Published in "Current Opinion in Plant Biology 10(1): 98–105, 2007"
which should be cited to refer to this work.

Programming good relations — development of the arbuscular mycorrhizal symbiosis

Didier Reinhardt

The majority of plants live in symbiotic associations with fungi or bacteria that improve their nutrition. Critical steps in a symbiosis are mutual recognition and subsequently the establishment of an intimate association, which involves the penetration of plant tissues and, in many cases, the invasion of individual host cells by the microbial symbiont. Recent advances revealed that in the arbuscular mycorrhizal symbiosis with soil fungi of the order *Glomeromycota*, plant-derived signals attract fungal hyphae and stimulate their growth. Upon physical attachment of the fungal symbiont to the root surface, an active plant developmental program prepares the epidermal cells for penetration by the fungus. Thus, plants actively help symbiotic fungi to colonize their roots rather than just tolerating them.

Addresses
University of Fribourg, Rte Albert Gockel 3, CH-1700 Fribourg, Switzerland

Corresponding author: Reinhardt, Didier (didier.reinhardt@unifr.ch)

Introduction

Once upon a time, a heterotrophic eukaryotic cell incorporated a photosynthetic prokaryote for dinner. But the prey survived and evolved into an endosymbiont, now known as the chloroplast [1], an interaction that was so successful that it gave rise to the evolution of an entire new kingdom, the plants. During this success story, the tendency towards symbiotic interactions continued and resulted, among other associations, in the arbuscular mycorrhizal symbiosis (AMS), probably the most widespread symbiosis of plants, which has its origin more than 400 million years ago (Mya) [2,3]. The AMS between most taxa of land plants and fungi of the phylum *Glomeromycota* shares some evolutionary and developmental aspects with the root nodule symbiosis (RNS). This is the best-studied symbiotic interaction of plants, involving members of the legume family and bacteria, which are referred to collectively as rhizobia [4].

Symbiosis can be defined as an intimate, usually mutually beneficial, interaction between two organisms, in which benefits of some sort, in many cases mineral nutrients and photosynthates, are exchanged. In the case of the RNS, the interaction results in the formation of a new type of symbiotic organ, the nodule, which harbours the bacteria and is specialized for nitrogen fixation and nutrient exchange between plant and bacteria. In the AMS, colonization of the root does not result in the formation of a new symbiotic organ. The interacting partners influence each other in many ways, however, and the coordinated developmental programs on both sides result in a symbiotic functional unit, the mycorrhiza, which achieves more than the sum of the activities of the two isolated symbionts [5]. This review focuses on recent advances in deciphering the molecular dialog and the genetic programs that regulate the AMS, with reference to the RNS where appropriate.

The first word in the symbiotic dialog: the seductive call of the plant

How do the symbiotic partners find each other? Apparently, the first step is the recognition of plant signals by the microbe. Plant roots constitutively release a complex mixture of substances, the root exudates, which contain signals that are perceived by symbiotic microbes. In the RNS, flavonoids attract the rhizobia and initiate a symbiotic program that results in the transcriptional induction of several bacterial symbiosis genes, the *Nod* genes [4,6].

In the case of the AMS, the initial signals of the plant are the constitutively released strigolactones [7••], which induce the hyphal branching [8] and metabolic activity of arbuscular mycorrhizal (AM) fungi [9,10•], thereby promoting their growth and increasing the chance of an encounter with the root. In addition, hyphae of AM fungi have recently been shown to grow chemotropically towards roots [11•].

Plant exudates might attract not only symbionts but also saprotrophs or potential pathogens. In this 'background' of microbial life in the rhizosphere, the plant has to recognize potential symbionts and to promote their growth and colonization of the root selectively, whereas it must reject pathogens by mounting a rapid defence response. Thus, a symbiosis signal is of primary importance as the 'door-opener' in symbiotic interactions.

The reply of microbes with good intentions, and how they are welcomed by the plant

In the RNS, flavonoid perception triggers the formation of the bacterial Nod factor, which signals back to inform the plant of the presence and identity of the rhizobia [4,6].

The Nod factor initiates a complex series of events that results in the active uptake of the bacteria into root hairs, in their translocation to the root cortex, and in the establishment of a new symbiotic organ, the nodule.

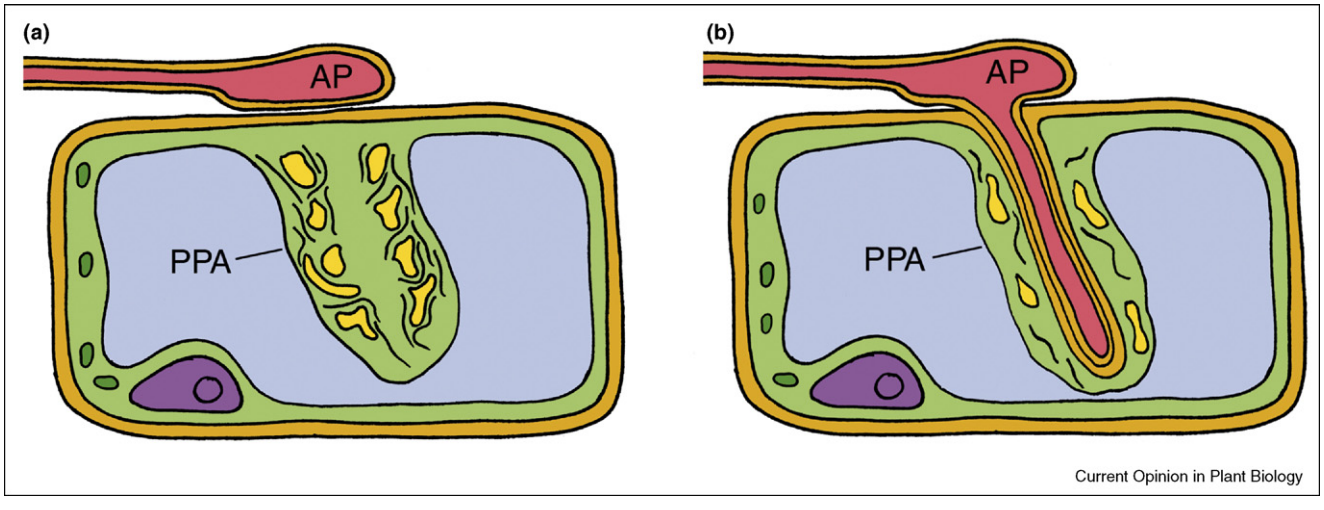
In the case of AM development, fungal hyphae that have started to branch in the vicinity of a host root emit a first signal of unknown nature, which activates the promoter of the symbiosis-related gene *ENOD11* in roots [12]. This response does not require direct physical contact between hyphae and roots, indicating that it is triggered by a diffusible substance.

When the fungus reaches the root surface, it forms appressoria: infection structures that can be triggered by physical contact with isolated cell walls of epidermal root cells in the absence of additional diffusible signals [13]. During appressorium formation, but preceding the first signs of penetration, the underlying epidermal cell responds with a striking program of cellular reorganization [14••]. First, the nucleus rapidly migrates to a position just below the appressorium, then it moves away, leaving behind it an aggregation of microtubules, actin microfilaments, and ER cisternae. This aggregation becomes organized into a finger-shaped structure, the pre-penetration apparatus (PPA), which projects into the cell lumen. The PPA defines a trajectory through the cell, which presages the path of the invading fungal hypha (Figure 1). These observations show that developing appressoria emit a second, local, signal that allows the underlying epidermal cell to detect their position with precision. In interactions in which the AM fungus penetrates the root through a cleft between epidermal cells, and the subsequent intracellular penetrations through the anticlinal cell walls, follow a similar sequence of events (A Genre, pers. comm.). In agreement with these cellular rearrangements, an active contribution of the plant to root penetration is also supported by genetic evidence from mutants that do not permit the fungus to gain access between epidermal cells [15].

The common sym pathway

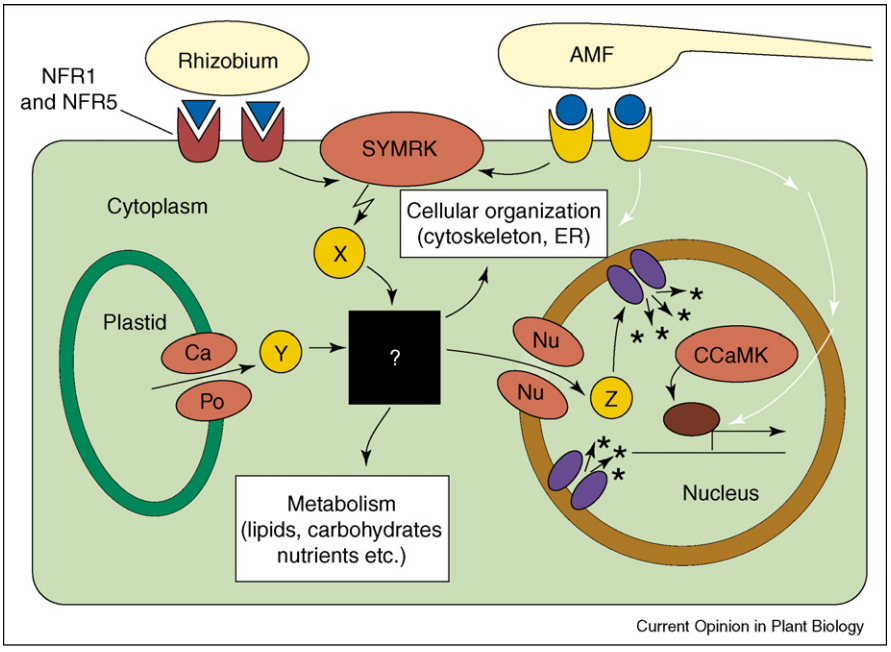
Interestingly, the establishment of a functional interaction in the RNS and the AMS involves a shared signalling pathway, here referred to as the 'common sym pathway' [16,17•]. The first common component is a receptor kinase (SYMRK) [18,19,20•], which is thought to integrate secondary signals from the perception of the Nod factor and putative Myc factors (Figure 2). A second component of the common sym pathway is a putative ion channel that is localized in the plastids [21,22••], which might release a plastidic factor that is involved in signal transduction. Symbiotic signalling further requires a nucleoporin, a protein that has homology to a component of the nuclear pore complex [23•]. Ultimately, symbiotic signalling triggers a nuclear calcium signal (i.e. calcium spiking), which is thought to be read by a calcium- and calmodulin-dependent protein kinase (CCaMK) [24,25], which in turn induces the transcription of symbiosis genes. Besides the induction of gene expression, symbiotic signalling leads to specific changes in cellular organization (e.g. the formation of the PPA) and metabolism. How the events at the plasmalemma, the plastid, and the nucleus are connected remains uncertain (black box in Figure 2).

Figure 1



Penetration of an epidermal cell by an AM fungus. **(a)** During appressorium (AP) formation, the underlying cell generates the pre-penetration apparatus (PPA). It contains large amounts of endoplasmic reticulum (yellow), microtubules and actin filaments (black lines). **(b)** The fungus penetrates along the trajectory delimited by the PPA. Note that the plant plasmalemma invaginates, hence the fungus develops in an apoplastic pocket that contains remnants of the plant cell wall. Colour code: red, fungal cytoplasm; brown, plant and fungal cell wall; green; plant cytoplasm; dark green, plastids; purple, plant nucleus; blue, central vacuole.

Figure 2



Model of early signalling in symbiosis. Rhizobia produce nod factors (blue triangles), which are perceived by a receptor complex involving NFR1 and NFR5. An analogous pathway is predicted for the perception of AMF-derived hypothetical Myc factors (blue circles). Both signals are integrated by SYMRK and transduced via phosphorylation of an unknown substrate (X). Symbiotic signalling also requires a putative plastidic ion channel consisting of Castor (Ca) and Pollux (Po), which might release a plastidic factor (Y) that is required for signal transduction. Ultimately, a second messenger (Z) is translocated to the nucleus in a NUP133-dependent fashion (Nu), where it triggers calcium channels (purple) to release calcium (stars) from the nuclear envelope (i.e. calcium spiking). The calcium signal activates a calcium- and calmodulin-dependent protein kinase (CCaMK), which induces the transcription of symbiosis genes. Symbiotic signalling also leads to changes in cellular organization and metabolism. How the events at the plasmalemma, the plastid, and the nucleus are connected is unknown (black box). Some plant responses to AMF, involving transcription and the cytoskeleton, are independent of the common sym pathway (white arrows). Genetically defined signalling components are coloured in red. (For details see [17•].)

The formation of the PPA and successful root colonization depend on the common sym pathway, but some early responses, such as ENOD11 induction [12], epidermal opening [15], and early nuclear migration [14••] are observed in mutants that are defective in the common sym pathway, indicating that independent signalling pathways exist for these responses [16]. Such parallel signalling pathways could provide the specificity that is required for the initiation of specific developmental events in the AMS and RNS downstream of the common sym pathway. Alternatively, downstream specificity could be mediated by modulating the activity of the common sym pathway in specific ways (e.g. by specific calcium-spiking signatures in AMS and RNS).

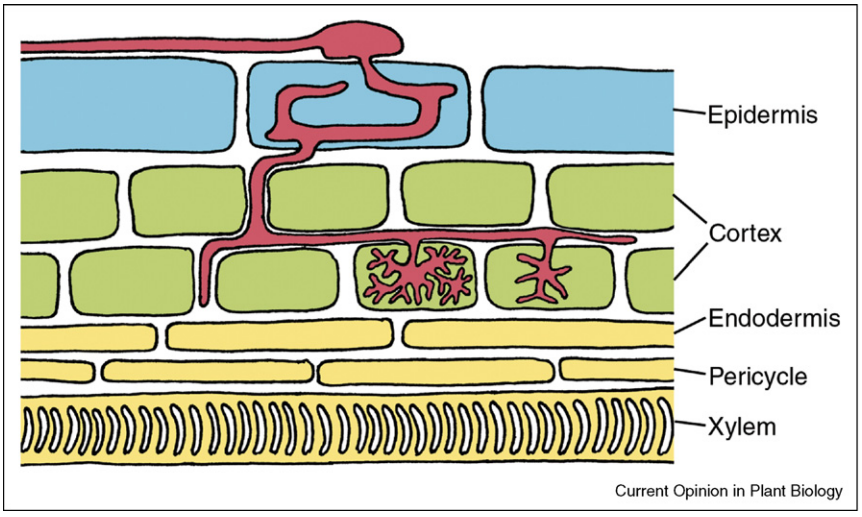
Root colonization and establishment of a functional symbiotic interface

Intracellular colonization of the first epidermal cell is followed by a phase of intercellular progression of the fungus into the root cortex, and along its longitudinal cell files (Figure 3). Whether this phase requires active help from the plant is uncertain, but several observations indicate that the fungus continues to be under the control of the plant (see below).

Symbiotic development culminates in the establishment of a symbiotic interface that serves to exchange nutrients and possibly signals [26,27]. The AM fungi have invented a specialized intracellular structure, the arbuscule, for nutrient exchange with the plant. It consists of highly ramified hyphae with very fine terminal tips, resulting in a surface-to-volume ratio that is greater than that of normal hyphae ([28]; Figure 4) and making them particularly efficient for nutrient transfer.

The establishment of the arbuscules, like the entry into epidermal cells, is accompanied by dramatic changes in the cellular organization of the host cell, such as vacuole fragmentation, nuclear migration from the periphery to the centre, cytoskeleton rearrangement, and plastid modification [29,30]. Although the arbuscules are intracellular, they remain surrounded by a plant membrane, the peri-arbuscular membrane (PAM), which is continuous with the plasmalemma (Figure 4).

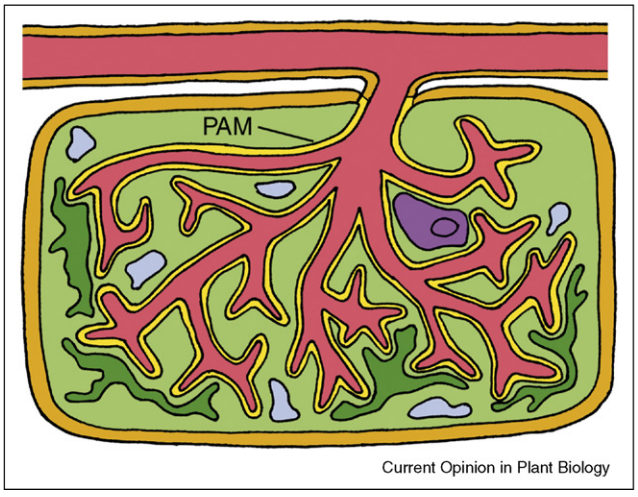
Figure 3



Colonization of the root cortex. After the formation of an intracellular coil in the first epidermal cell, the fungus grows intercellularly to colonize the underlying cortex. It advances to the inner cortex cells where it forms the intracellular arbuscules. Note that the fungus never penetrates the endodermis. The cell walls are ignored for clarity.

Cells that are inhabited by arbuscules develop an elaborate machinery for active nutrient transfer. They express phosphate transporters that reside in the PAM and are likely to take up the phosphate that is delivered by the arbuscule [31,32]. Induction of P-transporter genes appears to be triggered by a third, cell-autonomous, fungal signal [33]. The PAM and the fungal membrane of the arbuscule contain H^+-ATPases for the generation of an electro-chemical gradient that is required to energize nutrient transport [34]. Indeed, arbuscules are surrounded by an acidic environment [35], consistent with a role in active nutrient transfer.

Figure 4



Development of the arbuscule and its host cell. During fungal colonization, the central vacuole becomes fragmented (blue), and the nucleus (purple) moves to a more central position. The plastids (dark green) form tubular structures that associate with the fine branches of the arbuscule. These remain surrounded by the periarbuscular membrane (PAM), which is continuous with the plasmalemma. The space between the PAM and the fungal plasmalemma, the symbiotic interface (yellow), comprises remnants of the fungal cell wall and apoplastic material of the plant. Ultimately, the arbuscule occupies a large fraction of the cell volume.

Reprogramming of gene expression during AM development

The development of the AMS is associated with major changes in fungal and plant gene expression [36]. Fungal gene expression is changed upon perception of the branching factor [9], and the stage of appressorium formation [37,38]. On the other side, the gene-expression profile of plant roots changes dramatically during the AMS [39–44]. Some of the affected genes have a function in nutrient transport [26], whereas others might be involved in the reorganization of the cytoskeleton [45] or in cell wall modification [46]. Several induced genes, among them genes coding for phosphate transporters and for putative cell wall remodelling proteins, are expressed exclusively or primarily in the cells that contain arbuscules [31,32,39,47–50].

The early stages of AM fungal colonization are generally accompanied by a transient induction of genes that encode pathogenesis-related (PR) proteins and of other markers of defence [51,52]. In a conceivable scenario, this phenomenon might represent a first reaction of the plant to unspecific microbial signals (elicitors) from the AM fungus before the recognition of the Myc factor triggers the switch to the symbiotic program, resulting in the

suppression of the defence response. However, in some cases, things might be a bit more complex: a detailed study in *Medicago truncatula* of the PR protein chitinase, which occurs as a gene family, revealed that one member was induced specifically during symbiosis [53]. Constitutive overexpression of this gene in transgenic roots stimulated AM spore germination [54]. Consequently, this chitinase should be regarded as a 'symbiosis-related' protein rather than as a PR protein.

Control of the symbiotic interaction by the plant

In the RNS, bacterial uptake, colonization of the cortex, and nodule development appear to be controlled largely by a genetic program of the plant. This is suggested by (i) the fact that application of the Nod factor alone can trigger many of the symbiotic responses in the plant, resulting in the development of empty nodules [55]; (ii) by the large number of plant mutants, in which a genetic lesion abolishes the development of the symbiosis [56]; and (iii) by the way in which the bacteria are 'guided' through the plant tissues and accommodated in the nodule [4,6].

As in the RNS, the AMS appears to be controlled primarily by the plant. The formation of the PPA is an active process of the plant cell [14••]. Mutants in the common sym pathway, and possibly at other stages of symbiotic development, show an active contribution of the plant [16]. Depending on the combination of host plant and fungal symbiont, the morphology of the intracellular fungal structures varies, indicating that fungal development is influenced by the host [57].

Furthermore, in both the RNS and the AMS, the plant can limit the level of microbial colonization [58–61]. Interestingly, negative regulation of the AMS and the RNS influence each other [62]. Whether this reflects regulation at the level of the common sym pathway, or whether the two developmental pathways converge at a second point remains to be seen.

Nutrient supply can also influence the level of root colonization. If nutrients are available at such high levels that the plant alone can acquire enough for optimal growth, then fungal colonization is reduced [63]. Interestingly, the same happens if a defect in the symbiosis-specific phosphate uptake mechanism of the plant prevents nutrient acquisition ([64•]; M Harrison, pers. comm.). In both cases, the plant would provide carbohydrates to the fungus without receiving any benefit, thus the fungus would act like a parasite. This misbalance is apparently perceived by the plant, which then inhibits fungal colonization. Hence, the plant lets the fungus feed on its carbohydrates only if the bill is paid with nutrients. These observations show not only that the AMS influences plant nutrition but also that the nutritional status influences symbiotic development, demonstrating the operation of a feedback mechanism in symbiosis.

The peculiar genetics of AM fungi — even better than sex?

AM fungi are asexual, and might have evolved asexually for hundreds of millions of years [65,66]. They are coenocytic at all stages of their life cycle, that is, their nuclei are not packaged in individual cells. Surprisingly, the individual nuclei of a single spore turned out to be genetically different, a phenomenon referred to as heterokaryosis [67,68•]. How is this possible after hundreds of millions of years of clonal propagation (which would be expected to lead to a gradual loss of diversity)? The solution to this enigma might be the combination of accumulated mutations that have not been purged by recombination, coupled with a phenomenon referred to as anastomosis. During anastomosis, fungal hyphae come together and 'open their mouths' (from Greek στομα = mouth), or in other words: they kiss. During this process, the hyphae fuse and establish cytoplasmic continuity, allowing the exchange of genetic material between the anastomosing hyphae [69]. Thus, in natural populations with diversity within and between individual clones, anastomosis could lead to a continuous reshuffling of genomes. In this way, heterokaryotic diversity could be preserved over long time periods, a scenario that is supported by theoretical models [70]. Hence, for AM fungi, kissing may be even better than sex.

Is the heterogeneity of the genetic material in the fungus relevant for symbiotic development? The different nuclei could, for example, provide genetic diversity that is required for the recognition of, or the signalling to, a variety of potential host plants. Or is it not the diversity but the redundancy between the coexisting genomes that is important? The lack of selection through the bottleneck of a monokaryotic stage could have led to the gradual degeneration of the individual genomes, so that only the multigenomic (heterokaryotic) status allows the fungus to survive. These questions are challenging issues for future studies on the evolution, ecology, and development of AM fungi.

Conclusions

The establishment of the AMS involves specific developmental adaptations in both symbiotic partners, and coordination of their developmental programs with specific signals. Plants produce an early signal to initiate the interaction, whereas the fungus produces at least three signals, an early diffusible signal, a subsequent local signal that allows the plant to detect the position of appressoria, and later, a cell autonomous signal in colonized cells that induces gene expression. The identity of these signals, and the molecular regulation of the symbiotic program in the fungus, remains to be elucidated. On the plant side, major progress has been made with the recent identification of the initial signal, strigolactone,

and with the genetic and anatomical characterization of parts of the symbiotic program. However, many components that are involved in the signalling between the symbiotic partners and in the establishment of the functional symbiosis remain to be identified.

Acknowledgements

I thank Paola Bonfante, Ian Sanders, and Felix Mauch for comments on the manuscript. Our work is supported by the Swiss National Science Foundation (Grant 3100A0-101792) and the National Centre of Competence in Research 'Plant Survival'.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Kutschera U, Niklas KJ: **Endosymbiosis, cell evolution, and speciation**. *Theory In Biosciences* 2005, **124**:1-24.

2. Kistner C, Parniske M: **Evolution of signal transduction in intracellular symbiosis**. *Trends Plant Sci* 2002, **7**:511-518.

3. Redecker D: **Molecular identification and phylogeny of arbuscular mycorrhizal fungi**. *Plant Soil* 2002, **244**:67-73.

4. Stougaard J: **Regulators and regulation of legume root nodule development**. *Plant Physiol* 2000, **124**:531-540.

5. Harrison MJ: **Signaling in the arbuscular mycorrhizal symbiosis**. *Annu Rev Microbiol* 2005, **59**:19-42.

6. Broughton WJ, Jabbouri S, Perret X: **Keys to symbiotic harmony**. *J Bacteriol* 2000, **182**:5641-5652.

7. •• Akiyama K, Matsuzaki K, Hayashi H: **Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi**. *Nature* 2005, **435**:824-827.

Previous studies had shown that plant roots release a branching factor (BF) that stimulates the growth and branching of AM fungal hyphae [8]. This paper describes the identification of the active component in the BF of *Lotus japonicus*. It belongs to a class of molecules known as strigolactones, which had previously been found to stimulate the germination of the parasitic plant *Striga*.

8. Buee M, Rossignol M, Jauneau A, Ranjeva R, Bécard G: **The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates**. *Mol Plant Microbe Interact* 2000, **13**:693-698.

9. Tamasloukht M, Séjalon-Delmas N, Kluever A, Jauneau A, Roux C, Bécard G, Franken P: **Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea***. *Plant Physiol* 2003, **131**:1468-1478.

10. • Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G, Séjalon-Delmas N: **Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria**. *PLoS Biol* 2006, **4**:1239-1247.

The authors describe the physiological effects of the branching factor strigolactone and of its structural analogues on an arbuscular mycorrhizal fungus. Strigolactone rapidly activates mitochondria, suggesting that the growth-promoting effect of strigolactone could be due to increased β-oxidation of lipids, which are the main storage form of energy in arbuscular fungi.

11. • Sbrana C, Giovannetti M: **Chemotropism in the arbuscular mycorrhizal fungus *Glomus mosseae***. *Mycorrhiza* 2005, **15**:539-545.

This paper documents that hyphae grow directionally towards host roots. Chemotropism provides a second mechanism in addition to the hyphal-branching response to increase the chance of an interaction. The nature of the chemotropic signal remains to be identified, but it could be strigolactone.

12. Kosuta S, Chabaud M, Lougnon G, Gough C, Dénarié J, Barker DG, Bécard G: **A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula***. *Plant Physiol* 2003, **131**:952-962.

13. Nagahashi G, Douds DD: **Appressorium formation by AM fungi on isolated cell walls of carrot roots**. *New Phytol* 1997, **136**:299-304.

14. •• Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG: **Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection**. *Plant Cell* 2005, **17**:3489-3499.

This paper describes striking evidence for an active cellular program in epidermal host cells, which is required for the AM symbiosis. Using fluorescently labelled proteins that associate with F-actin, microtubules and the ER, the authors document the assembly of a tube-like structure beneath the fungal appressoria. The fungal penetration hypha follows this predetermined path exactly.

15. Demchenko K, Winzer T, Stougaard J, Parniske M, Pawlowski K: **Distinct roles of *Lotus japonicus* SYMRK and SYM15 in root colonization and arbuscule formation**. *New Phytol* 2004, **163**:381-392.

16. Parniske M: **Molecular genetics of the arbuscular mycorrhizal symbiosis**. *Curr Opin Plant Biol* 2004, **7**:414-421.

17. • Oldroyd GED, Downie JA: **Nuclear calcium changes at the core of symbiosis signalling**. *Curr Opin Plant Biol* 2006, **9**:351-357.

This nice review describes in detail the central position of calcium as a second messenger in the common sym pathway. It also considers the interesting question of how calcium signalling in the common sym pathway could be linked to specific downstream events in the arbuscular mycorrhizal and the root nodule symbiosis, respectively.

18. Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K *et al.*: **A plant receptor-like kinase required for both bacterial and fungal symbiosis**. *Nature* 2002, **417**:959-962.

19. Endre G, Kereszt A, Kevei Z, Mihacea S, Kaló P, Kiss GB: **A receptor kinase gene regulating symbiotic nodule development**. *Nature* 2002, **417**:962-966.

20. • Yoshida S, Parniske M: **Regulation of plant symbiosis receptor kinase through serine and threonine phosphorylation**. *J Biol Chem* 2005, **280**:9203-9209.

This paper shows that the SYMRK of *L. japonicus* exhibits serine/threonine kinase activity, and that several *symrk* mutant alleles carry mutations that abolish kinase activity. This evidence suggests that signalling through SYMRK involves phosphorylation.

21. Ané J-M, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Lévy J, Debellé F, Baek JM, Kaló P *et al.*: ***Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes**. *Science* 2004, **303**:1364-1367.

22. •• Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K *et al.*: **Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots**. *Nature* 2005, **433**:527-531.

This and the previous paper [21] describe the identification of a putative cation channel as a component of the common sym pathway in *L. japonicus* and *M. truncatula*, respectively. In *L. japonicus*, the putative channel is encoded by a pair of similar genes, *CASTOR* and *POLLUX*, whereas the related legume *M. truncatula* has only one homolog, *DMI1*, indicating that *CASTOR* and *POLLUX* might have resulted from a gene duplication. Interestingly, CASTOR and POLLUX are expressed in plastids.

23. • Kanamori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EMH, Miwa H, Downie JA, James EK, Felle HH, Haaning LL *et al.*: **A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis**. *Proc Natl Acad Sci USA* 2006, **103**:359-364.

The authors describe the isolation of a new component of the common sym pathway, NUP133, which shows homology to proteins of the nuclear pore complex. Interestingly, the *nup133* mutant has no phenotype besides its defect in symbiosis, suggesting that NUP133 has a specific function in symbiosis. NUP133 might be involved in the translocation of a second messenger that is required for subsequent downstream events in the nucleus.

24. Levy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ané JM, Lauber E, Bisseling T *et al.*: **A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses**. *Science* 2004, **303**:1361-1364.

25. Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, Oldroyd GED, Long SR: **A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning**. *Proc Natl Acad Sci USA* 2004, **101**:4701-4705.

26. Karandashov V, Bucher M: **Symbiotic phosphate transport in arbuscular mycorrhizas**. *Trends Plant Sci* 2005, **10**:22-29.

27. Genre A, Bonfante P: **Building a mycorrhizal cell: how to reach compatibility between arbuscular mycorrhizal fungi**. *J Plant Interaction* 2005, **1**:3-13.

28. Dickson S, Kolesik P: **Visualisation of mycorrhizal fungal structures and quantification of their surface area and volume using laser scanning confocal microscopy**. *Mycorrhiza* 1999, **9**:205-213.

29. Genre A, Bonfante P: **Actin versus tubulin configuration in arbuscule-containing cells from mycorrhizal tobacco roots**. *New Phytol* 1998, **140**:745-752.

30. Fester T, Strack D, Hause B: **Reorganization of tobacco root plastids during arbuscule development**. *Planta* 2001, **213**:864-868.

31. Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M: **A phosphate transporter expressed in arbuscule-containing cells in potato**. *Nature* 2001, **414**:462-466.

32. Harrison MJ, Dewbre GR, Liu JY: **A phosphate transporter from *Medicago truncatula* involved in the acquisiton of phosphate released by arbuscular mycorrhizal fungi**. *Plant Cell* 2002, **14**:2413-2429.

33. Karandashov V, Nagy R, Wegmüller S, Amrhein N, Bucher M: **Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis**. *Proc Natl Acad Sci USA* 2004, **101**:6285-6290.

34. Ferrol N, Gianinazzi S, Gianinazzi-Person V: **Arbuscular mycorrhiza induced ATPases and membrane nutrient transport mechanisms**. In *Mycorrhizal Technology in Agriculture: from Genes to Bioproducts.* Edited by Gianinazzi S, Schüepp H, Barea JM, Haselwandter K. Birkhäuser; 2002:113-122.

35. Guttenberger M: **Arbuscules of vesicular-arbuscular mycorrhizal fungi inhabit an acidic compartment within plant roots**. *Planta* 2000, **211**:299-304.

36. Balestrini R, Lanfranco L: **Fungal and plant gene expression in arbuscular mycorrhizal symbiosis**. *Mycorrhiza* 2006, in press.

37. Breuninger M, Requena N: **Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage**. *Fungal Genet Biol* 2004, **41**:794-804.

38. Requena N, Breuninger M, Franken P, Ocón A: **Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the mycorrhizal fungus *Glomus mosseae***. *Plant Physiol* 2003, **132**:1540-1549.

39. Liu J, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ: **Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis**. *Plant Cell* 2003, **15**:2106-2123.

40. Brechenmacher L, Weidmann S, van Tuinen D, Chatagnier O, Gianinazzi S, Franken P, Gianinazzi-Pearson V: **Expression profiling of up-regulated plant and fungal genes in early and late stages of *Medicago truncatula–Glomus mosseae* interactions**. *Mycorrhiza* 2004, **14**:253-262.

41. Grunwald U, Nyamsuren O, Tamasloukht M, Lapopin L, Becker A, Mann P, Gianinazzi-Pearson V, Krajinski F, Franken P: **Identification of mycorrhiza-regulated genes with arbuscule development-related expression profile**. *Plant Mol Biol* 2004, **55**:553-566.

42. Hohnjec N, Vieweg ME, Puhler A, Becker A, Kuster H: **Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza**. *Plant Physiol* 2005, **137**:1283-1301.

43. Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ *et al.*: **Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis**. *Plant Cell* 2005, **17**:2217-2229.

44. Guimil S, Chang HS, Zhu T, Sesma A, Osbourn A, Roux C, Ionnidis V, Oakeley EJ, Docquier M, Descombes P *et al.*: **Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization.**. *Proc Natl Acad Sci USA* 2005, **102**:8066-8070.

45. Timonen S, Peterson RL: **Cytoskeleton in mycorrhizal symbiosis**. *Plant Soil* 2002, **244**:199-210.

46. Balestrini R, Bonfante P: **The interface compartment in arbuscular mycorrhizae: a special type of plant cell wall?** *Plant Biosyst* 2005, **139**:8-15.

47. Balestrini R, Perotto S, Gasverde E, Dahiya P, Guldmann LL, Brewin NJ, Bonfante P: **Transcription of a gene encoding a lectin-like glycoprotein is induced in root cells harboring arbuscular mycorrhizal fungi in *Pisum sativum***. *Mol Plant Microbe Interact* 1999, **12**:785-791.

48. van Buuren ML, Maldonado-Mendoza IE, Trieu AT, Blaylock LA, Harrison MJ: **Novel genes induced during an arbuscular mycorrhizal (AM) symbiosis formed between *Medicago truncatula* and *Glomus versiforme***. *Mol Plant Microbe Interact* 1999, **12**:171-181.

49. Gianinazzi-Pearson V, Arnould C, Oufattole M, Arango M, Gianinazzi S: **Differential activation of H^+-ATPase genes by an arbuscular mycorrhizal fungus in root cells of transgenic tobacco**. *Planta* 2000, **211**:609-613.

50. Journet EP, El-Gachtouli N, Vernoud V, de Billy F, Pichon M, Dedieu A, Arnould C, Morandi D, Barker DG, Gianinazzi-Pearson V: ***Medicago truncatula ENOD11*: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells**. *Mol Plant Microbe Interact* 2001, **14**:737-748.

51. Dumas-Gaudot E, Gollotte A, Cordier C, Gianinazzi S, Gianinazzi-Pearson V: **Modulation of host defence systems**. In *Arbuscular Mycorrhizas: Physiology and Function.* Edited by Kapulnik Y, Douds DDJ. Kluwer Academic Publishers; 2000:173-200.

52. Garcia-Garrido JM, Ocampo JA: **Regulation of the plant defence response in arbuscular mycorrhizal symbiosis**. *J Exp Bot* 2002, **53**:1377-1386.

53. Salzer P, Bonanomi A, Beyer K, Vögeli-Lange R, Aeschbacher RA, Lange J, Wiemken A, Kim D, Cook DR, Boller T: **Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation, and pathogen infection**. *Mol Plant Microbe Interact* 2000, **13**:763-777.

54. Elfstrand M, Feddermann N, Ineichen K, Nagaraj VJ, Wiemken A, Boller T, Salzer P: **Ectopic expression of the mycorrhiza-specific chitinase gene *Mtchit 3-3* in *Medicago truncatula* root-organ cultures stimulates spore germination of glomalean fungi**. *New Phytol* 2005, **167**:557-570.

55. Denarie J, Cullimore J: **Lipo-oligosaccharide nodulation factors — a minireview. New class of signaling molecules mediating recognition and morphogenesis**. *Cell* 1993, **74**:951-954.

56. Stougaard J: **Genetics and genomics of root symbiosis**. *Curr Opin Plant Biol* 2001, **4**:328-335.

57. Smith FA, Smith SE: **Tansley Review No. 96 Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses**. *New Phytol* 1997, **137**:373-388.

58. Morandi D, Sagan M, Prado-Vivant E, Duc G: **Influence of genes determining supernodulation on root colonization by the mycorrhizal fungus *Glomus mosseae* in *Pisum sativum* and *Medicago truncatula* mutants**. *Mycorrhiza* 2000, **10**:37-42.

59. Shrihari PC, Sakamoto K, Inubushi K, Akao S: **Interaction between supernodulating or non-nodulating mutants of**

soybean and two arbuscular mycorrhizal fungi. *Mycorrhiza* 2000, **10**:101-106.

60. Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F *et al.*: **Shoot control of root development and nodulation is mediated by a receptor-like kinase**. *Nature* 2002, **420**:422-426.

61. Nishimura R, Hayashi M, Wu GJ, Kouchi H, Imaizumi-Anraku H, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M *et al.*: **HAR1 mediates systemic regulation of symbiotic organ development**. *Nature* 2002, **420**:426-429.

62. Catford JG, Staehelin C, Lerat S, Piché Y, Vierheilig H: **Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors**. *J Exp Bot* 2003, **54**:1481-1487.

63. Amijee F, Tinker PB, Stribley DP: **The development of endomycorrhizal root systems. 7. A detailed study of effects of soil-phosphorus on colonization**. *New Phytol* 1989, **111**:435-446.

64. • Maeda D, Ashida K, Iguchi K, Chechetka SA, Hijikata A, Okusako Y, Deguchi Y, Izui K, Hata S: **Knockdown of an arbuscular mycorrhiza-inducible phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis**. *Plant Cell Physiol* 2006, **47**:807-817.
The phosphate transporters (PT) of plants occur in families that have members that are induced specifically during the AM symbiosis. The authors show that the RNAi-mediated knockdown of the AM-specific PT3 of *L. japonicus* (LjPT3) leads to a reduction in arbuscule formation. This shows that the plant can evaluate the efficiency of the symbiosis and limit fungal growth when it does not receive phosphate. A similar effect has been observed in *M. truncatula* (M Harrison, pers. comm.).

65. Sanders IR: **Ecology and evolution of multigenomic arbuscular mycorrhizal fungi**. *Am Nat* 2002, **160**:S128-S141.

66. Pawlowska TE: **Genetic processes in arbuscular mycorrhizal fungi**. *FEMS Microbiol Lett* 2005, **251**:185-192.

67. Kuhn G, Hijri M, Sanders IR: **Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi**. *Nature* 2001, **414**:745-748.

68. • Hijri M, Sanders IR: **Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei**. *Nature* 2005, **433**:160-163.
AM fungi show unexpected diversity in ribosomal and protein-encoding genes. It has been a matter of debate whether this diversity is due to divergent gene copies within individual nuclei (with the nuclei being all identical, a scenario called homokaryosis), or to divergent copies on different nuclei (causing the nuclei to be genetically different, called heterokaryosis). The authors show compelling evidence that supports a heterokaryotic organization of AM fungi.

69. Giovannetti M, Azzolini D, Citernesi AS: **Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi**. *Appl Environ Microbiol* 1999, **65**:5571-5575.

70. Bever JD, Wang M: **Arbuscular mycorrhizal fungi — hyphal fusion and multigenomic structure**. *Nature* 2005, **433**:E3-E4.