SYMBIOSIS

Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria

Francis M. Martin,*† Stéphane Uroz, David G. Barker*†

BACKGROUND: Among the extensive cortège of plant-associated microorganisms (the so-called plant microbiota), mutualistic fungal and bacterial symbionts are striking examples of soil microorganisms that have successfully coevolved with their hosts since plants adapted to terrestrial ecosystems. They promote plant growth by facilitating the acquisition of scarce nutrients. In these associations, plant root colonization requires complex molecular cross-talk between symbiotic partners to activate a variety of host developmental pathways and specialized symbiotic tissues and organs. Despite the evolutionary distances that separate mycorrhizal and nitrogen-fixing symbioses, recent research has identified certain highly conserved features associated with early stages of root colonization. We focus on recent and emerging areas of investigation concerning these major mutualistic symbioses and discuss some of the molecular pathways and cellular mechanisms involved in their evolution and development.

ADVANCES: Phylogenomic analyses and divergence time estimates based on symbiotic plant fossils are shedding light on the evolution of mutualistic symbioses. The earliest land plants [~407 million years ago (Ma)] were associated with fungi producing mycorrhiza-like intracellular structures similar to extant symbioses involving Glomeromycotina and Mucoromycotina. Arbuscular mycorrhizal endosymbioses then diversified by the Late Carboniferous. Pinaceae species from the Late Jurassic and Early Cretaceous (~180 Ma) formed the first ectomycorrhizal associations involving Dikarya. More recently, certain angiosperms evolved a "predisposition" for the evolution of nitrogen-fixing root nodule symbioses (~100 Ma) with bacteria.

A conserved core module of the "common symbiotic signaling pathway" (CSSP) is shared by all host plants that establish endosymbioses, including arbuscular mycorrhizal, rhizobial, and actinorhizal associations. This striking conservation among widely divergent host species underlines the shared evolutionary origin for this ancient symbiotic signaling pathway. Furthermore, chitin-based signaling molecules secreted by both arbuscular mycorrhizal fungi and rhizobia activate the host CSSP after perception by related receptor-like kinases. Downstream signal transduction pathways then lead to the apoplastic intracellular infection modes that characterize the majority of these associations and, finally, to the coordinated development of sophisticated bidirectional symbiotic interfaces found in both arbuscules and nitrogen-fixing nodules. A common feature of all these mutualistic associations is phytohormone-associated modifications of root development, which lead to an increase in potential colonization sites as well as major structural and functional changes to the root during the establishment of symbiotic tissues.

OUTLOOK: Although we are at last beginning to understand how mutualistic microorganisms communicate with plants, how associated root developmental pathways are modulated, and how plant immune responses are successfully circumvented, many important questions remain. For example, little is currently known about more primitive modes of intercellular apoplastic colonization, whether for ectomycorrhizal fungi or for certain nitrogen-fixing symbioses. Neither do we know whether the CSSP has a key role in ectomycorrhizal associations, nor how host plants distinguish between structurally similar chitin-based "symbiotic" and "pathogenic" microbial signals. Answering these questions should contribute to our understanding of the underlying mechanisms that govern the relationships between plants and their entire microbiota. On a broader level, improved understanding of how environmental and genetic cues, together with plant metabolism, modulate microbial colonization will be crucial for the future exploitation of the microbiota for the benefit of sustainable plant growth. ∎
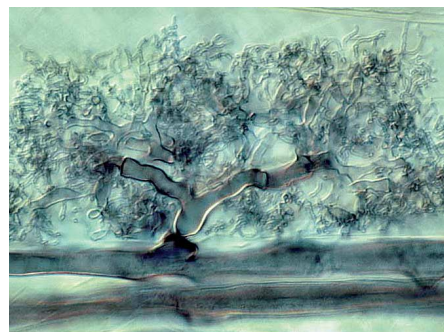
The major root symbioses established by land plants with soil microorganisms are arbuscular mycorrhizal, ectomycorrhizal, and nitrogen-fixing associations. Top left: Image of a mature arbuscule of the arbuscular mycorrhizal fungus *Funneliformis mosseae* in a plant root cell. Top right: Ectomycorrhizal rootlets of beech (*Fagus sylvatica*) in symbiosis with the ochre brittlegill fungus (*Russula ochroleuca*). Bottom left: Symbiotic root N-fixing legume nodules on a fava bean (*Vicia faba*) plant. Bottom right: Symbiotic N-fixing actinorhizal nodules on the root of an alder tree.

REVIEW

SYMBIOSIS

Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria

Francis M. Martin,[1]*† Stéphane Uroz,[1] David G. Barker[2]*†

Within the plant microbiota, mutualistic fungal and bacterial symbionts are striking examples of microorganisms playing crucial roles in nutrient acquisition. They have coevolved with their hosts since initial plant adaptation to land. Despite the evolutionary distances that separate mycorrhizal and nitrogen-fixing symbioses, these associations share a number of highly conserved features, including specific plant symbiotic signaling pathways, root colonization strategies that circumvent plant immune responses, functional host-microbe interface formation, and the central role of phytohormones in symbiosis-associated root developmental pathways. We highlight recent and emerging areas of investigation relating to these evolutionarily conserved mechanisms, with an emphasis on the more ancestral mycorrhizal associations, and consider to what extent this knowledge can contribute to an understanding of plant-microbiota associations as a whole.

Evolution is dependent on interactions, and in particular on cooperation and mutual dependence among organisms (*1*). As such, mutualistic symbiotic microbes are central to the evolution, biology, and physiology of land plants because they promote plant growth by facilitating the acquisition of scarce and essential nutrients (e.g., phosphorus and nitrogen). As coined originally by de Bary (*2*), "symbiosis is the living together of unlike organisms," thus encompassing close and often long-term interactions between different biological species. There are symbioses with advantage to both partners (mutualistic relationships), those involving the exploitation of one (or multiple) partner(s) by another partner (parasitism), or mutualistic and detrimental associations of different partners at the same time.

Since their origin in the Mid-Ordovician period [460 to 470 million years ago (Ma); see below], land plants have coevolved with a large variety of microorganisms (*3*, *4*). Plants represent a stable, nutrient-rich niche for associated microbes to thrive, and these interactions take place either in the immediate vicinity or within plant tissues and cover the full spectrum from beneficial symbioses to plant disease (*2*). The importance of symbiosis is now recognized across the biological sciences, and understanding the various types of symbiotic associations that can be established between plants and their microbial cortège—the so-called microbiota (Box 1) (*5*)—has become a priority for researchers in plant and microbial sciences, evolutionary biology, and ecology. One of the main

reasons for this is the urgent need to develop effective microbe-based strategies for sustainable agriculture and forest management (*6*, *7*).

Thanks to the unprecedented resolution provided by high-throughput meta-barcoding technologies, plants are more appropriately viewed not as autonomous entities, but rather as an assemblage comprising the host plant and its associated microorganisms (bacteria, archaea, fungi, oomycetes. and viruses) (*7*, *8*). These microorganisms inhabit the rhizosphere (i.e., the zone of soil directly influenced by root exudation), adhere to the root or leaf surface (rhizoplane and phylloplane), or colonize the interior of roots and leaves (endosphere) (see Box 1). Certain bacterial and fungal species associated with plants can extend their hosts' phenotypes, including many host life history traits such as physiological processes, disease susceptibility, reproduction, and fitness (*9*, *10*). This can also lead to improved growth through increased acquisition and assimilation of nutrients and superior resistance to various environmental stresses. But it remains unclear why only a small proportion of soil microbes are able to colonize plant tissues and why only a few of these have evolved the capacity to establish mutualistic symbioses.

Microbes entering plant tissues and establishing mutualistic symbioses need to avoid plant immunity responses while at the same time activating host developmental switches and establishing coordinated metabolic activities. Important questions are therefore being addressed regarding the signaling and developmental pathways that mediate the establishment and maintenance of mutualistic symbioses, such as the N-fixing rhizobial and mycorrhizal associations, as well as the complex molecular cross-talk that occurs between symbiotic partners and between the host plant and the other associated microbiota. The knowledge so far gained for certain of these

beneficial symbiotic interactions is already extensive (*11*–*14*) and will no doubt provide useful examples for deciphering the interactions between the prominent members of the microbiota and their host plants. This knowledge should also enable us to answer pressing questions about how microbial symbioses have shaped plant morphology and development, microbial genomes and their evolution, and how endospheric microbes are selected among the myriads of microorganisms proliferating in the vicinity of the plant. More generally, we need to better understand the primary mechanisms driving host-microbe as well as microbe-microbe interactions in the host plant. What are the relative contributions of plant immunity, hormone homeostasis, and physiological activities in driving host microbial community composition and function, and how do plants promote mutualistic symbiotic associations while restricting the establishment of pathogenic associations?

Here, we examine recent and emerging areas in the study of the major mutualistic symbioses through the lens of the more ancient mycorrhizal associations, focusing on key signaling and developmental features that have been conserved and remodeled throughout evolutionary history. (Although the primary role of these mutualistic symbioses is to promote plant growth through improved nutrient acquisition, we do not address the molecular bases of these metabolic exchanges.) After placing the mycorrhizal and N-fixing symbioses in their evolutionary contexts, we discuss the conserved molecular pathways and colonization strategies that underpin the successful establishment of mutualistic symbioses, in addition to the various strategies used to circumvent host immunity responses. Deciphering the principal signaling and developmental pathways that give rise to these highly specialized associations should facilitate the characterization of key mechanisms shaping the plant microbiota (Box 1), as well as future studies of the interactions between plants and their endospheric microbes. Harnessing alliances between plants and their beneficial microbiota should also facilitate the development of the novel crop-plant production systems needed to cope with the increasing frequency of extreme climatic events, expanding pest and pathogen pressures, and limited resource availability (*7*).

Evolution of mutualistic symbiotic lifestyles

Evolution of mycorrhizal symbioses

Early soil-forming communities colonizing terrestrial ecosystems during the early Paleozoic (419 to 470 Ma) were likely similar to modern soil biological photoautotrophic crusts (algae, mosses, and liverworts), which form symbioses with fungi and cyanobacteria (*15*, *16*). Primitive plant-microbe associations developed when the earliest plants made the transition to the land surface. Phylogenetic analyses indicate that all extant land plants derived from a common multicellular ancestor in a single freshwater algal charophyte lineage, following a single land

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colonization event in the Middle to Upper Ordovician (~443 to 470 Ma) (*16, 17*). These phylogenetic analyses also suggested that arbuscular mycorrhizal (AM) symbiosis emerged in the Early Devonian (~393 to 419 Ma) (*3, 4, 18, 19*). During this era, the flora was highly diverse (*18–22*) and the diminutive herbaceous vegetation that colonized the land at this time was characterized by small, rootless, leafless plants with simple rhizoid-based absorbing systems (*21, 23*) (Fig. 1). These early plants, especially those found in the Rhynie Chert fossil site in Aberdeen, Scotland (~407 Ma), were associated with fungi producing intracellular structures similar to extant liverwort symbioses, including arbuscules characteristic of the Glomeromycotina

and swellings and hyphae reminiscent of Mucoromycotina (*18, 20, 24*) (Fig. 1).

It has been hypothesized (*3, 4, 19, 23*) that the earlier-diverging AM-like fungal and plant lineages coevolved from early plant colonization of terrestrial ecosystems because, in the absence of existing soil, plant hosts faced major issues of nutrient and water limitation (*21, 23*). These harsh conditions may have driven the alliance of early land plants and fungi toward symbiotic associations (*3, 4*), such that the fungus provided inorganic nutrients and water to the host plant and in return received carbohydrates from the host. Phylogenomic analyses and divergence time estimates indicate that the early-diverging fungi belonging to the Glomeromycotina and Mucor-

omycotina diverged from a common ancestor between 358 and 508 Ma (*25*). Studies of the signaling pathways and trophic interactions involved in the symbiotic associations between extant bryophytes and their Glomeromycotina and Mucoromycotina symbionts would no doubt provide important insights into these ancient interactions (*26–28*). These early plants likely hosted nonsymbiotic rhizospheric and endophytic bacteria and fungi, but unfortunately there is no documented trace of these associations in fossils.

Primitive forest ecosystems expanded on land during the Mid-Devonian (~385 Ma), but the nature of the fungal associations inhabiting these early trees is currently unknown. Arborescent lycopsids that formed the first extensive swamp

Box 1. The plant microbiota. In recent years, the resolving power provided by high-throughput sequencing has given unprecedented access to the diversity and composition of the plant microbiota (*106*). The real challenge now is to determine how the endospheric microbiota is recruited and how it affects plant biology, since each plant species is characterized by defined rhizospheric and endospheric microbial communities (*106, 107*). The partial overlap observed between the endospheric and rhizospheric microbiota suggests that only part of the former originates from the soil and rhizosphere, whereas a second part derives from seed microbes via vertical transfer and atmospheric deposits. It appears that plants actively select the microbes from the rhizosphere (*106–108*). Although much of microbial taxonomic diversity remains unexplained and depends on the plant tissues considered, it is now established that both abiotic factors [e.g., soil type, pH, nutrient availability (*106, 107*)] and biotic factors [e.g., microbe-microbe interactions, host genetics and symbiosis establishment (*108, 109*)] are important in structuring the microbiota. The most prevalent bacterial taxa found in the rhizosphere and endosphere belong to Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and, to a lesser extent, Firmicutes (*106–108*). The dominant rhizospheric and endospheric fungal taxa differ widely (*108, 109*). Ascomycota are among the most abundant taxa colonizing the phyllosphere, whereas Ascomycota and Glomeromycotina are the most abundant species in the rhizospheric communities of most land ecosystems, except forests. In the latter, Basidiomycota are very abundant in ECM tree rhizospheric communities (*107, 110*). Surveys of endophytic communities indicate that Ascomycota are likely to be the most abundant species hosted in plant tissues (*107, 110*). With the exception of mutualistic associations discussed in this review, the molecular mechanisms controlling the recognition and colonization of plant tissues by endospheric bacteria and fungi and their accommodation in planta are poorly understood. However, recent studies suggest that the endospheric microbiota are strongly regulated by defense phytohormones such as salicylic acid (*111*), interactions with other rhizospheric microbes (*108*), and the availability of host carbon compounds (*112*).

Environmental drivers of the plant microbiota. Microbiota composition is determined by (**1**) soil conditions (e.g., soil type, pH, nutrient availability), (**2**) plant exudates varying with plant genotype and metabolism, and (**3**) the development of mutualistic symbiotic associations (blue circles) [e.g., N-fixing nodules (**3a**) or mycorrhizal root tips (**3b**)]. (**4**) A subset of the microbiota inhabiting plant surfaces is able to internally colonize root and leaf tissues to establish endophytic symbioses.
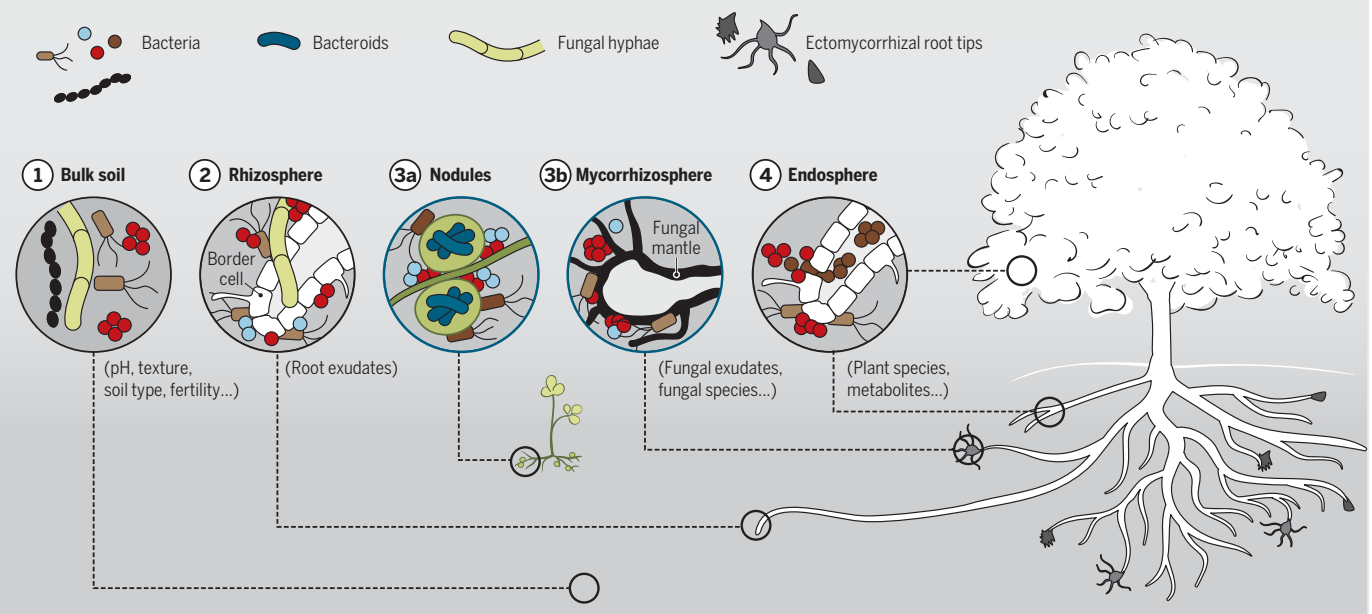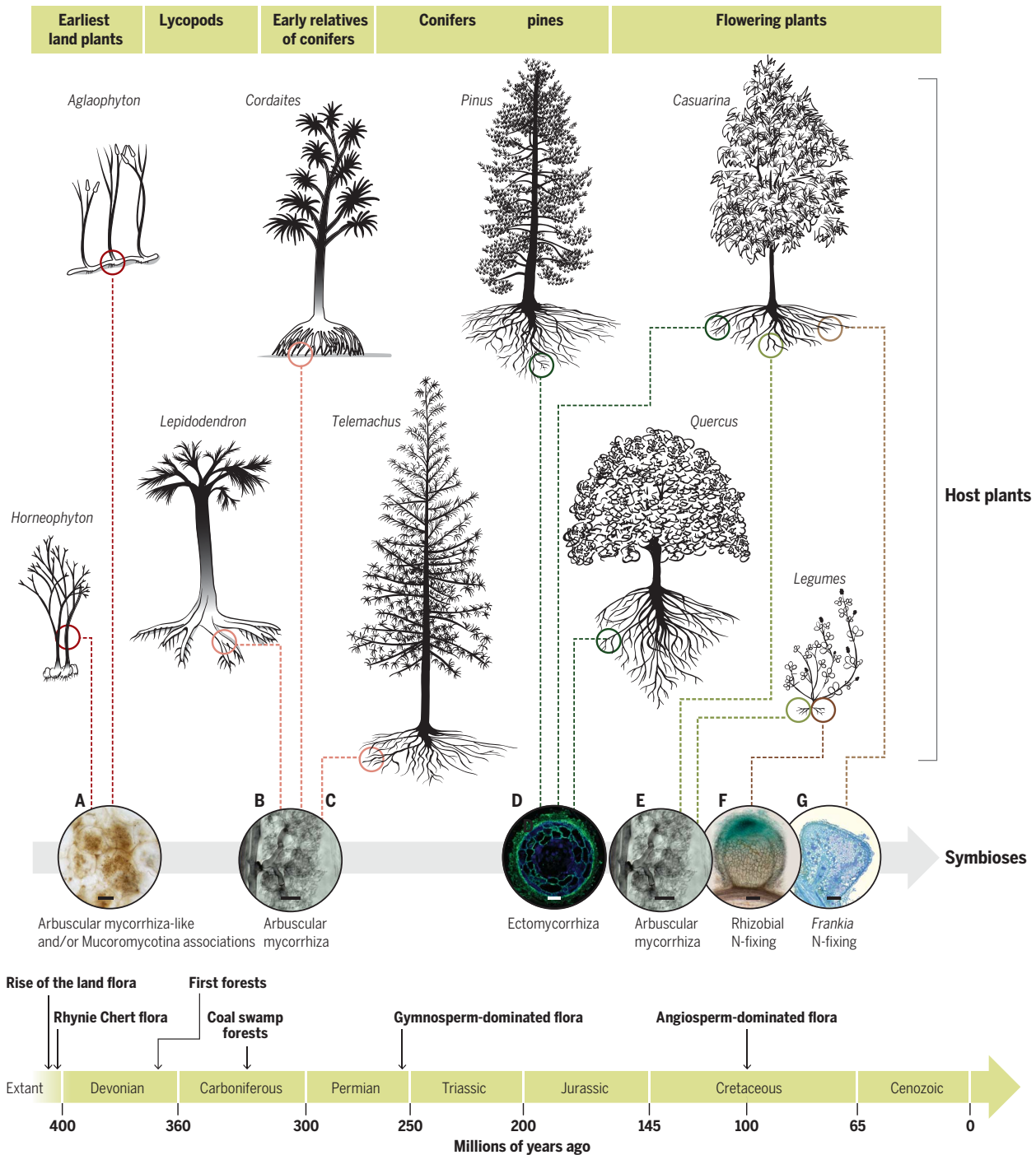
Fig. 1. The evolution of mutualistic symbiotic associations: A possible scenario. Land colonization by early plants started during the Mid-Ordovician, ~470 Ma. Evidence for mutualistic microbial associations with these plants remains inconclusive. (**A**) Fossilized plants from the Rhynie Chert flora (~407 Ma) were colonized by Glomeromycotina and Mucoromycotina fungi forming intracellular structures similar to mycorrhizae of extant bryophytes and lycophytes (18, 24). Image shows a fungal endophyte of the Glomeromycotina type in *Aglaophyton major* from the Lower Devonian Rhynie Chert; scale bar, 10 μm. (**B**) By the Late Carboniferous, continents were covered by large forests of seed ferns, lycophytes, and early relatives of conifers (e.g., Cordaites); the last two groups formed mycorrhizal symbioses with AM fungi (29, 30) similar to extant AM symbiosis. Image shows a mature arbuscule of *Funneliformis mosseae* in an extant host plant; scale bar, 10 μm. (**C**) Symbioses between gymnosperms (e.g., cycads, conifers) and AM fungi evolved during this period

and dominated the land flora between the Triassic and the Cretaceous periods (19). (**D**) The first ECM fungal species plausibly evolved with the earliest Pinaceae in the Jurassic (14, 33). Image shows a section of an extant *Cenococcum-Pinus* ectomycorrhiza; scale bar, 100 μm. (**E**) Basal angiosperms, early monocots, and early eudicots appeared almost simultaneously during the Early Cretaceous and later became dominant in a majority of terrestrial habitats from the Late Cretaceous until the present day. The majority of these nontree species establish AM symbiosis. Image shows a mature arbuscule of *F. mosseae* in an extant host plant; scale bar, 10 μm. (**F** and **G**) At ~100 Ma, angiosperms within the rosids I (Fabidae) evolved the ability to form a symbiosis with N-fixing rhizobial proteobacteria and *Frankia* actinomycetes (37, 39, 40). Shown are *Medicago-Sinorhizobium* (F) and *Casuarina-Frankia* nodules (G); scale bars, 100 μm. [Images, C. Strullu-Derrien (A), M. Brundrett [(B), (C), (E)], M. de Freitas (D), F. de Billy (F), and H. Gherbi (G) with permission]

forests in the Late Carboniferous period (299 to 323 Ma) harbored AM-like associations in the fine rootlets that developed from their rhizomes (Fig. 1) (29, 30). Later, cycads and conifers from the Middle Triassic (237 to 247 Ma) and Middle Eocene periods (~48 Ma) were colonized by AM fungi that formed symbioses that resemble those observed in extant AM symbioses (20) (Fig. 1). Pinaceae species, which became established in the Late Jurassic and Early Cretaceous periods (140 to 180 Ma), formed a new type of mycorrhizal association, known as ectomycorrhizal (ECM) associations, involving roots and Dikarya (Fig. 1). The oldest fossils of permineralized ECM roots of Pinaceae and Dipterocarpaceae are from the Early Eocene (41 to 56 Ma) (31, 32), but evidence from phylogenetic and paleogenomic analyses hints at much earlier origins during the Jurassic (~180 Ma) (33). ECM symbioses likely emerged in semi-arid forests dominated by conifers under tropical to subtropical climates and diversified in angiosperms and conifer forests driven by a change to cooler climate during the Cenozoic (34). As revealed by phylogenetic and phylogenomic analyses, ECM fungi have arisen repeatedly from saprotrophic fungal ancestors in several independent lineages, as a result of the loss of plant cell wall degradation enzymes and the emergence of effector-like secreted proteins (14, 33). The ericoid mycorrhizal symbiosis involves the youngest lineages of ericaceous heath plants (Ericaceae) associating with Leotiomycetes fungi (Ascomycota). Fossils of Ericaceae-like plants date back to the Cretaceous, and recent molecular phylogenetic analysis dates the earliest Ericaceae species to ~117 Ma (35).

Evolution of N-fixing bacterial symbioses

The ability to fix molecular nitrogen is widespread among various groups of eubacteria and archaea (36). Two groups of N-fixing soil bacteria can establish mutualistic symbioses with angiosperms and induce the formation of root nodules (37). Rhizobia, a polyphyletic group of proteobacteria, can associate symbiotically with legumes (Fagales) and with one nonlegume genus, *Parasponia* (Cannabaceae, Rosales). Filamentous actinobacteria of the genus *Frankia* can induce nodulation on a diverse group of plants belonging to the orders Fagales, Rosales, and Cucurbitales. The wide host taxonomic distribution of N-fixing bacteria might at first suggest multiple independent regulatory molecular mechanisms that evolved de novo through convergent evolution. However, an alternative hypothesis proposes that around 100 Ma, certain angiosperms (the so-called N-fixing clade) evolved a "predisposition" toward the evolution of nodulation (38–40). Recruitment of the signaling pathways required for the formation of AM symbioses may have been one of these key steps (12). Quantitative reconstruction of the major phylogenetic events driving the origin of symbiotic N fixation has now provided evidence that a single and necessary evolutionary innovation—the differentiation of N-fixing symbiotic organs—took place within the rosids I (Fabidae) at >100 Ma (40). This major event was then followed

by multiple gains and losses of N-fixing symbioses (41). Subsequently, N-fixing root nodule symbioses evolved several times independently among the plants with the common predisposition; their independent origins are reflected by differences in root colonization strategies as well as in nodule ontology and development (11, 12).

Although nodule development is outside the scope of this article, the following sections address recent findings relating to host-microbe communication, how symbiotic fungi and bacteria enter and are accommodated in host tissues (including the creation of symbiotic interfaces), the central role of phytohormones in symbiosis-associated root development, and the various mechanisms by which host defense responses are circumvented during microbial colonization.

Coordinated host and microbe development during root symbiotic associations

Host-microbe communication: A prerequisite for successful symbiotic root colonization

Considerable research in recent decades has been devoted to understanding how host plants recognize appropriate microbial symbionts among the extremely diverse population of microbes present in the soil rhizosphere (Box 1) and how plant developmental pathways are harnessed by the colonizing microbes to accommodate symbiotic structures and metabolism. Studies focused primarily on model legume species (notably *Lotus japonicus* and *Medicago truncatula*) have revealed a unique signal transduction pathway activated in host cells in response to appropriate microbial symbiotic signals, leading to the elaborate cell reprogramming necessary for apoplastic microbial root entry (see below). This specialized pathway is now generally referred to as the "common symbiotic signaling pathway" (CSSP), because it has been shown that a conserved core module of the CSSP is shared by all host plants establishing endosymbioses, including AM, rhizobial, and more recently actinorhizal associations (42). The mode of action and the various components of the CSSP, including the highly characteristic triggering of nuclear Ca^{2+} spiking (oscillations), are already well described in several recent reviews (43, 44) (fig. S1).

The remarkable conservation of the core CSSP between widely divergent angiosperm host species underlines the shared evolutionary origin for this ancient symbiotic signaling pathway. Indeed, a recent phylogenomic study has revealed that extant freshwater algal charophyte species corresponding to the immediate ancestors of the earliest plant lineages also possess these conserved CSSP components (45), which suggests that certain features of this pathway may well have predated the evolution of the first terrestrial plants in the Middle to Late Ordovician and thereby contributed to the establishment of the earliest AM associations with ancestral land plants (27, 45, 46). However, in contrast to the AM, rhizobial, and actinorhizal endosymbioses, there is no evidence to date that

the CSSP is required for fungal ECM associations. In this context, it is noteworthy that the conserved core CSSP genes are absent (presumably lost via genome erosion) from the Pinaceae lineage (46).

In most rhizobial/legume N-fixing associations, secreted legume flavonoids stimulate the synthesis of rhizobial lipochito-oligosaccharide (LCO) signals known as Nod factors, which are perceived by legume LysM-containing receptor-like kinases (LysM RLKs), leading in turn to the triggering of the CSSP (43, 47) (fig. S1). That this molecular dialogue is central to symbiosis was demonstrated by phenotypic studies of bacterial mutants that failed to produce Nod factors or host plant mutants that were defective for Nod factor perception or transduction.

In contrast to the rhizobial/legume association, the absence of genetic approaches for either Glomeromycotina AM fungi or filamentous *Frankia* has greatly hampered the identification of the respective microbial symbiotic factors. Nonetheless, recent findings have revealed that chitin-based molecules present in AM fungal exudates are also able to activate the conserved CSSP. These include Myc-LCOs, whose structures resemble rhizobial Nod factors (48), as well as short-chain chitin oligomers (Myc-COs) lacking lipid decorations (49). Interestingly, Myc-CO levels are enhanced in fungal exudates in the presence of the host-secreted phytohormone strigolactone; this observation provides the best evidence to date for a pre-infection molecular dialogue between host and fungal partners (49, 50). Although it is not yet possible to unequivocally ascribe signaling roles for either Myc-COs or -LCOs during the establishment of the AM association in legume hosts, recent studies in non-nodulating plants, including rice (51, 52) and tomato (53), have confirmed that the initial stages of fungal colonization are indeed dependent on LysM RLKs. The case of rice is particularly intriguing, because a mutation in the bifunctional chitin receptor kinase *OsCERK1* gene turned out to be defective not only for chitin-triggered immunity but also for AM fungal entry (51); this finding implies that this LysM RLK participates in receptor complexes binding different chitin-based ligands and activating distinct downstream signaling pathways. Indeed, a recent study has revealed that the rice *Oscerk1* mutant is defective in perceiving the Myc-COs, which are able to activate the CSSP in target root epidermal cells (54). Finally, *Frankia* spp. also secrete symbiotic factors capable of activating the *Casuarina glauca* CSSP in the actinorhizal N-fixing association (55, 56), although preliminary characterization indicates that these factors are unlikely to be chitin-based (55).

Accommodation of symbiotic microbes: Apoplastic modes of root colonization

The establishment of functional microbial root symbioses is complex, often requiring sequential developmental steps necessitating codifferentiation of both partners. We address several characteristic features of concerted microbe-host differentiation, whether during initial root colonization, the establishment of the symbiotic

interfaces, or the accompanying modifications to host root development.

Initial root entry of fungal and bacterial symbionts is always apoplastic, whether intercellular or via de novo constructed infection compartments (Fig. 2). As a result, the microbe is always separated from the host cytoplasm by a membrane–cell wall matrix interface. Even during later developmental stages (e.g., during arbuscule or bacteroid development), the intracellularly housed microbe is continuously surrounded by a specialized plant membrane. These various colonization strategies, usually referred to as accommodation (12), permit the regulation of microbial growth and development within the host tissues in addition to limiting the activation of antimicrobial defense mechanisms (see below).

Whereas little is currently known about the mechanisms that regulate intercellular colonization, intracellular apoplastic infection has been studied in considerable detail during rhizobial and AM fungal root colonization. In vivo confocal imaging has shown that the fundamental cellular mechanisms leading to the construction of the specialized infection compartments are highly conserved, irrespective of the host cell type and the colonizing microbe (12, 57–59) (Fig. 2), further underscoring the close evolutionary relationship between these two endosymbiotic associations. In parallel, molecular genetic studies of rhizobial infection have uncovered a plethora of host proteins and regulators with key functions in the infection process (60, 61). Recent studies have

shown that certain of these symbiosis-specific transcriptional regulators are present in multiple copies with partially redundant activities (62, 63), presumably reflecting the importance of genome duplication/rearrangement events in legumes that accompanied the evolution of the N-fixing symbiotic association with rhizobia (64). Certain of these actors are also known to have roles during both rhizobial and AM fungal infection, including a presumed membrane-trafficking protein known as vapyrin (50, 65, 66), as well as proteins associated directly with exocytosis (67, 68). Finally, recent studies using model legume species are now providing evidence for phytohormonal (auxin/cytokinin) control of rhizobial root entry (69, 70). This is in line with earlier findings indicating a role for auxin during *Frankia* root hair–mediated infection of the actinorhizal host *Casuarina glauca* (71). As discussed below, phytohormones and growth regulators of microbial origin are also important players during other key developmental processes leading to successful symbiotic associations.

Symbiotic membrane–cell wall interface development

The establishment of functional root symbioses after initial microbe entry also requires the coordinated development of symbiotic exchange interfaces within host root and nodule tissues. To illustrate this, we focus on the AM fungal symbiosis, in which the microbial partner is concomitantly present both within and outside the

root, mobilizing soil nutrients in exchange for access to host photosynthate. As a consequence of this particular lifestyle, glomeromycetes possess both a unidirectional extraradical interface associated with hyphal soil exploration and a bidirectional interface exchanging signals and nutrients localized within the cortical cell arbuscules (12, 13).

The most striking visual feature of arbuscules is the extent of the hyphal ramification that follows initial fungal entry into the host cortical cell. This ramification increases the potential exchange surface and occurs simultaneously with the progressive formation of the host periarbuscular membrane (PAM)–cell wall interface, which surrounds the branched hyphae (13). Recent findings are beginning to reveal the molecular mechanisms orchestrating arbuscule development. In particular, several members of the GRAS-domain family of transcription factors are pivotal in creating regulatory hubs between AM fungal signaling and phytohormone (gibberellic acid) signaling, which coordinate arbuscular hyphal branching (72–76) and associated host cortical cell expansion (77). Further information about the multiplicity of roles played by phytohormones during the establishment and regulation of the AM association can be found in two complementary reviews (78, 79). Finally, the establishment of the periarbuscular interface also requires coordinated regulation and membrane localization of the suite of metabolite transporters necessary for efficient bidirectional

Fig. 2. Apoplastic modes of root entry by symbiotic soil microbes. Initial intracellular root infection by either AM fungi (left) or N-fixing rhizobia (right) occurs within de novo host-constructed apoplastic compartments formed within both epidermal and outer cortical tissues. Host nuclear migration and associated intracellular remodeling have been shown to play a key role in compartment construction in advance of microbial cell entry (57, 58), and high-frequency nuclear Ca^{2+} spiking has been observed to occur specifically within these nuclei, concomitant with the passage between adjacent cell layers (59). The fact that this nuclear Ca^{2+} response is a well-characterized hallmark for the triggering of the common symbiotic signaling pathway [CSSP (43, 47) (fig. S1)] argues for active microbe-host communication throughout these early root colonization stages. In the case of ECM intercellular entry (center), there is strong evidence that major cell wall remodeling

AM intracellular root entry

ECM intercellular root entry

Rhizobial intracellular root entry

AM fungal hyphopodium — Peri-fungal infection compartment

Extraradicular fungal mycelium — Apoplastic fungal penetration

Rhizobia — Infection thread

Epidermis

Cortex

Pre-penetration apparatus (PPA) — Nuclear Ca^{2+} spiking

Pre-infection thread (PIT) — Nuclear Ca^{2+} spiking

occurs at sites of fungal mycelia root entry during the formation of the intraradicular Hartig net (99). However, it is not yet known to what extent microbe-host communication is associated with ECM entry, or whether host Ca^{2+} signaling is involved in this process. [Images of rhizobial, ECM, and AM infection, F. de Billy, F. Zhang, and A. Genre, respectively, with permission] Scale bars, 20 μm.
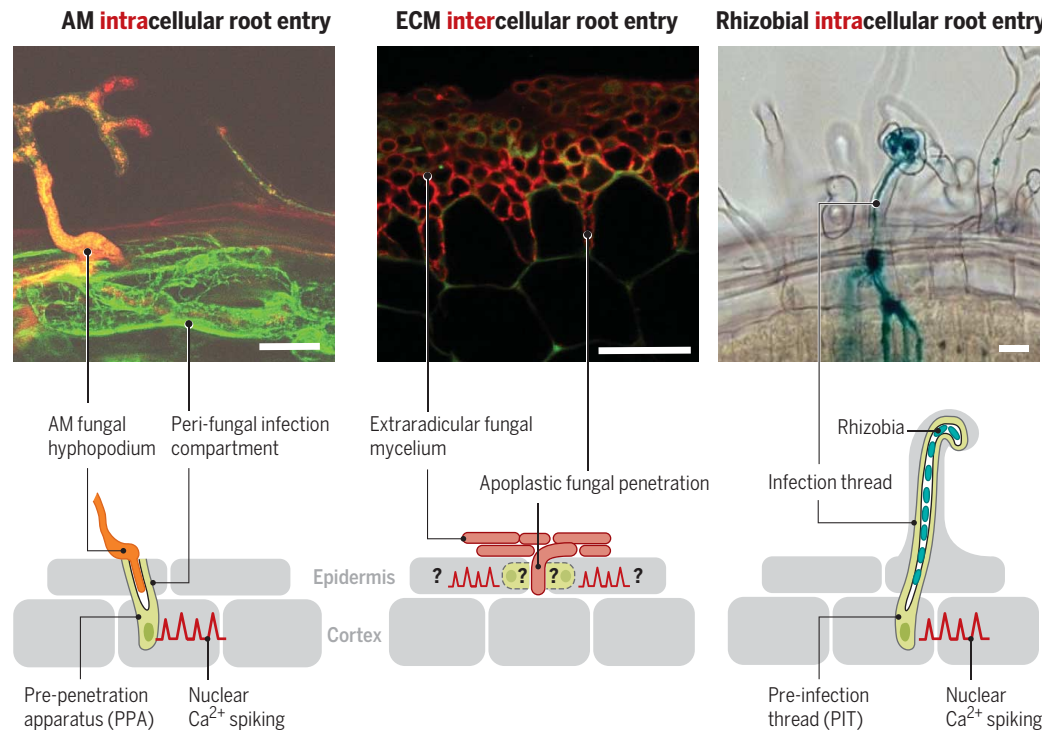
exchange (80). The recent development of large-scale comparative phylogenomic approaches based on evolutionary conservation patterns has also yielded several novel AM-associated candidate genes (81–83), contributing to the identification of proteins specifically involved in the targeted exocytotic delivery of symbiosis-related components to the PAM (84, 85).

The modern-day cortical arbuscule is thus an exquisite example of >400 million years of intimate coevolution between fungal and plant partners, providing the sophisticated bidirectional interface that regulates metabolic fluxes while simultaneously maintaining host integrity. In this context, we emphasize that more recently evolved ECM associations also possess sophisticated membrane-rich symbiotic interfaces within the highly ramified intercellular hyphal net, although in ECM the fungus remains within the extracellular apoplast (14).

Phytohormone-related root development associated with the formation of symbiotic tissues

A common feature of these mutualistic associations is the modification of root development. This includes the stimulation of lateral root growth to increase potential colonization sites in response to rhizobial and AM fungal LCOs (48), major structural and functional changes to the root during the establishment of the associations, and the development of the highly specialized N-fixing root nodules in legume and actinorhizal host plants. As mentioned, phytohormones are important actors in these various processes; to illustrate this further, we focus here on certain features of legume nodule organogenesis, as well as on the major root developmental responses associated with the ECM symbiosis.

Studies in legume hosts have shown that apoplastic intracellular rhizobial infection and nodule organogenesis are tightly coordinated processes and that both require Nod factor–dependent activation of the CSSP (86). The central role of plant hormones during nodule organogenesis is to integrate bacterial and plant signaling cues (11, 87). For example, cytokinin receptors (LHK1/CRE1) are directly involved in nodulation, and loss-of-function mutations in the genes encoding them lead to defects in nodule formation, whereas gain-of-function mutations lead to the formation of spontaneous nodules in the absence of rhizobia (88, 89). Furthermore, cytokinins positively regulate the expression of key regulatory genes involved in nodule organogenesis, as well as the expression of auxin influx carriers that lead to local auxin accumulation in the dividing nodule primordia (88, 90, 91). In contrast to legume nodules, which are initiated by cell divisions in cortical tissues and possess a peripheral vascular system, actinorhizal nodules elicited in response to Frankia are modified lateral roots with a central vasculature (42). Nonetheless, despite these striking differences, the conserved CSSP also has a pivotal role in actinorhizal nodulation (92).

During host root colonization by ECM fungi, the root often undergoes major lateral root de-velopment (LRD). A well-studied example is the association between poplar and the auxin-secreting basidiomycete Laccaria bicolor. Poplar auxin homeostasis and signaling are modified in the presence of the fungus, and stimulation of host LRD is dependent on the expression of the polar auxin efflux carrier PtaPIN9 and other auxin-related gene products (93). Evidence suggests a major role in this process for the regulation of auxin homeostasis at the root tip (94). More recently, it was shown that volatile sesquiterpenes generated by L. bicolor can also stimulate poplar LRD, although the mechanism of action is not yet elucidated (95). In all these cases, the presence of the ECM fungus secreting a mixture of diffusible and volatile morphogens results in modifications in root development that favor the extent of root colonization. In contrast, during the more advanced stages of the poplar-Laccaria association, other phytohormones, such as ethylene and jasmonic acid, appear to have a role in limiting the intercellular apoplastic development of the fungal Hartig net within outer root tissues (96).

Circumventing and attenuating host defense responses

The successful establishment of these complex multistep mutualistic associations requires that the activation of host defense responses should be kept minimal. This is achieved by several distinct and complementary mechanisms. One is the host-regulated construction of intracellular apoplastic infection compartments (Fig. 2), as observed for AM fungal root entry as well as rhizobial and Frankia root hair–mediated colonization. However, as mentioned earlier, colonization of the outer root tissues can also occur intercellularly, as observed for certain legume hosts such as Aeschynomene spp. (97), the nonlegume Parasponia (98), the majority of nodulating actinorhizal genera (42), and, of course, all ECM host plants. It is currently unclear to what extent microbial-host signaling and the activation of the CSSP play a role prior to and during these more rudimentary modes of root colonization, nor is it known whether they are equally efficient at avoiding immunity activation. In the particular case of Aeschynomene, it has been shown that both infection and nodulation by certain Bradyrhizobia species are Nod factor–independent (97). Furthermore, Lotus japonicus mutants defective in intracellular root hair infection can nonetheless be inefficiently colonized via an ancient default pathway involving intercellular entry (60). Although the focus of limited research until recently, major membrane–cell wall interface remodeling is also likely to accompany intercellular microbial colonization (see Fig. 2); indeed, for the intraradicular ECM Hartig net, the root apoplast is drastically altered during fungal ingress (14, 99).

The second mechanism, particularly well studied for ECM fungi, is the evolutionary loss of genes involved in degrading the plant cell wall. The sequencing of ECM fungal genomes has revealed the convergent loss of various components of the ancestral saprotrophic decay machinery, including plant cell wall–degrading enzymes and lignin peroxidases (33). Hence, ECM fungi have evolved to depend on their plant hosts for photosynthetic carbon, and the loss of their organic matter decomposition potential has contributed to the evolutionary stability of this type of association (14). In the case of obligate AM fungi, the situation is even more extreme, because sequencing of the Rhizophagus irregularis genome (100) revealed no sign of plant cell wall–degrading enzymes. These findings argue that both ECM and AM fungi have evolved to minimize the release of molecules that could elicit plant immune responses.

Fungal symbionts also secrete effector-like small proteins that modulate host defenses to facilitate microbial colonization. Sequencing of the R. irregularis genome has highlighted the presence of a notable repertoire of potential mycorrhiza-induced small secreted protein (MiSSP) effectors (100, 101). One of these MiSSPs (known as SP7) targets the plant nucleus, where it interacts with the pathogenesis-related MtERF19 transcription factor (102). A combination of deletion and overexpression experiments for both proteins indicates that the SP7 effector attenuates host responses during AM colonization. Likewise, in ECM, there is also strong evidence that MiSSP effectors can interfere with the host defenses. In the case of L. bicolor (103), silencing by RNA interference revealed that MiSSP7 is required for the establishment of the symbiotic Hartig net within the poplar host root. Subsequently, it was found that MiSSP7 interacts with key regulators of the jasmonate signaling pathway to suppress defense responses (104).

Future challenges

Detailed studies of mutualistic root symbioses in model legumes have revealed how secreted microbial signal molecules are able to activate the symbiosis-specific host signaling pathway known as the CSSP, leading to the remarkable cell remodeling required for controlled bacterial or fungal entry within root cells and tissues. The striking evolutionary conservation of this pathway initially led to the idea that rhizobial-like LCOs might be universal microbial signals recognized by plants hosting other microsymbionts such as AM fungi and filamentous Frankia. However, recent findings indicate that this is clearly not the whole story, because nonlipidic short-chain COs can also serve as AM fungal signals (49, 50, 54); in the case of Frankia, we are awaiting chemical characterization of the corresponding factors that do not appear to be either LCOs or COs (55). Major challenges for the future therefore include the identification of the plant receptors that perceive the novel Frankia signals and, in the case of the chitin-based signals, a deeper understanding of how the host receptors are able to distinguish between symbiotic and pathogenic elicitors (105). Finally, it is still unclear to what extent the CSSP and associated epidermal cell remodeling are required for initial microorganism entry of the host root via an intercellular pathway, as in the case of certain rhizobia (60, 86) and Frankia (42) as well as

ECM fungi (*99*). Research in this area is of particular importance because this more rudimentary mode of root entry probably represents a more appropriate model for early stages of root colonization by the majority of endophytes.

We have examined the various strategies used by the host and microsymbiont to avoid or minimize host immunity responses. In the case of the two major mycorrhizal symbioses (AM and ECM), we discussed two striking examples that underscore the roles played by effector-like fungal proteins in modulating plant defense activation (*102*, *103*). However, whole-genome sequencing has revealed a multitude of potential effectors for both AM and ECM fungi (*14*, *100*, *101*), and future research will need to be directed to uncovering other host cellular targets that facilitate fungal entry and subsequent symbiotic development within the host root tissues. In addition to immunity response avoidance (*105*), one of the most important developments in current research on mutualistic associations has been the uncovering of certain of the molecular mechanisms that allow direct links to be established, via key regulatory hubs, between microbial/host symbiotic signaling and phytohormonal regulation of root development. The pursuit of this exciting field of research will be essential if we wish to fully understand the place of these beneficial symbioses in influencing host root architecture, phytohormone homeostasis, and tolerance to both biotic and abiotic challenges.

In our opinion, detailed studies now need to be developed on a selected set of taxonomically and ecologically relevant bacterial and fungal endophytic interactions. The challenges facing plant and microbial ecologists include determining the mechanisms that regulate nutrient trade dynamics, as well as characterizing how mutualistic symbionts affect the establishment of the rhizospheric and endospheric microbiota (and vice versa), how the signaling pathways triggered by multiple interactions are integrated by host cells, how perturbations of the recognition and accommodation systems influence plant responses, and how metabolic responses are regulated in multiple interactions. The exploitation of a wide range of tools, including comparative genomics, metatranscriptomics, newly created mutants to probe effector/receptor interactions, genome-wide association studies, and experimental manipulation of the microbial communities, will all contribute to this effort.

A better understanding of how genetic variation in both hosts and microbes affects the structure of microbial communities will also facilitate future efforts to obtain the full benefit of mutualistic symbionts and other members of the microbiota. The heritability of beneficial microbiota also needs to be investigated, because this genetic feature determines whether the extended host plant phenotype can evolve in response to host plant selection, and this may play an important role in host speciation. Finally, among other future benefits, manipulating the plant microbiota should permit the modulation of plant development, as well as promoting the sustainable growth of crop plants by influencing plant processes such as nutrient acquisition, drought and salt tolerance, and disease resistance (*6*, *7*). This exciting and rapidly developing area of plant-microbe interaction research is clearly poised for substantial breakthroughs in the years to come.

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Editor's Summary

Taking a look at plant-microbe relationships

Ever since plants colonized land, they have evolved a range of mutualistic associations with bacteria and fungi. Indeed, such associations were probably required for plants to grow on harsh, nutrient-poor surfaces. Martin *et al.* review the spectrum of plant-microbe symbioses and their evolution, including evidence from the Rhynie Chert of the Devonian period and modern associations. Surprisingly, diverse functional plant-microbial symbioses have several common conserved features, including signaling pathways, immune evasion, and root development.

Science, this issue p. eaad4501

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