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At the root of plant symbioses: Untangling the genetic mechanisms behind mutualistic associations

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Introduction

The successful adaptation and diversification of plants in almost all biomes of our planet required the emergence of various functional innovations. One key step in plant diversification, and terrestrial life in general, took place 450MYA when the first land plant emerged from water and conquered the land [1]. Adaptation to land required plants to overcome new constraints such as UV radiation, drought, or nutrient limitations [2]. One of the key innovations has been the ability to form intimate intracellular mutualistic association with fungi, the arbuscular mycorrhiza symbiosis (AMS) [3]. In extant land plants, the ability to establish mutualistic interactions with microorganisms has diversified to include both intra- and extra-cellular association with various fungi and bacteria. The classical trade-off between partners consists of the exchange of the plant's carbohydrates for soil macro- and micro-nutrients scavenged by the microorganism. These associations can also confer tolerance against biotic and abiotic stresses [4]. In natura, interactions often involve multiple microbial partners, and it has been demonstrated that partners can control the amount of transferred resources [5]. With the era of bioinformatics, molecular, and genetic studies, new light has been shed on the underlying genetic mechanisms regulating plants-microorganism interactions. In this review we aim to summarize the recent advances made in our understanding of these mutualistic plant-microbe interactions and their evolution.

Intracellular symbioses: an ancestral origin and shared signaling pathway

Intracellular symbioses are the most intimate interactions where microorganisms penetrate within the cell host where exchanges occur. In the last decade, multiple genomes and transcriptomes have been produced covering the different symbiotic statuses of plants among nonvascular plants and Streptophyte algae [6-11]. However, genomic coverage of plants outside Angiosperms remains scarce and this should be addressed in the coming sequencing projects. In 2015, Delaux et al. found that all genes required for AMS were present in the common ancestor of all land plants and, for a few of them, in Streptophyte algae, suggesting the ancestral character of the ability to engage this symbiosis (Figure 1a) [12]. Later on, a phylogenomic study based on a large-scale analysis (including more than 300 plant species) covering the diversity of beneficial intracellular symbioses identified three genes (SymRK, CCaMK, and CYCLOPS) present only in land plants able to form intracellular mutualistic associations [13]. Their functional analysis confirmed the conservation of their biochemical properties between vascular and non-vascular plants [13]. Upstream and downstream of this universal pathway are genetic mechanisms specific or partially shared between the different types of intracellular symbioses. These mechanisms are, to date, mainly genetically characterized for AMS and nitrogen-fixing nodule (NFN) symbiosis, whereas their actual function in other mutualistic symbioses, such as the orchidoid, ericoid, or jungermanniales mycorrhizae, remains elusive, paving the road for new scientific discoveries. Processes that allowed the emergence of the symbiotic genes are still poorly characterized but the recent analysis of Zygnematophyceae genomes, the closest algae relative to land plants, revealed the presence of GRAS transcription factors deriving from horizontal gene transfer from bacteria (Figure 1a) [10]. The GRAS family underwent multiple rounds of duplication in land plants leading to the emergence of crucial genes for mutualistic symbioses such as RAD1, NSP1, and NSP2 or DELLA [10,14-17].

The arbuscular mycorrhizal symbiosis relies on genetic processes conserved in land plants

AMS is considered the most ancient intracellular interaction with fossil data dated back to ca. 400 MYA [18]. In the last three years, two processes of the AMS have been demonstrated as regulated by conserved genetic mechanisms in land plants: the molecular dialogue between partners and the transfer of lipids from the host to the fungus. The molecular dialogue starts with the release of strigolactones (SLs) by the plants [19]. These hormones, synthesized through key genes D27, CCD7, CCD8, and MAX1, act as rhizosphere signals and developmental regulators (reviewed in Ref. [20]). In 2022, a new SL compound called BSB, required for AMS was discovered through the biochemical characterization of the rhizoid exudates of the AMS-forming non-vascular plant *Marchantia paleacea* [21]. In *M. paleacea*, BSB does not play any role in the developmental processes since non-producing BSB mutants did not exhibit abnormal phenotypes or gene expression profiles [21]. Investigation of BSB in other species revealed its presence in other non-vascular plants, ferns, and even seed plants [21]. This pattern associated with its role during AMS in *M. paleacea* places BSB as a likely ancestral SL in land plants, inducing AMS (Figure 1b, left panel). In addition, studies identified that under phosphate starvation, the flowering plants SL transporter PDR1 and its homologs in non-vascular plants are upregulated to increase the release of SL in the rhizosphere [22,23]. Taken together, these results support an ancestral origin in land plants of the SL biosynthesis and transport to enable AMS.

Arbuscular mycorrhizal fungi (AMF) are lipid auxotroph since the Fatty Acid Synthase (FAS) encoding gene has been lost in their common ancestor [24,25] and then rely exclusively on the plant host to acquire this precious resource [26,27]. The central role of lipids in the AMS establishment and in the regulation of its development appears as an essential key point and benefited from significant advances in recent years (reviewed in Ref. [28]). From the genetic perspective, phylogenomic comparison of AMS-forming and their AMS-incapable counterparts among flowering plants associated with transcriptomic analysis identified multiple genes involved in the lipid metabolism during AMS [7,27,29-31]. Briefly, lipids are synthesized by the plants through a pathway composed by KAS, FatM, and RAM2 genes then likely transferred to the AMF by STR and STR2 ABC transporters [29,31,32] and/or via parallel processes such as exocytotic vesicles as recently suggested by Ref. [33]. The transcriptomic response of the non-vascular plant *M. paleacea* and five other flowering plants during AMS were compared using phylotranscriptomics, a method that combines phylogenomic and multi-species transcriptomic data. This comparison led to the identification of a shared response among these species [7]. These common upregulated genes during AMS in evolutionarily distant species fulfill various functions such as SL biosynthesis, infection, or lipid metabolism regulation. Among these genes, the transcription factor WRINKED (WRI) arose as an ancestral lipid transfer regulator. Indeed, it emerged from duplication before the land plants divergence and is absent in algal genome. In Angiosperms, it underwent multiple rounds of duplication leading to different paralogs, some of which are involved in the lipid metabolism regulation during AMS [30,34]. Functional analysis of the single WRI copy in *M. paleacea* revealed its crucial role in the regulation of lipid production and transfer to AMF [7].

Combined, the analysis of SL biosynthesis and regulation of lipid metabolism pathway revealed that they are shared among evolutionary distant land plants and likely emerged in their common ancestor at the time of land conquest. These results bring the first genetic evidence of the ancestral character of AMS in land plants.

Nitrogen-fixing nodulation symbiosis: an ancestral origin driven by transcriptomic rewiring

Besides AMS, the NFN symbiosis is the first most studied intracellular symbiosis. The NFN symbiosis is restricted to four orders of flowering plants (Cucurbitales, Fabales, Fagales, and Rosales) forming a monophyletic clade. The evolution of NFN symbiosis has recently intensified between two scenarios: (1) a convergent evolution and (2) an ancestral gain followed by multiple losses. Phylogenomics brought the first genetic evidence in favor of the ancestral gain hypothesis [35-37], while the convergent evolution hypothesis is based on ancestral state reconstruction method. The latter recently proposed a scenario where the NFN symbiosis arose from a precursor ancestral state followed by shifts in evolutionary rate leading to the multiple emergences of NFN symbiosis [38].

To further explore the evolution of NFN symbiosis a recent study performed a phylotranscriptomics analysis. This study included the expression data of nine species belonging to three of the four NFN clades during NFN symbiosis [39]. This approach led to the identification of a common gene set likely deregulated during the NFN symbiosis in the common ancestor of the NFN clade. All the essential genes

needed for a functional association were retrieved in this approximative ancestral transcriptome, bringing new genetic evidence for the ancestral gain hypothesis. The integration of expression data from other processes (AMS and lateral root formation) and an experimental evolution assay identified that 46% of genes with an NFN-transcriptomic response were co-opted from other processes (Figure 1b, right panel). These findings are also supported by recent functional analyses that demonstrated the effective recruitment of lateral program by the NFN-symbiosis master regulator NIN [40]. Subsequent investigations of the transcriptome at different evolutionary nodes of NFN clade also support the recently proposed hypothesis of a convergent evolution of symbiosome release (a structure which hosts bacteria in the Mimosoid and Papilionoid subfamilies) and could be associated with the remarkable evolutionary stability of the NFN symbiosis in these legume sub-families [39,41]. There are no identified genetic causes explaining such rewiring yet. However, non-coding regions could hold part of the answer. Indeed, regulation of traits such as mutualistic interactions also relies on non-coding RNA and cis-regulatory elements. Such elements have been identified as associated with both AMS and NFN symbioses [42-46], with the striking example of the CYCLOPS response element in the promoter of master regulator NIN. It likely emerged in the common ancestor of NFN clade to connect CYCLOPS from the universal signaling pathway with NIN and play a role during the establishment of the interaction [47,48]. Such findings urge for a more systematic investigation of non-coding regions and their role in regulating mutualistic interactions.

Altogether, these analyses support the hypothesis of an ancestral gain in the common ancestor of NFN clade followed by multiple losses and functional optimization in the extant nodulating species.

Not only the intracellular associations matter: the extracellular interactions

Although less studied than AMS or NFN symbiosis, extracellular associations such as ectomycorrhizal (EcM) or lichens (fungi-chlorophyte algae) are essential in natural ecosystems. Contrary to intracellular symbiosis, both EcM and lichens symbioses derive from convergent evolution in both the plant and fungal partners (Figure 1a) [49-54]. Although most of the data have been generated from the fungal partner side, recent findings have shed new light on their evolution and genetic regulation. Among chlorophyte algae, only two classes, namely Trebouxiophyceae and Ulvophyceae, contain species able to form lichen associations. Ancestral state reconstruction and phylogenomic approaches inferred at least one gain in these both classes for the lichenization [52,55,56]. In Trebouxiophyceae, expansions of gene families involved in carbohydrate metabolism or stress tolerance as well as the upregulation of desiccation-related have been found associated with lichens-forming algae [56,57]. In addition, Trebouxiophyceae able to lichenize harbor a glycoside hydrolase (GH8) able to degrade lichen polymers that have been acquired through horizontal gene transfer and could play a role in the membrane remodeling to facilitate the interaction between partners, which is consistent with the thinner membrane at the contact point [56,58].

Ectomycorrhiza symbiosis is restricted to 30 monophyletic seed plant lineages (Figure 1a) [50], and remains largely unknown from the genetic perspective. Driven by its model association between *Populus trichocarpa* and *Laccaria bicolor*, mechanisms regulating the EcM symbiosis have been proposed (for a review see Ref. [59]). Among the most striking recent discoveries, one can note the role of a G-type lectin receptor [60] or the possible involvement of the intracellular universal pathway (SymRK, CCaMK, CYCLOPS) [61]. However, this latter point needs further investigation since many EcMcapable species lack this common pathway and impairment of this pathway in *P. trichocarpa* only results in a partial defect in EcM establishment [61]. Recently the expression analysis of *Eucalyptus grandis* roots colonized by various isolates of the fungus *Pisolithus microcarpus* found that the transfer of carbon from plant to fungus involved defense as well as stress-related genes of the host [62]. From the non-coding region perspective, another recent study discovered that hypomethylated mutants of *Populus tremula* x *Populus alba* exhibit a lower mycorrhiza formation rate compared to the wild type [63]. As for the other symbioses, this encourages a broader investigation of the non-coding region's role in the regulation of mutualistic interactions. The evolution of EcM in fungi is also convergent and associated with major genomic rearrangements linked to transposable elements expansions, the reduction of the CAZymes repertoire, and the implication of small secreted proteins in the symbiosis establishment [49,64,65].

Both lichens and EcM symbioses derive from convergent evolution and have been mainly studied through the prism of the fungal partner and species-specific approaches that started to uncover the genetic mechanisms behind these interactions. Both of them offer a lot to discover and will benefit from large-scale without a priori phylogenomic comparisons to better understand their evolution and regulation.

Conclusion & perspectives

Plant-microbe interactions continuously shape the adaptation and diversification of both partners. With the era of genomics and integrative biology, mechanisms underpinning these associations have been extensively studied at a scale never reached before. It has especially shed light on the different evolutionary trajectories of these interactions and characterized different processes controlling their emergence and evolution. However, gaps in our knowledge of these processes remain and the recent progress also opens the door to new questions and research opportunities.

Most of the studies focused on bipartite systems (one plant, one symbiont) and gave an unprecedented understanding of these interactions although a lot of questions remain open. However, plants have to deal with a great diversity of microbes, including non-cultivable ones, and many studies pointed out the variability of beneficial associations even at the intrapopulation or individual levels [66-69]. To address this limitation, a rising number of studies explored the whole environment using meta-genomics and transcriptomics, but technical barriers remain.

In addition, mutualism is the result of multipartite associations that infer on the outcome of the interaction. For example, specific bacterial communities have been found in the extraradical hyphae of AMF [70,71]. Thus, understanding the genetic mechanisms regulating the complexity of these associations appears as a crucial challenge, especially in the urge to develop a more sustainable agriculture.

In mutualistic intracellular symbioses, the up and downstream genes of the common signaling pathway are only characterized in the AM and NFN symbioses while they are unknown in other interactions such as ericoid, orchidoid, or jungermanniales mycorrhization. Thus, exploring the genetic components surrounding the common signaling pathway appears as a logical perspective that will require new data to be generated for these symbioses. In addition, investigating how plants unable to engage intracellular mutualistic symbioses such as Brassicaceae or Caryophyllaceae compensate for the absence of symbionts could open useful discoveries for environmentally responsible agricultural practices.

Finally, the capacity to engage in mutualistic symbioses with microbes is not restricted to the plant kingdom. Indeed, these interactions can be retrieved in arthropods, cnidarians, vertebrates, and fungi [72,73]. These associations are diverse in terms of regulation, symbiont transmission, and the benefits obtained by both or one of the partners and shape their evolution and diversification [74,75]. Although some genetic mechanisms have started to be discovered across these kingdoms [76-78], available knowledge remains scarce. In addition, some aspects such as partner recognition, exchange regulation, or even the formation of a dedicated organ seem to be shared. Thus, comparing intracellular mechanisms at a trans-kingdom level could enable the discovery of shared rules. This opens broad new perspectives in our understanding of organism-organism interactions in the entire tree of life.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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