Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease?

Martin Parniske

Plant cells engage in mutualistic and parasitic endosymbioses with a wide variety of microoganisms, ranging from Gram-negative (*Rhizobium*, *Nostoc*) and Gram-positive bacteria (*Frankia*), to oomycetes (*Phytophthora*), Chytridiomycetes, Zygomycetes (arbuscular mycorrhizal fungi) and true fungi (*Erysiphe*, ascomycete; *Puccinia*, basidiomycete). Endosymbiosis is characterised by the 'symbiosome', a compartment within host cells in which the symbiotic microorganism is either partially or completely enclosed by a host-derived membrane. The analysis of plant mutants indicates that the genetic requirements for the interaction with rhizobia and arbuscular mycorrhiza fungi are partially overlapping. The extent to which plants use similar or identical developmental programs for the intracellular accommodation of different microorganisms is, however, not clear. For example, plant cells actively weaken their cell wall to facilitate bacterial colonisation, whereas penetration by fungal symbionts appears not to be assisted in this manner. Moreover, different transport requirements are imposed on the symbiotic interface of different interactions indicating that additional system-specific components are likely to exist.

Addresses
The Sainsbury Laboratory, Colney Lane, Norwich NR4 7UH, UK; e-mail: martin.parniske@bbsrc.ac.uk

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Abbreviations

AM	arbuscular mycorrhiza
PBM	peribacteroid membrane
PBS	peribacteroid space
SM	symbiosome membrane

Introduction

In its broadest sense, symbiosis refers to organisms living together, whether the interaction is mutualistic, commensal or parasitic. Plant endosymbioses are characterised by the penetration of living plant cells by a microbial symbiont, followed by a period during which the symbiont lives partially or entirely within plant cells (Figure 1). Endosymbiotic interactions play a significant role in agriculture and natural ecosystems. The most widespread interactions are formed between plants and fungi, producing the mildew and rust diseases and the mutualistic symbiosis of plant roots known as arbuscular mycorrhiza (AM). The fungi within these interactions are classified as 'biotrophic' because they rely on living plant tissue to support their growth. Biotrophic fungal pathogens are among the most devastating plant pests. The molecular analysis of plant diseases caused by biotrophic fungi has so far focussed on the characterisation of genes and pathways leading to resistance (see the review by Jeff Ellis *et al.* in this issue [1]). In contrast, surprisingly little is known about the molecular processes that allow these fungi to infect and exploit living plant cells during disease in compatible interactions.

Although the common feature of endosymbioses is the penetration of the plant cell wall and the apparent colonisation of the plant cell, the invading microorganisms are separated from the cytoplasm by a plant membrane, the symbiosome membrane (SM), which is, at least initially, *in continuum* with the plasmamembrane (Figure 1). Often, cell-wall-like matrix material is deposited between the SM and the microorganism. Thus, the microsymbiont may be intracellular, but it is always extracytoplasmatic because of the integrity of the SM. The invasion of plant cells opens up an exclusive niche, whereas the nutrients in the intercellular space of plant tissue can also be accessed by opportunistic competitors. Because the SM and the plant plasmamembrane are physically separated, their transport capabilities can be distinct. This potentially allows for vectorial nutrient flow, which is spatially confined to the site of microbial penetration.

The symbiosome: the unifying feature of endosymbioses

The term 'symbiosome' was originally suggested for the cellular compartment formed during endosymbiotic interactions in which the entire microorganism is taken up by the host cell and becomes completely engulfed by a host-derived membrane [2]. Owing to the homologies between different endosymbioses (Figure 1), it is also used in this review to encompass similar cellular compartments formed during interactions between plant cells and fungal hyphae, in which only a partial uptake of the microsymbiont occurs, but in which a symbiotic interface comprising a plant-derived perimicrobial membrane is formed. Following this definition, haustorial and arbuscular complexes are also symbiosomes (Figure 1).

During endosymbiosis, the engulfment of the microorganism by a plant-derived membrane occurs in a manner that resembles phagocytosis in animal cells [3]. In animal macrophages, material taken up by phagocytosis ends up in phagosomes and is usually digested: phagosomes fuse with lysosomes leading to the acidification of the resulting phagolysosomes and degradation of their contents [4]. Similarly, in plant endosymbioses with rhizobia and AM fungi, digestion of the microbe eventually occurs, but only after a significant period of symbiotic existence. The peribacteroid space (PBS) of soybean root nodules, that is, the space between the SM and the bacteroid harbours several hydrolytic enzymes including proteases and glycosidases.

The analogies between the PBS and lytic cell compartments have led to speculation that bacteroid survival depends on their ability to counteract their digestion. This view is supported by the premature degradation of bacteroids formed by some mutant rhizobia, whereas wild-type bacteroids only become digested during nodule senescence [5,6]. Small GTP-binding proteins have been implicated in vesicle trafficking and the control of vesicle membrane fusion. Expression of antisense constructs of *Rab1p* or *Rab7p* in infected cells of soybean nodules resulted in the fusion of symbiosomes with the vacuole and the degradation of bacteroids, suggesting that the small GTP-binding proteins encoded by these genes are critical in determining vesicle fate in infected nodule cells [7].

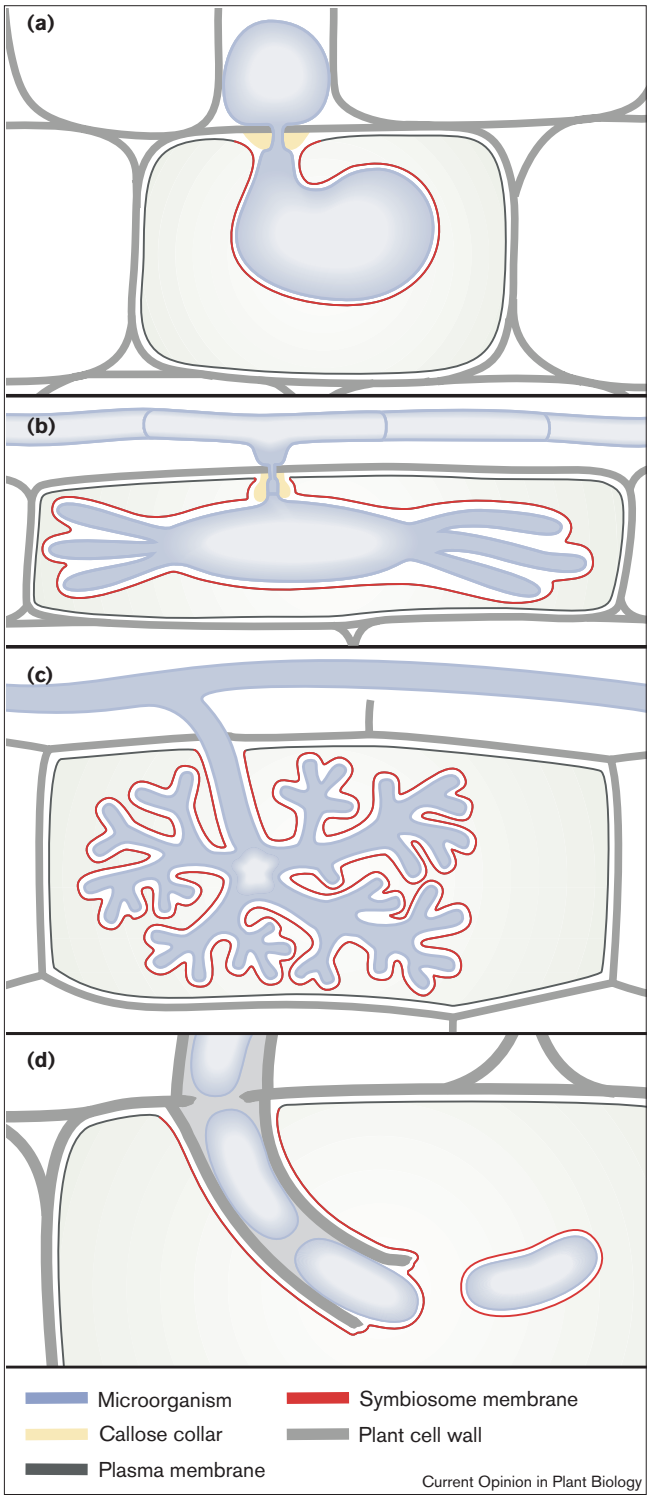
It is probable that other plant endosymbionts also live within a potentially lytic compartment. Arbuscules are ephemeral structures; after a relatively short life-span they disappear, and the infected host cell will eventually be able to host another arbuscule [8]. Arbuscule degradation presumably involves hydrolytic activities within the periarbuscular space. The signals of the plant endosymbionts that modulate symbiosome fate are of major interest. The lifestyle of plant endosymbionts within a potentially lytic compartment has striking similarities to some mammalian pathosystems. Although macrophages usually digest microorganisms upon phagocytosis, a large number of taxonomically diverse parasites have evolved mechanisms that allow them to live within so-called parasitophorous vacuoles without being digested by influencing the fate of the compartment that they inhabit [9]. The mechanisms employed to circumvent digestion are diverse [10]. For example, genes of *Legionella* have been identified that are required to modulate phagosome biogenesis to create an organelle in which intracellular growth is permitted. This is a phagosome-autonomous process because infected macrophages retain the ability to digest non-virulent microorganisms [11].

The SM is, at least initially, an invagination of the plasma membrane [12] and, therefore, it is not surprising that some typical plasma-membrane-associated enzymes are present within it. Indeed, a latent enzymatic activity of the peribacterial membrane (PBM) is 1,3-β-glucan (callose) synthase, a typical plasma membrane marker enzyme. No callose can, however, be detected within normal symbiosomes [13]; rhizobial exopolysaccharides inhibit this enzyme *in vitro*, suggesting they might be implicated in preventing callose accumulation in the PBS [14]. Callose is not deposited in the *Phytophthora* extrahaustorial matrix but is deposited in cell-wall appositions [15], suggesting that callose synthase activity is suppressed in the haustorium. These results indicate the significance of the suppression of host defence responses, other than digestion, at the symbiotic interface.

Evolution of plant endosymbioses

The ability of cells to take up microorganisms is as least as old as the event that gave rise to mitochondria within the

Figure 1



Examples of endosymbiotic interactions. **(a)** The haustorial complex of *Peronospora* (oomycete) within a leaf mesophyll cell. **(b)** The haustorial complex of *Erysyphe graminis* within a leaf epidermal cell. **(c)** The arbuscule of *Glomus sp.* within a root cortical cell. **(d)** An infection thread and bacteroid within a root nodule cell. The perimicrobial membrane (symbiosome membrane) is a unifying feature of endosymbioses and is of plant origin (shown in red).

proeukaryote approximately three billion years ago [16]. A relative of α-proteobacteria [17] was probably taken up by a phagocytosis-related process; instead of being digested, the resulting proto-mitochondrion became a partner within a stable relationship. It now seems certain that all plastid-bearing organisms can be traced back to one endosymbiotic event between a cyanobacterium-like ancestor and an eukaryotic phagotroph [18]. It is therefore probable that ancient cellular functions, that characterise the eukaryotic lineage, are employed by endosymbioses that evolved more recently. Many endosymbiotic relationships exist today with hosts belonging to all three eukaryotic kingdoms: plants, fungi and animals. But how ancient are plant endosymbioses and how do they relate to each other?

Plant–fungus endosymbioses probably existed before the colonisation of land

Zygomycetes of the order Glomales are the fungal partners of the AM symbiosis. Both fossil evidence and DNA-sequence divergence within the Glomales suggest that the AM symbiosis is about 400 million years old, coinciding with the plant's colonisation of land. Therefore, and because of the selective advantage that this symbiosis confers, the vast majority of all extant land plant species engage in this symbiosis (reviewed in [8,19]). According to fossil records, some of the earliest land plants also formed intracellular interactions with parasitic fungi resembling extant chytridiomycetes [20]. These ancient plant interactions with Zygomycetes and Chytrids were already quite complex, suggesting that they might be even older than the fossil record shows [20]. Land plants and red algae are very distantly related [18] and diverged before the colonisation of land; nevertheless, red algae also support the biotrophic haustoria of chytridiomycetes [21]. Therefore, assuming a single origin, the plant program for intracellular accommodation of fungi evolved very early in plant evolution. The radiation of ascomycetes and basidiomycetes, which include the modern powdery mildews and rusts, probably occurred later but fossil evidence of these organisms is sparse [22,23].

One important feature of fungal endosymbioses concerns plant cell entry. Plants do not appear to contribute actively to fungal penetration of the cell wall. Most fungi have to force their way through the cell wall to initiate endosymbiosis, in some cases encountering countermeasures, such as callose deposition, by the plant. Many biotrophic fungal leaf pathogens develop appressoria that can build up sufficient mechanical pressure to penetrate the plant cell wall [24], this is often combined with hydrolytic enzyme attack [25]. As for pathogenic fungi, there is no evidence that plants contribute to cell-wall passage by Glomales; indeed *Gigaspora* forms appressoria and penetration hyphae (which do not fully develop) [26] on isolated cell-walls. The non-involvement of plants in cell-wall penetration by fungi is in marked contrast to bacterial endosymbioses in which *Rhizobia* and *Frankia* rely on plant activity to cross the cell wall (see Figure 2 and below).

Evolution of plant–bacteria endosymbioses

Several mosses, the fern *Azolla* and one group of gymnosperms (the cycads) form nitrogen-fixing symbioses with cyanobacteria, but in these associations the bacteria do not infect the plant cells [27]. Endosymbioses between bacteria and plants are apparently restricted to the angiosperms: bacteria within symbiosomes are only found in the root nodule and the *Nostoc–Gunnera* symbioses. The earliest angiosperms date back to the early Cretaceous period and their radiation occurred 110 million years ago [28]. Therefore, the earliest bacterial–plant endosymbioses probably evolved several hundred million years later than the fungal ones.

The evolution of nitrogen-fixing root nodule symbioses encompasses two novel plant responses, namely plant cell-wall invasion and root nodule development, which seem to be independent achievements. Infection is likely to have evolved independently of nodule formation, as legumes harbouring infection threads but no root nodules have been observed. It has recently been reported that root cells of the tropical ornamental tree *Gleditsia* contain infection-thread-like structures that are inhabited by *Rhizobium*-like bacteria that do react with antibodies against nitrogenase. These infections are sometimes associated with inconspicuous swellings of the root but not with true nodules [29]. This phenomenon might be more widespread: only 26% of the examined Cesalpinioideae species are nodulating but many non-nodulating legume species have a high nitrogen-content and their roots exhibit nitrogenase activity [30]. Nevertheless, additional evidence is required to show that nitrogen-fixation occurs within infected cells of non-nodulating legumes and is not carried out by bacteria associated with the root surface. Mutations in plant genes such as *sym5* of pea, which allow infection-thread formation in the absence of nodule-development, are additional evidence of the independence of infection and nodule formation [31].

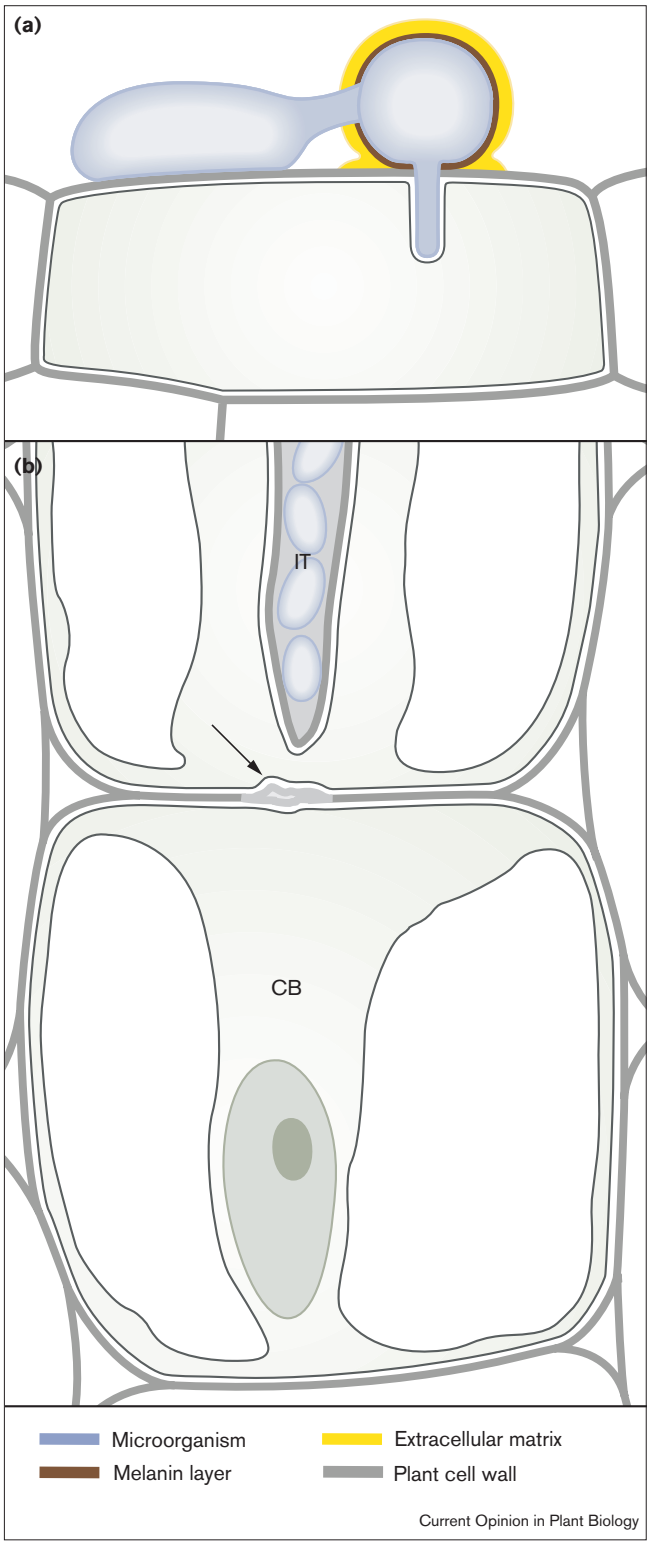
Root nodules are formed in symbioses between plants and Gram-positive *Frankia* or Gram-negative rhizobia [32]. Nodule formation has probably evolved several times independently, as nodule organogenesis and structure differs substantially not only between legumes and non-legumes but also within legumes. Moreover, nodulation occurs inconsistently with respect to phylogenetic relationships [33]. Nevertheless, all nodule-forming plants belong to the rosid I clade; their close relationship suggests that the predisposition to nodulate might have arisen only once [34,35]. It is tempting to speculate that the predisposition to nodulate involves the ability to take up bacteria through cell walls, as this phenomenon appears to be more widespread within the rosid I clade than the actual formation of nodules.

The active contribution of plants to cell-wall penetration during the formation of bacterial endosymbioses is an evolutionary novelty. In the legume–*Rhizobium* symbiosis, the plant itself weakens the cell wall at the site of

Figure 2 legend

Plant cell-wall penetration differs fundamentally among the evolutionarily ancient plant–fungus interactions and the more recently evolved plant–bacteria endosymbioses. **(a)** Fungi cross the plant cell wall using physical force in combination with hydrolytic enzyme attack. There is no evidence for an active contribution of the infected plant cell to that process. However, once the cell wall is negotiated, an accommodation structure is actively formed by the plant cell. **(b)** Cell-wall passage by rhizobia requires the activation of the division program of the plant cell. The growing infection thread (IT) crosses cell walls at preformed cell-wall weakenings (arrow) and subsequently grows within a cytoplasmic bridge (CB). Cell-wall lysis is a feature usually occurring along the cell-division plane. Cell division is not completed; however, endoreduplication within the nuclei of infected cells is likely to occur.

Figure 2



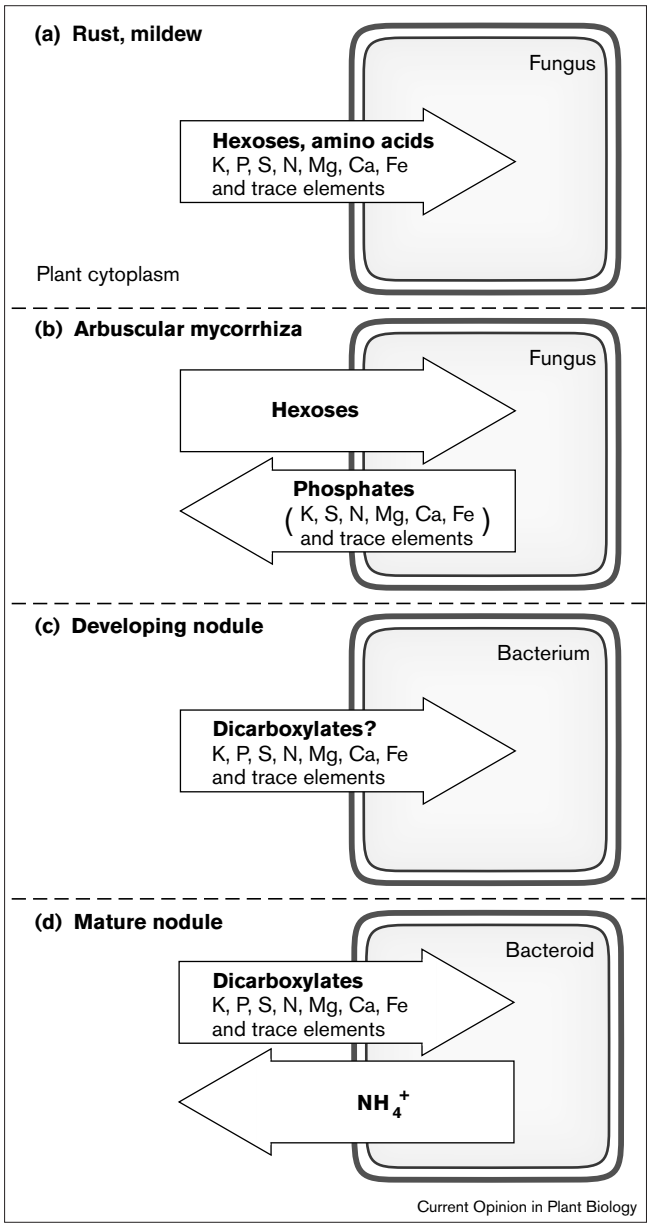
anticipated infection (Figure 2; [36]) by recruiting functions of the mitotic cell cycle. During cell division, the cell wall of the mother-cell is lysed along the division plane in order to separate the daughter-cells. In response to rhizobia, the cytoplasm of cortical cells aggregates to form phragmosome-like cytoplasmic bridges, the cell wall is locally weakened in the vicinity of the poles of these bridges (Figure 2). The bridges probably take part in infection-thread growth, directing its growth through the outer cortical cell layers towards the meristematic cells in the lower root cortex. Part of this program can be triggered in the absence of rhizobia by the addition of Nod-factors [37] and appears to involve the induction of a cell-division process that arrests subsequent to DNA replication and histone synthesis [38]. The cloning of *ccs52*, a plant gene involved in mitotic cyclin degradation and control of endoreduplication [39•], and the genes encoding *Rhizobium*-induced cell-wall hydrolysing enzymes [40] are important steps towards understanding the molecular basis of plant-cell entry by symbiotic bacteria.

The signals that trigger the uptake of rhizobia from the infection thread into SM-enclosed compartments are unknown (Figure 1d). *Salmonella* and *Shigella* use type-III secretion systems to inject specific effector proteins into the cytoplasm of the mammalian host, inducing dramatic cytoskeletal rearrangements and uptake of the pathogenic bacteria [9]. Type-III secretion systems have also been identified in rhizobia. Mutations in components of the type-III protein secretion system of the broad-host-range *Rhizobium spp.* NGR234 block the secretion of two proteins and strongly affect its ability to nodulate a variety of tropical legumes [41]. It will be interesting to identify the plant cellular functions that are affected by the injected rhizobial peptides.

Genetic commonalties between endosymbioses

Common evolutionary origins of different endosymbioses are possible. For example, the intracellular accommodation functions of the root nodule symbiosis could have evolved by recruiting functions of the much older AM symbiosis. Likewise, modern pathogenic rust and mildew fungi might exploit genetic programs similar or identical to those exploited by the more ancient chytrids. It is even possible that all plant endosymbioses share a common genetic program for the intercellular accommodation of microbes. Mutants that no longer support infection by both rhizobia and AM fungi were first isolated in pea and fababean [42], and subsequently in several other legume species. These

Figure 3



Hypothetical nutrient fluxes across the SMs (bold lines) of different endosymbioses. These nutrient fluxes are likely to be mediated by specific transporters within the SM. Therefore, SMs of different endosymbioses require different transporter composition.

mutants demonstrate a genetic overlap between these two endosymbioses that could also be involved in other symbiotic relationships (reviewed in [8,43]). Some of the mutated genes are probably involved in the regulation or development of the plant's endosymbiotic accommodation program. Consistent with this hypothesis, no perifungal membrane was detected in the epidermal cells of a *Ljsym4* mutant of *Lotus japonicus* that was infected with the AM fungus *Gigaspora margarita*. Likewise, this mutant does not form infection threads in response to rhizobia, although root-hair deformations are observed [44]. The use of legumes with small genome size such as *L. japonicus* or *Medicago truncatula* to isolate mutants that have impaired endosymbiotic interactions should facilitate the cloning of the genes involved in such interactions using positional cloning strategies [45,46]. Common functions between AM and the *Rhizobium* symbiosis are also suggested by the expression patterns of a growing list of genes and proteins (reviewed in [8,43]). Recent additions are first, a lectin-like glycoprotein (PsNLEC-1C) and the corresponding transcript, the expression of which is induced in both root symbioses [47,48] and second, the *L. japonicus LjCbp1* gene, which encodes a calcium-binding protein homologue that has been identified by promoter trapping and is induced in root hairs and nodules in response to *Rhizobium* in a *NodC*-dependent manner [49]. Strong GUS (β-glucuronidase) expression is also observed in root areas infected with *Glomus intraradices* (S Coomber, J Webb, M Parniske, unpublished data).

Surprisingly little is known about the plant genetic requirements for pathogenic interactions. For example, we do not know which plant genes are required for the accommodation of a rust or a powdery mildew haustorium and whether some are in common. Although structural similarities between fungal symbiotic interfaces have been recognised for a long time and functional comparisons have been reviewed [50], the genetic overlap of root symbioses with endosymbiotic pathogenic interactions has not been studied. Are the same plant genes involved in the formation of a haustorial and an arbuscular complex? The analysis of haustorial interfaces has largely employed cytological and biochemical approaches (reviewed in [50,51]). Comparisons between pathosystems have revealed significant differences in haustorium structure, one of the distinguishing features being the presence or absence of the 'neck band' that potentially seals the contents of the perihaustorial space from the extracellular space [52]. The morphology of the haustorium varies considerably among fungal–plant endosymbioses and appears to be determined by both plant and fungus (Figure 1). In contrast to the large number of published genes induced in the context of resistance responses, only a single gene, *fis-1* (*fungus induced sequence 1*) encoding a putative aldehyde dehydrogenase, which is specifically induced in flax leaves during a compatible interaction with the flax rust fungus *Melampsora lini*, has been described [53]. A number of *Arabidopsis* genes induced in a compatible interaction with the obligate biotrophic fungus *Peronospora parasitica* have been identified by cDNA-AFLP (amplified fragment length polymorphism) [54]. It will be rewarding to compare plant gene expression patterns among different endosymbioses using genome-scale transcriptome analysis to define potential overlaps in their genetic make-up.

Nutrient transport across the SM

Different symbiotic interfaces fulfil different functions and therefore require different sets of specific transporters (Figure 3). Biotrophic leaf pathogens are entirely dependent

on the host to provide all of their macro- and micronutrients, including carbon, nitrogen and phosphate as well as water. Being entirely surrounded by the host plant cell, rhizobia also rely on the host to provide all of their nutrients; before the onset of nitrogen fixation this also includes nitrogen sources (reviewed in [55]). In contrast to rhizobia, AM fungi have similar or even better access to the soil substratum that provides the plant with nutrients. Presumably, the flow of many ions and also water across the periarbuscular membrane is directed towards the plant. This flow is in the opposite direction to that across the SMs of other endosymbioses, and is probably achieved by a different set of transporters (Figure 3).

Transporters for phosphate-uptake by the plant have been postulated to be active in the periarbuscular membrane (Figure 3). cDNAs encoding phosphate-transporters have been cloned from *M. truncatula* roots. The expression of such cDNAs is suppressed in mycorrhizal roots, suggesting that the phosphate transporters that they encode are probably involved in non-symbiotic phosphate uptake by roots, rather than being localised in the periarbuscular membrane [56]. In other endosymbioses, phosphate-containing molecules have to be transported towards the microbial partner, again suggesting a different protein composition of the SMs (Figure 3).

It is generally accepted that the main energy sources consumed by rhizobia to fuel nitrogen fixation are dicarboxylates [55]. The carbon sources used by biotrophic pathogenic fungi might be different for rust and powdery mildew fungi. The cloning of a plasmamembrane-located putative amino-acid transporter from the rust fungus *Uromyces fabae* suggests that amino acids constitute at least part of its diet [57]. It is currently believed that *Erysiphe* uses hexoses rather than sucrose or organic acids [58] and that AM fungi take up glucose and fructose, but only within plant roots [59]. It therefore appears that in both the haustorial complexes of *Erysiphe* and AM, hexoses are transported towards the fungus (see also [50]). In interactions where extensive extracellular mycelia are formed within the host tissue, however, the extrahaustorial flow of nutrients between the symbiotic partners might also be significant [19,52].

Development of the SM

Endosymbiosis is accompanied by a massive membrane proliferation: an infected root nodule cell harbours a plasmamembrane area of 2800 μm^2 but an SM area of 21,500 μm^2 [12]. The biosynthesis of an SM is common to all endosymbioses and constitutes a core part of the accommodation program that is likely to provide a functional and genetic overlap between endosymbiotic systems. Nevertheless, the different transport requirements of SMs (Figure 3) should result in differences in protein composition in the various endosymbioses. In addition, the ATPase activity of the peribacteroid [60] and periarbuscular membranes is generally lacking from the SMs of pathogenic interactions (reviewed in [52,61]). Therefore, symbiosome membrane development can conceptually be dissected into two components, a common core program for the synthesis of the microbe-engulfing membrane and a specific program that determines the precise protein composition in each system.

It is conceivable that different endosymbionts can modulate the protein composition of the SM to adapt it to their specific requirements. The PBM protein pattern changes during nodule development [62], possibly as a reflection of the different transport requirements of the PBM during bacteroid maturation. The PBM protein composition appears to be modulated in response to signals emanating from the bacterial microsymbiont (reviewed in [61]). *Bradyrhizobium japonicum* possesses a bacteroid-specific respiratory chain [63], and mutants in components of this system do not develop into nitrogen-fixing bacteroids, probably because of their inability to adapt to the low-oxygen environment required. For example, the *B. japonicum* mutant T8-1 carries a transposon in a region homologous to the *cycH* gene, which encodes an inner-membrane-anchored periplasmic protein required for the formation of most c-type cytochromes [64]. Mutant T8-1 is not released from the infection thread [65]. Although PBM synthesis is not induced, the transcription of a plant gene encoding Nodulin 26, a normally PBM-localised protein, is induced in T8-1 infected nodules [65]. Another mutant of *B. japonicum*, strain 2960, which is defective in biogenesis of cytochromes, is also unable to develop into bacteroids [66]. This mutant carries a transposon insertion in the *cycW* gene, which encodes the membrane-bound subunit of an ABC-type transporter homologue with unknown substrate [67]. In contrast to T8-1, strain 2960 does induce PBM synthesis, resulting in the presence of host plant cells filled with empty PBM vesicles. Interestingly, the transcription of four genes encoding PBM nodulins is induced by strain 2960, although not the full set, which is induced by wild-type *B. japonicum* [68]. It is possible that T8-1 and 2960 induce the same subset of PBM nodulins, but that their gene expression has been analysed by different methods. Mutations in either of two signal peptidase genes of *B. japonicum* also result in altered PBM protein composition [69,70•]. The substrates of the signal peptidases have not been determined, but cytochrome *bc*1 of the bacteroid respiratory chain is probably post-translationally cleaved by a signal peptidase and is, therefore, a potential target [71]. These results indicate that PBM development can be uncoupled from bacteroid development. They also suggest that induction of the full protein complement of the PBM requires a different signal to the induction of membrane synthesis and that PBM protein composition is partly under the control of the microsymbiont. One of the most interesting challenges in this area is to identify the signals involved in PBM development and modification. Plant mutants in genes like *sym13*, *sym31*, *sym33* and *sym40* of pea that affect infection thread and PBM development are important tools in analysing these processes [47,72,73].

Conclusions

The endosymbiotic program is deeply rooted in the eukaryotic lineage. Therefore, the enormous variety of extant interactions is unlikely to have arisen from processes that have evolved completely independently. More likely, endosymbioses have evolved by exploiting some common core components. Some of these constitute basic cellular functions such as wall reorganisation, membrane synthesis and cytoskeleton rearrangements. These processes are likely to be similar if not identical between systems; in fact, accumulating evidence suggests that the AM and the root nodule symbioses of legumes build upon some common core components.

Nevertheless, some aspects of the development and physiology of endosymbioses differ significantly and require specific adaptations in different systems. The plasticity of the accommodation program has been documented in the root–nodule symbiosis and the identification of the molecular triggers that modify the endosymbiotic compartment will be interesting. Of high priority are mechanisms that prevent the symbiosome from turning into a lytic compartment. Will it be possible, by employing genetics, to switch the development of haustoria-bearing compartments to become lytic, thereby managing disease? Future research strategies will exploit functional genomics and genetics to define the minimal set of common plant functions and to explore system-specific differences. These will be important for the development of agrochemicals that target pathogen-specific functions of the accommodation program. Mutant screens that identify accommodation genes have to be carefully designed because the core program appears to overlap largely with functions that are indispensable for eukaryote survival. Nevertheless, the first mutants in *Arabidopsis* that are impaired in their interaction with *Erysiphe* have been isolated [74•].

The analysis of plant endosymbiosis has so far been spearheaded by work involving the legume-*Rhizobium* symbiosis. The interactions of plant cells with obligate biotrophic fungi has lagged behind because of obvious experimental restrictions. Nevertheless, stable transformation of *Erysiphe* [75•] and transient transformation of rusts [76] and AM fungi [77] has been achieved using ballistic DNA-delivery methods.

The formation of a whole new organ such as the root nodule is a very complex process, and transferring the ability to form nodules to non-legumes might prove difficult. However, as all major crop species form AM symbiosis, the basic accommodation functions are already in place. If nodule formation were not a prerequisite for nitrogen-fixation by bacteria within root cells, as is suggested by recent observations in non-nodulating legumes [29,30], and the accommodation program for AM fungi and rhizobia were indeed very similar, then this would have significant implications for the prospect of engineering non-legumes to successfully fix nitrogen through endosymbiosis. It may be that relatively few additional functions are required to trigger cell invasion by rhizobia or *Frankia* on non-leguminous crop plants. This view is further supported by the finding that a number of genes that are upregulated during the *Rhizobium*-symbiosis, for example *enod40*, have homologues in tobacco, maize and rice [78•]. The search for missing functions will be aided by technologies such as expression profiling of symbiosis-related plant genes [79], which, using micro-array-technology, will be possible on a genome-wide scale. The recent cloning of the first plant nodulation gene that is required for infection thread formation, *LjNin1* from *Lotus japonicus* [80••], is a breakthrough in that direction.

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