-pathogenic, i.e. mutualistic or commensal interactions, the host is likely to distinguish analogous non-self, non-cultivar-dependent determinants. For want of a better name, I will call these determinants SAMPs (Symbiont-Associated Molecular Patterns) because hosts in commensal and mutualistic relationships are just as likely to recognize non-self, as are hosts in pathogenic/parasitic interactions. SAMPs are also proposed to be involved in animal mutualisms and commensal interactions (M. McFall-Ngai, pers. comm.). SAMPs overlap with PAMPs, but there is clearly a level of specificity that is involved, such that PAMPs are distinguishable from SAMPs by specific PRRs. For example, the conserved peptide found in flagellin proteins, if derived from *Rhizobium* and then presented to a host, does not activate FLS2 as does the comparable peptide from a pathogenic bacterium (Felix et al., 1999). In other words, different PRRs are proposed to exist for microbe-associated molecules that trigger non-cultivar-dependent commensal, mutualistic, or pathogenic interactions (Fig. 2).

If such an initial recognition system exists for mutualism and commensalism, it brings up some interesting questions. Are these molecules, as are PAMPs, generalized elicitors such as small peptides, oligochitins, glucans or LPS? Chitin oligomers, which are known elicitors, i.e. non-self determinants, provoke calcium spiking in legume root hairs if provided at a high enough concentration, although the spiking is qualitatively different from that elicited by substituted Nod factors at significantly lower concentrations (Walker et al., 2000). Chitin-binding proteins are common to many plants, and in *Arabidopsis*, there over 600 members of the RLK family, but the function of the vast majority is unknown. Specialized RLKs or other proteins are thus predicted to exist in the host to distinguish chitin oligomers functioning as a SAMP from chitin oligomers functioning as a PAMP.

In addition to distinct PRRs, the response to symbionts, at least in the case of *Rhizobium*, may be qualitatively different from that of pathogens in that related elicitors complete for one or more receptors. Besides host-specific Nod factor and chitin oligomers, several forms of Nod factor (plus and minus the various substitutions on the reducing and non-reducing ends of the molecule) are produced. In support of this idea, Müller et al. (2000) reported that certain forms of Nod factor interfered with chitooligomer perception in cultured soybean cells. The identification and characterization of proteins used for recognition of SAMPs should be a fruitful area of pursuit. It is highly likely that SAMPs are involved not only in *Rhizobium*-legume recognition, but also in other plant symbioses. Lectins are known to recognize PAMPs in mammalian systems, and as described earlier, are important for recognition both in cyanobacterial symbioses and the *Rhizobium*-legume interaction. The SAMPs in question are likely to be cell surface glycans produced by the microbial partner. Similarly, N-acetyl-glucosamine fragments from mycorrhizal fungal cell walls (Salzer et al., 1996) should be considered as being perceived by the host as SAMPs, not as PAMPs. The identification of the proteins that perceive these signals as well as those involved in the downstream signal transduction pathways should be targets for future research.

The highly substituted Nod factors of rhizobia, which interact only with a particular host legume (and concomitantly with its specific Nod factor-binding protein and receptor kinase), differ from the generalized SAMPs just described. This argues that NORK/SymRK and the putative Nod factor-binding proteins (see Fig. 2) are functionally similar to the specialized R-gene encoded LRR-RLKs, and concomitantly supports the case that other proteins must exist for the recognition of SAMPs and PAMPs. Gómez-Gómez and Boller (2002) have cogently argued that gene-for-gene resistance and its associated *avr* and *R* gene products and downstream signal transduction pathway should not be equated with innate immunity. Rather, a connection should be made between the perception of generalized elicitors that provoke a plant defense response and the recognition of PAMPs by PRRs and elicitation of innate immunity in insect and mammalian systems. Whether or not SAMPs elicit any semblance of innate immunity in plants is speculative at this time, but it seems likely that they could do so in animal systems.

6. A Marriage of Convenience and More

Commensal and mutualistic interactions allow organisms to survive in new environmental niches and to attain biochemical functions that they would not have been able to have on their own. This cooperative arrangement or "marriage" between organisms has been suggested to be the major driving force for the evolution of eukaryotic cells (Margulis and Bermudes, 1985). However, symbiosis *sensu lato* is not only a driving force for evolution, but also for development.

Stappenbeck et al. (2002) showed that the common gut bacterium, *Bacteroides thetaiotaomicron*, induced angiogenesis within 10 days in germ-free mice, which prior to inoculation had a poorly vascularized intestinal capillary system. The resulting increase in absorptive capacity of the intestine brought about by angiogenesis also resulted in an increase in bacterial density and species complexity (Stappenbeck et al., 2002). In the *V. fischeri-Euprymna* symbiosis, inoculation with the symbiont results in the development of the light organ, which provides a niche for the bacteria to reproduce and also makes light for the host (McFall-Ngai, 2000). Major changes occur in the squid including apoptosis, crypt swelling, and an increase in microvillar density, all before the actual formation of the light organ.

Frankia, a Gram-positive actinomycete, and *Rhizobium*, a Gram-negative α-proteobacterium, both elicit the formation of a new organ, the nitrogen-fixing nodule, on plant roots. In contrast, as described earlier for cyanobacteria symbioses, less obvious remodeling takes place in other mutualistic plant-microbe interactions, e.g., in mycorrhizae where the fungal partner differentiates to form arbuscules in the case of AM fungi, but no obvious morphological changes occur in the root other than the elaboration of the host membrane around the arbuscule (Fig. 1G). For ectomycorrhizae, the fungi establish the Hartig net, an elaboration of hyphae that penetrates between epidermal and root cortical cells, while simultaneously on the plant side, root hair development is suppressed or root hairs become incorporated into the fungal sheath (Massicotte et al., 1987; 1990; Thomson et al., 1989). In lichens, both organisms cooperate to form a thallus, which can exhibit considerable morphological diversity, being crustose (Fig. 1A), foliose, or fruticose. The fungus can also become biochemically altered, synthesizing pigments that presumably protect the photobiont, which is located in a layer near the upper surface (insert, Fig. 1A), from ultraviolet irradiation.

7. Evolution towards Dependence on one Partner?

As in animal symbioses, the symbionts of plants can either be facultative or obligate. The best-known example of the latter is that of arbuscular mycorrhizae whereby the fungal symbiont cannot live separately from its plant host. Numerous attempts have been made to culture the mycobiont separately from its host, but so far none have been successful, strongly suggesting that the fungus is completely dependent on its host for the synthesis of certain molecules. The AM symbiosis is also one of the oldest plant-microbe interactions, dating from the Devonian, which was more than 390 million years ago (see reviews by Hirsch and Kapulnik, 1998; Bonfante, 2003). Hence, this symbiosis has been under evolutionary selection for a very long time. In the case of obligate symbionts of non-plant hosts, there is frequently a reduction in genome size (Moran, 2002). The reduction in genome size and with it, diminished biosynthetic capabilities suggest that the host is the primary source of various molecules supplied to the symbiont. It will be of interest to determine whether the obligate AM fungal symbionts also show gene loss compared to non-symbiotic relatives. Genomic studies of the AM fungi will be challenging, however, because these organisms are multinuclear and possibly genetically variable.

Faster rates of nuclear substitution have been observed for symbiotic associations, e.g., for lichen fungi (Lutzoni and Pagel, 1997) and for the endosymbiotic bacteria that associate with aphids (Wilcox et al., 2003). Like the AM symbiosis, lichens represent an ancient mutualism. They are also difficult to grow in axenic culture, which makes studies on lichens particularly taxing.

8. Conclusions and Prospects

Commensalism, mutualism, and to a more limited extent, parasitism/ pathogenesis, can be considered as a continuum in photosynthetic symbioses (Fig. 3). Numerous reports in animal symbioses show that commensal bacteria can shift from their relatively benign state to a pathogenic one depending on the genetic background of the host (see Hooper and Gordon, 2001). Mutualists such as *Rhizobium* can live as commensals on plant roots and can also establish biofilms on abiotic surfaces (N.A. Fujishige and A.M. Hirsch, unpublished results). These growth habits may serve as survival mechanisms for these non-spore forming bacteria in the absence of a suitable host. The transfer to a Nod$^-$Fix$^-$ *Mesorhizobium loti* of a "symbiotic island" enabling the nodulation of *Lotus* sp. is an excellent example of how a commensal species can relatively rapidly become a mutualistic one (Sullivan et al., 1995; Sullivan and Ronson, 1998).

If plants are considered in their natural habitats, then one quickly becomes aware that symbiosis is the fundamental state of existence. Plant roots are covered with microbes, and even leaves possess their share of hardy microorganisms. Symbiotic organisms are important even in artificial environments such as agricultural soils. Zero-tillage restores soil microbes to

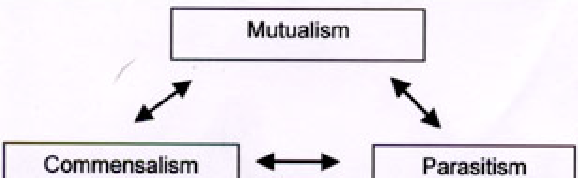

Figure 3. The symbiosis continuum includes commensalism, mutualism, and parasitism.

levels where they can beneficially impact crop growth, at the same time decreasing the need for pesticides and herbicides that pollute groundwater.

The existence of SAMPs and PAMPs that interact with their cognate RLKs is another way to link these different manifestations of symbiosis sensu lato together. In commensalisms, the interaction may only involve the initial non-self recognition whereas for mutualisms and parasitisms, especially where the microbial partner is encapsulated by host membrane, non-self recognition may encompass multiple steps and with it, additional levels of signaling and complexity.

Yet research in commensal and mutualistic symbioses has lagged significantly behind that of pathogenesis/parasitism in both plant and animal biology in spite of the fact that numerous studies have shown that the health of the host depends on the right mix of commensal and mutualistic microbes. This improvement in host health is often accomplished in such profound ways as by influencing the development of host tissues or by complementing the biochemistry of the host. Moreover, as more and more eukaryotic genome sequences become annotated, it becomes clear that some of the DNA sequences represent the symbiotic organisms housed within the host. However, our knowledge of how this "symbiome" influences host evolution and development is woefully inadequate. Future research needs to be focused on these fundamental relationships with the ultimate goal of maintaining the balance of symbiotic organisms within their hosts, thereby lessening the onslaught of opportunistic pathogens. Studying the mechanisms whereby the balance is maintained will also bring us to a better understanding of how organisms interact with each other on an evolutionary and developmental scale.

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