7 Genetics and Genomics Decipher Partner Biology in Arbuscular Mycorrhizas

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CONTENTS

I. Introduction

Arbuscular mycorrhizas (AMs) are often defined as the most widespread plant symbiosis: 72% of vascular plants (Brundrett and Tedersoo 2018) interact at root level with a group of early-diverging fungi, **Glomeromycotina**, originating a symbiosis which is not detectable at naked eye, but has deep consequences on a global scale, from nutrient cycles and soil structure to plant health, photosynthesis, and productivity. In addition, several non-vascular plants, including many bryophytes, also host Glomeromycotina in the cells of their haploid thalli. Being able to colonize both sporophytes and gametophytes, AM fungi are therefore central for land plant biology, making AM symbiosis a major scientific topic in diverse fields, from mycology to botany, microbiology, ecology, agronomy, and bioinformatics, also involving modeling and economics studies. For this reason it is not surprising to obtain millions of hits when entering the keywords *arbuscular mycorrhizas* in any search engine on the World Wide Web (December 2018). However, adding the term *Genetics* strongly reduces the number of hits, and the scientific papers that have both *Genetics and AMs* among their keywords are only a few. This scenario mirrors the history of mycorrhiza studies: while the so-called endotrophic mycorrhizas have been discovered and then studied since the end of the nineteenth century (Bonfante 2018), application of molecular techniques to AMs required the advent of PCR (Mullis 1990), almost exactly 100 years later. The first papers reporting the application of this powerful tool to mycorrhizal research were focused on the development of molecular probes based on RNA ribosomal genes and aimed at the identification of fungal symbionts in ectomycorrhizas (White et al. 1990; Gardes and Bruns 1993). Only later, molecular tools as well as -omics approaches were successfully applied to AM fungi, originating in the two main trajectories (Ferlian et al. 2018) that still characterize mycorrhizal studies: on the one hand, molecular approaches have provided a wealth of information on the ecology, distribution, and diversity of AM fungi; on the other hand, they have represented the starting point to decipher the molecular mechanisms underlying plant-fungal interactions.

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Differently from model fungi (*Neurospora*, *Aspergillus*) and plants (*Arabidopsis*), the **genetics of arbuscular mycorrhizas**, intended as the sum of interacting plants and fungi, **is** therefore **a very recent domain of science**. Parniske (2004) was one of the first to use the term genetics in his highly quoted review "Molecular genetics of the arbuscular mycorrhizal symbiosis."

In this context, the aim of this chapter is to provide a review of the multiple interactions that are included in the term "arbuscular mycorrhizas" and present an updated view of our knowledge on the molecular genetics of AMs, covering the genomes of AM fungi, the cellular and molecular responses of the host plant, as well as the fungal and plant natural variation that contributes to the outcome of this fascinating interaction.

II. A New Look at the Interacting Partners: From Two to Many

Arbuscular mycorrhizas (AMs) are traditionally described as the symbiosis resulting from the interaction between the roots of land plants and soil fungi. Even if AM host plants can survive if deprived of their fungal symbionts, this condition is virtually unknown in natural ecosystems, where AM fungi are associated as helper microorganisms in most of the environments so far investigated (Davison et al. 2015, 2018). On the other hand, **AM fungi are still considered to be unculturable in the absence of their host: being auxotrophic for lipids** (Jiang et al. 2017; Luginbuehl et al. 2017; Keymer et al. 2017), they strictly depend on their green hosts for growth and reproduction, which gives them the status of obligate biotrophs. From an evolutionary point of view, the ecological success of AM fungi demonstrates that the advantages of such a strict association with plants have overcome the risks arising from the loss of saprotrophic capabilities (Bonfante and Genre 2010). Recent findings have however demonstrated that the interaction is more complex than a *one-to-one* relationship, since **roots may simultaneously host many AM fungi and also other microbes**. In addition to the Glomeromycotina, some members of the related subphylum of Mucoromycotina (Spatafora et al. 2016) have been demonstrated to colonize early-diverging plants, i.e. liverworts and hornworts, and lycophytes (Bidartondo et al. 2011; Desirò et al. 2013; Rimington et al. 2015). In addition, a typology of AM fungi, usually identified as *Glomus tenuis* (the fine endophyte), has been recently suggested to be related with the Mucoromycotina (Orchard et al. 2017), broadening therefore the taxonomic spectrum of aseptate fungi which colonize land plants.

The colonization process of AM fungi belonging to the Glomeromycotina has been described in detail by using light and electron microscopy (Bonfante 2018), while more recent information is based on the use of in vivo confocal microscopy (McLean et al. 2017; Lanfranco et al. 2018; Pimprikar and Gutjahr 2018). By contrast, the colonization processes by Mucoromycotina are still to be defined: these fungi may establish different interactions with plants; some of them also establish ectomycorrhizas (Fassi et al. 1969) and have been detected mostly by using molecular tools, while morphology suggests that they form characteristic intracellular swellings (Bidartondo et al. 2011). In the light of the hypothesis that *G. tenuis* is actually belonging to Mucoromycotina, a few observations dating back to the 1980s described how the cellular features of these fungi and their interaction with the plant host are impressively similar to those of Glomeromycotina (Gianinazzi-Pearson et al. 1981). Since many reports revealed that **a single plant may be simultaneously colonized by both Mucoromycotina and Glomeromycotina** (Desirò et al. 2013), it will be crucial to develop cellular tools to clearly distinguish between the two fungal subphyla during their growth *in planta*. Lastly, **many AM fungi host obligate endobacteria** which live inside their cytoplasm and have an impact on fungal biology (Bonfante and Desirò 2017; Salvioli et al. 2016). **The bacteria living in Glomeromycotina appear to be common to many Mucoromycota**, since related microbes have been detected in *Rhizopus*, *Mortierella*, as well as *Endogone* species, suggesting that their presence predates the

Mucoromycota divergence (Bonfante and Desirò 2017).

On the basis of the current data, Glomeromycotina can therefore be defined as a stable component of the plant microbiota, since they are found in most of the environments so far investigated (Davison et al. 2015, 2018), but on the other hand, they also host their own microbiota, given by the intracellular endobacteria as well as by the bacteria which are commonly associated to the surface of their extraradical hyphae (Turrini et al. 2018).

Interestingly, the molecular investigations, including the exploitation of transcriptomics data, have also allowed the description of **viral sequences hosted within AM fungi** (Turina et al. 2018). Mycoviruses can therefore be considered an additional component of the AM microbiome with the potential to influence the biology of AM fungi and their host plant (Ikeda et al. 2012).

III. Lessons from the Genome Sequencing of AM Fungi

Our knowledge of the AM symbiosis mainly mirrors a plant-centric view. This is due to (1) the obligate biotrophic status of Glomeromycotina, which cannot be cultivated in the absence of their host plants; (2) their multinuclear condition, i.e., hundreds of nuclei coexist within one continuous cytoplasm; and (3) the absence of observable sexual reproduction and a uninucleated life stage (Chen et al. 2018). All these aspects hamper the use of the classical genetic tools which have, by contrast, allowed to study model fungi like *Neurospora* or *Aspergillus*, or their host plants which offer genetically tractable systems. In addition, protocols to obtain a stable genetic transformation of AM fungi are not yet available.

In the first decade of the new century, the development of -omics approaches and the first sequencing of an AM fungal genome have offered novel groundbreaking insights in their biology. However, achieving **the first sequenced genome of a Glomeromycotina, *Rhizophagus irregularis***, was not an easy task and required many years (Martin et al. 2004; Tisserant et al. 2013). The strain DAOM-197198 was selected for several reasons: it had been hypothesized to possess a very small genome; it easily grows in association with root organ cultures, producing a large amount of non-contaminated fungal material; and – as a last key feature – it does not host endobacteria, thus representing a potentially more amenable scenario. The sequence of its 153-Mb haploid genome showed a repertoire of about 30,000 genes and revealed a low level of polymorphism offering for the first time a reply to the crucial question: do the nuclei of AM fungi possess multiple, highly diverged genomes? The data strongly suggested the inconsistency of such a hypothesis, which was also elegantly refuted by the whole sequence of isolated single nuclei (Lin et al. 2014). Mating (MAT)-related genes were found to be expanded, suggesting the **existence of cryptic sex-related processes** and opening the possibility that a non-observable mating does not mean absence of sex. Genomic analyses of several *R. irregularis* isolates clearly proved that some strains are **homokaryotic** (containing genetically identical nuclei with one putative MAT locus) while other strains are **dikaryotic** (harboring two distinct nuclear genotypes each with a different MAT locus; Ropars et al. 2016; Corradi and Brachmann 2017). Moreover, Chen et al. (2018), following the single-nucleus sequencing approach (Fig. 1), demonstrated that nuclei with distinct genotypes in their MAT alleles can undergo recombination, originating genetic diversity. Despite evidence of recombination, however, **clonality still appears to be the prevalent mode of reproduction** (Chen et al. 2018).

A. The Biotrophism of AM Fungi

The expectations of the researchers involved in genome sequencing of *Rhizophagus irregularis* were first focused on another crucial question: why are AM fungi unculturable? At a first glance, their obligate biotrophy was not explained by genome erosion or any related loss of metabolic complexity in central metabolism. Only later it was clear that **AM fungi do**

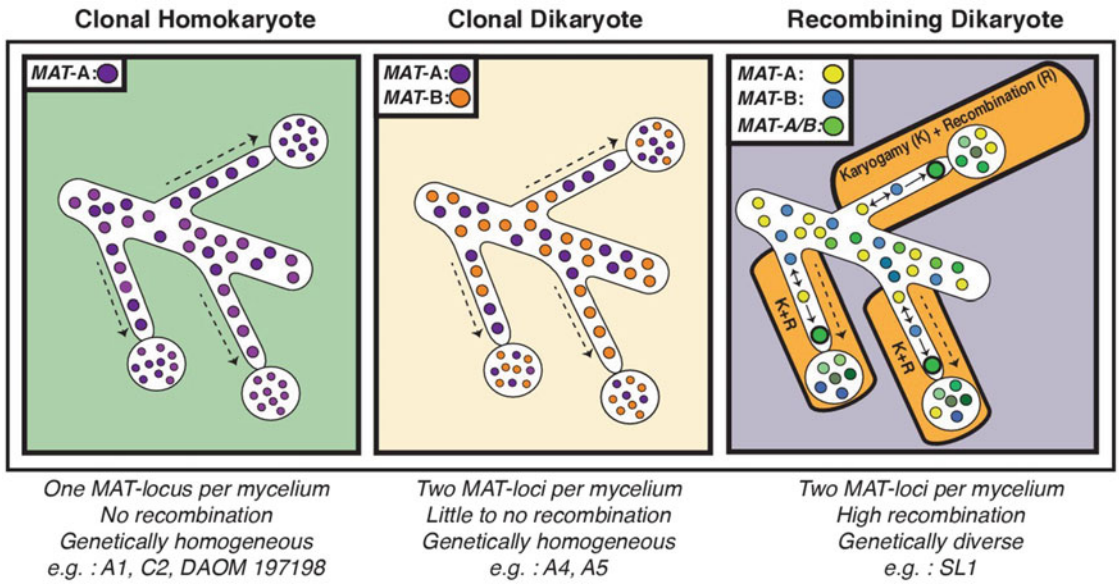

Fig. 1 Schematic representation of the three genome organizations found to date in the model AM fungus *Rhizophagus irregularis*. Left: Most isolates analyzed using genome analysis and PCR targeted to the MAT locus have been found to carry nuclei with the same MAT locus. In these isolates, genetic variability is lower than dikaryotic relatives, and recombination is undetectable. Middle: The *R. irregularis* isolates A4 and A5 carry nuclei with two distinct MAT loci. Evidence for recombination is very rare, and two divergent genotypes appear to coexist in the cytoplasm. Right: In some cases, strains can harbor nuclei with two distinct MAT loci that undergo frequent karyogamy. The frequency of karyogamy increases nuclear diversity within the mycelium. From Chen et al. (2018), licensed under CC BY 2.0 (https://creativecommons.org/licenses/by/2.0/)

not possess fatty acid synthetases (FAS; Wewer et al. 2014), resulting to be auxotroph for this essential group of compounds which are totally received from the host plant (Luginbuehl et al. 2017; Jiang et al. 2017; Keymer et al. 2017). Interestingly, Tisserant et al. (2013) firstly pointed to the **almost total lack of genes encoding plant cell wall-degrading enzymes** as well as of genes involved in toxin and thiamine synthesis. The limited number of cell wall-degrading enzymes is currently considered one of the signatures of AM fungal genomes, since this feature has been found in all other genomes sequenced later: *R. clarus* (Kobayashi et al. 2018), *Rhizophagus cerebriforme*, and *Rhizophagus diaphanum* (Morin et al. 2018) all belonging to the same *Rhizophagus* genus, as well as members of the Diversisporales, *Diversispora epigea* (Sun et al. 2018), *Gigaspora rosea* (Morin et al. 2018), and *Gigaspora margarita* (Venice et al. 2019). Taken in the whole, these results highlight a still unsolved question: how do AM fungi penetrate within their host tissue, being for a large part of their life cycle intracellular endophytes? How do they cross the plant cell wall? On one hand, the limited number of such hydrolytic enzymes well fits with the life strategy of AM fungi: they do not activate plant defenses, since they have to keep their host alive and prone to accommodate the fungus, but, on the other hand, they enter the root cells probably thanks to a finely tuned regulation of the plant metabolism. Among the battery of mycorrhiza-induced secreted proteins which are mainly expressed in symbiotic tissues (Tisserant et al. 2013; Sędzielewska Toro and Brachmann 2016), some of them can act as effectors which might manipulate plant regulatory pathways (see next paragraph). It can be hypothesized that some effectors may induce the weakening and swelling of plant cell walls, which may compensate for the absence of hydrolytic enzymes and is indeed observed by

morphological analyses (Balestrini and Bonfante 2014).

The repertoire of *R. irregularis* genes has therefore provided an excellent basis for understanding not only the fungal biology but also the genetics mechanisms underlying the AM symbiosis. The genomes of AM fungi which have been recently sequenced thanks to the impressive advances in DNA sequencing technologies have provided the opportunity to develop **comparative genomics analyses** (Morin et al. 2018; Venice et al. 2019). The genome size of AM fungi can be very diverse, ranging from about 150 Mb of *Rhizophagus* species (Chen et al. 2018) to 600 Mb of *G. rosea* and 784 Mb of *G. margarita*. The genome expansion seems to be strictly correlated with the presence of **transposable elements** (TE) which, in the case of *G. margarita*, represent more than 80% of the whole genome. Interestingly, also ectomycorrhizal fungi belonging to the truffle groups and characterized by the production of hypogeous fruitbodies have genomes very large and rich in TE (Murat et al. 2018).

Differently from Glomeromycotina, the ecological role of their sister group, Mucoromycotina, is much more enigmatic. In the order Endogonales, the family Endogonaceae contains some ectomycorrhizal fungi producing small hypogeous truffle-like fruitbodies (Fassi et al. 1969; Yamamoto et al. 2017), while the other family, Densosporaceae, groups fungi that live associated to early-diverging plants (Desirò et al. 2013; Rimington et al. 2015) as well as the fine root endophytes (Orchard et al. 2017). Remarkably, the genome sequencing of four Endogonaceae fungi (Chang et al. 2018) has detected the symbiotic signatures already identified in the other mycorrhizal fungi such as large genome size, high repetitive DNA content, and low diversity of plant cell wall-degrading enzymes but without elevated small secreted proteins/secretome ratios. Notwithstanding the absence of fungi belonging to the Archeosporaceae, all the genomes of these mycorrhizal Mucoromycota fungi share similar features. AM fungi, however, have some further specific traits which well explain their unculturability (lipid auxotrophism), their TE abundance (TE burst), their high compatibility with host plants (strong reduction of cell wall-degrading CAZymes), and expansion of some metabolic pathways (chitin synthesis and degradation) allowing therefore a fine-tuning of the molecular dialogue with their host (Venice et al. 2019).

The genomes of *D. epigea* (Sun et al. 2018), *G. margarita* (Venice et al. 2019), and Endogonales (Chen et al. 2018) also allowed to gain new information on **endobacteria** living in their cytoplasm. The genome sequence led to the detection of Mollicutes-related endobacteria (MREs) in *D. epigea* and in three of the four sequenced Endogonaceae. Their genomes can therefore be read as "metagenomes." By contrast, *G. margarita* genome confirmed the presence of *Candidatus* Glomeribacter gigasporarum, which was already sequenced (Ghignone et al. 2012). The presence of such endobacteria, which have also been discovered in the phylogenetically related Mortierellomycotina (Uehling et al. 2017), strongly suggests that endobacteria may be an evolutionary marker of Mucoromycota. The intimate contact between bacteria and fungi may have favored horizontal gene transfer (Torres-Cortés et al. 2015; Naito et al. 2015; Sun et al. 2018), potentially leading to an impact on the fungal biology (Salvioli et al. 2016).

B. From Structure to Function

The genome sequencing of AM fungi has so far provided relevant information concerning their genome structure and evolution, even if data from some more distantly related members, such as *Archeospora*, would be essential to better define their ancient relationships. By contrast, functional genomics study of AM fungi is still at its infancy. Many genes, and mostly those expressed during the symbiotic phase, are orphan genes (i.e., do not show similarities with genes listed in databases), and the lack of genetic transformation procedures further hampers their characterization.

Following the studies on pathogenic interactions, attention has been given to the **secretome, the pool of proteins characterized by the**

presence of a signal peptide that guides proteins toward the endomembrane system for secretion (Kloppholz et al. 2011; Tisserant et al. 2013; Lin et al. 2014; Sędzielewska Toro and Brachmann 2016; Kamel et al. 2017; Zeng et al. 2018) since the secretome includes proteins called effectors which are of crucial relevance in host-microbe interactions. **Effectors** are microbial molecules that, once delivered to the host cells, can manipulate cellular mechanisms often leading to an attenuation of innate immune response or a promotion of nutrient exchange and thus favoring host colonization (Lo Presti et al. 2015).

AM fungi possess a rather rich secretome with hundreds of candidate **secreted proteins** which, in the different publications, may vary in number according to the criteria used to define them. Comparative analyses of genomes and transcriptomic data from AM fungi showed that many secreted proteins are conserved in phylogenetically related AM species; however, in analogy to other fungal groups with different lifestyles/nutritional strategies (Schirawski et al. 2010; Heard et al. 2015; Pellegrin et al. 2015), there is a prevalence of lineage-specific proteins, suggesting specific biological roles. Indeed, AM effectors have also been hypothesized to be important factors to control symbiotic efficiency and/or host preferences (Zeng et al. 2018), two aspects of AM fungi biology whose molecular mechanisms are still largely unknown.

Interestingly, two studies have clearly demonstrated that, while some secreted proteins showed similar gene expression levels in different host plants, suggesting that they fulfill conserved roles, a subset of them were differentially expressed depending on the host species (Kamel et al. 2017; Zeng et al. 2018). Host-specifically expressed secreted proteins, candidate effectors, also have been observed for the endophyte *Piriformospora indica* (Lahrmann et al. 2013). Evidence that these secreted proteins can play a significant role in host specificity also comes from plant pathogens where their evolution seems to be under host-directed selection (Zhong et al. 2016).

On the other hand, a small set of secreted proteins, also shared by distantly related AM fungi (*Rhizophagus irregularis* and *Gigaspora rosea*), showed similar expression patterns in different host plants (Kamel et al. 2017). These genes, described as the AM symbiotic core secretome, encode proteins with unknown function or proteases. The proteolytic activity may play a role in the production of oligopeptides and amino acids with nutritional roles, the inactivation of plant defense proteins (Jashni et al. 2015), or the generation or turnover of fungal/plant signaling proteins.

Induced expression *in planta* is a commonly applied additional predictive criterion to identify effectors among secreted proteins. Gene expression profiles from laser microdissected cells even allowed to identify a set of genes most specifically expressed at the arbuscule stage (Zeng et al. 2018). Although the majority of them are orphan genes, some secreted proteins could be associated to lipid signaling (which is of particular interest considering the finding of fatty acid auxotrophy of AM fungi) or show homology, again, to endopeptidases (Zeng et al. 2018). But, so far, **only three AM effectors have been characterized in detail**. The first, called SP7, was shown to target the host cell nucleus where it counteracts the function of the pathogenesis-related transcription factor MtERF19 (Kloppholz et al. 2011). The putative secreted protein SIS1 from *R. irregularis* was found among those genes upregulated in strigolactones-treated germinating spores (Tsuzuki et al. 2016) and strongly expressed in the intraradical mycelium, including arbuscules (Zeng et al. 2018), in line with its predicted role in intraradical colonization (Tsuzuki et al. 2016). Recently, a crinkler (CRN) effector (RiCRN1) that belongs to a subfamily of secreted CRN proteins from *R. irregularis* was also characterized (Voß et al. 2018). As CRNs were originally described in plant pathogenic oomycetes (Schornack et al. 2009), this finding extends the similarity between AM fungi and plant pathogens also outside the fungal kingdom. Although not yet defined, the mechanism of action of RiCRN1 does not involve cell death processes as often described for CRNs from oomycetes.

In all these three abovementioned cases, **host-induced gene silencing** (HIGS) has been

used to specifically silence the fungal genes during the symbiotic phase allowing the description of an impaired colonization pattern. These examples also highlight how, in the absence of protocols for stable genetic transformation for AM fungi, genetic manipulation tools developed for the host plants can be successfully applied to study the function of AM fungal genes at least in the *in planta* phase.

The availability of genome and transcriptomic data also allowed the large-scale analysis of Cu, Fe, and Zn transporters: beside an expansion of some gene families, it has been observed that some genes were upregulated in the intraradical phase suggesting that metals are important for plant colonization (Tamayo et al. 2014), in analogy to what has been observed for the endophytic fungus *Epichloë festucae* (Johnson et al. 2013).

Analogous investigations led to an inventory of conserved proteins of the **RNA interference machinery (RNAi)** in *Rhizoglomus irregulare* with the discovery of putative ancient events of horizontal gene transfer involving two class I ribonuclease III protein-coding genes possibly from cyanobacterial genomes (Lee et al. 2018). From an evolutionary perspective, this finding may reflect an ancient symbiosis history of AM fungi with cyanobacteria. Remarkably, this type of interaction can still be observed today between *Geosiphon pyriforme*, an AM fungus assigned to the basal order Archaeosporales and the cyanobacterium *Nostoc punctiforme* (Gehrig et al. 1996). The presence of a RNA silencing machinery suggests that *R. irregulare* has the potential to produce small RNAs and, hypothetically, to use them also as effectors, in a process so called cross-kingdom RNAi, as it has been shown in the pathogenic interaction of *Botrytis cinerea* and *Arabidopsis* (Wang et al. 2017). Some evidences in this direction have been recently obtained (Silvestri et al. 2019). Further investigations are needed to verify whether also AM fungi use RNA effectors to regulate plant processes; on the other hand, the inverse phenomenon, that is, the delivery of small RNA with gene silencing purposes from the plant to the fungus, is also likely to occur as again it has been demonstrated in the *Botrytis cinerea-Arabidopsis* interaction (Cai et al. 2018). The success of the HIGS approach as a tool to silence fungal genes in the AM symbiosis (Helber et al. 2011; Tsuzuki et al. 2016) is a strong clue toward the occurrence of such a process.

IV. Molecular Tools Reveal Plant Responses to AM Fungi

A. Cellular and Molecular Changes in the Host Plant

In analogy to most root-microbe interactions, AM establishment depends on finely tuned **recognition processes** (Bonfante and Genre 2015) through signal release and perception between both partners before their physical contact (Gianinazzi-Pearson 1996).

Root exudates (Fig. 2) contain several bioactive, low molecular weight compounds. Among them, strigolactones are a class of terpenic compounds deriving from the carotenoid metabolism. They are biosynthesized in the root and actively transported to the shoot or released in the rhizosphere (Gomez-Roldan et al. 2008). **Strigolactones**, whose release increases under phosphate starvation, rapidly undergo spontaneous hydrolysis in water solutions, which limits their diffusion in the rhizosphere and makes them reliable signals of root vicinity for several root-interacting organisms. Indeed, strigolactones were originally identified in plant root exudates as germination stimulants for parasitic plants of the family Orobanchaceae (Matusova et al. 2005). Later, strigolactones have been reported to be indispensable for the establishment of AM (Akiyama et al. 2005, 2010; Besserer et al. 2006, 2008) and more recently also to be involved in symbiotic nitrogen fixation (Soto et al. 2009; Foo and Davies 2011).

Strigolactone perception triggers a cascade of molecular and cellular events in AM fungi, such as nuclear multiplication, mitochondrial growth, and a fast increase of cytotoxic NADH and ATP content, indicating respiration as one of the primary metabolic targets (Akiyama and Hayashi 2006). Such cellular responses associ-

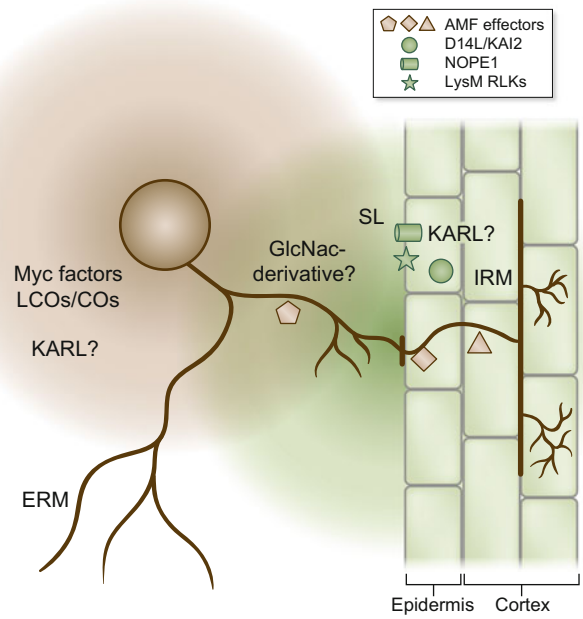

Fig. 2 Molecules involved in the communication between AM fungi and host plants. Plant roots release strigolactones (SL) which stimulate fungal metabolism and hyphal branching to promote colonization (Akiyama et al. 2005; Besserer et al. 2006, 2008). A rice mutant deficient for the D14L gene is characterized by an absence of hyphopodia (Gutjahr et al. 2015). The D14L/KAI2 protein localizes to the nucleus and cytoplasm. It is yet unclear whether the karrikin-like (KARL) ligand of D14L/KAI2 relevant for AM symbiosis is of plant or fungal origin. The recent finding that a plasma membrane-resident plant N-acetylglucosamine (GlcNAc) transporter (NOPE1) is required for early signaling in AM suggests the existence of GlcNAc-based diffusible plant molecules, which may trigger pre-symbiotic fungal reprogramming (Nadal et al. 2017). Also AM fungi use GlcNAc-based molecules, which include lipo-chito-oligosaccharides (LCOs; Maillet et al. 2011) and short chitin tetra- and pentamers (COs; Genre et al. 2013); these are perceived by plant LysM-RLKs (Zipfel and Oldroyd 2017) and activate plant symbiotic responses. AM fungal effector candidates, thought to interfere with host cellular processes to favor colonization at early and/or late stages of the AM symbiosis, have been predicted from fungal genomes and transcriptomes (Sędzielewska Toro and Brachmann 2016; Kamel et al. 2017). SLs stimulate the production of chitin oligomers (Genre et al. 2013) and secreted proteins (Tsuzuki et al. 2016; Kamel et al. 2017) by AM fungi. Note that the tissue-specific expression of D14L/KAI2 and NOPE1 is currently unknown. IRM, intraradical mycelium; ERM, extraradical mycelium. From Lanfranco et al. (2018) with permission

ate with hyphal proliferation and repeated branching close to the root, facilitating contact with the host surface (Besserer et al. 2006, 2008).

The role of strigolactones as fungus-directed signals in AM interactions has indirectly been confirmed by the observation that rice mutants defective in the SLs receptor D14 are not perturbed in AM colonization (Yoshida et al. 2012; Gutjahr et al. 2015). The study of strigolactone perception by the host plant has anyway revealed intriguing crosstalk mechanisms with other signaling pathways. For strigolactone perception, D14 forms a receptor complex with the F-box protein MAX2/D3/RMS4 (Hamiaux et al. 2012). In turn, MAX2 was shown to also be involved with KAI2/D14LIKE in the receptor complex for karrikins, the butenolide molecules found in smoke extracts that promote seed germination of many plant species (Flematti et al. 2004; Nelson et al. 2010; Waters et al. 2012). Interestingly, rice *d3* and pea *rms4* mutants displayed important defects in AM colonization and arbuscule formation, respectively (Yoshida et al. 2012; Foo et al. 2013; Gutjahr et al. 2015); furthermore, a *d14l/kai2* rice mutant does not stimulate the formation of hyphopodia (Gutjahr et al.

2015) and does not respond transcriptionally to AM germinating spore exudates. Together these results suggest that the karrikin receptor complex plays a role in symbiotic signaling even if the involvement of karrikin-like molecules of fungal or plant origin remains to be investigated (Gutjahr et al. 2015; Waters et al. 2017).

The discovery of a rice and maize N-acetylglucosamine transporter (NOPE1) required for AM signaling and colonization points to the role of additional diffusible plant molecules in the activation of pre-symbiotic fungal reprogramming (Nadal et al. 2017). Elucidating the exact molecular function of NOPE1 and its substrate will shed light on long predicted new molecular actors in AM signaling (Bonfante and Requena 2011).

Even if fungal receptors for strigolactones remain unknown (Waters et al. 2017), recent data suggest the activation of a calcium-mediated pathway (Moscatiello et al. 2014) and – intriguingly – the release of pre-symbiotic fungal signals (Genre et al. 2013).

In fact, AM fungi release water-soluble molecules collectively known as **Myc factors** (mycorrhizal factors) (Bonfante and Genre 2015). Myc factor perception triggers plant symbiotic responses (Bonfante and Requena 2011) through a Ca^{2+}-mediated **signal transduction pathway**. Responses include transcriptional regulation, starch accumulation in roots, and lateral root formation (Kosuta et al. 2003, 2008; Oláh et al. 2005; Kuhn et al. 2010; Chabaud et al. 2011; Mukherjee and Ané 2011; Maillet et al. 2011; Genre et al. 2013), overall preparing the plant to a successful symbiotic association.

The first evidence of diffusible Myc factors was found in the exudates of germinated spores (GSE), which triggered a transient increase in cytosolic calcium concentration of soybean cultured cells (Navazio et al. 2007). The GSE was later shown to contain different chitin-related oligomers that are responsible for such plant responses: these include **lipo-chito-oligosaccharides** (Myc-LCOs; Maillet et al. 2011) and **tetra- and penta-chito-oligosaccharides** (Myc-COs; Genre et al. 2013).

Myc-LCOs were purified from sterile exudates of mycorrhizal carrot roots and identified as putative Myc factors by their induction of the early symbiotic gene *MtENOD11* in *Medicago* plants. Furthermore, such LCOs stimulate root hair branching (a typical Nod factor-related response) in *Vicia sativa* (Maillet et al. 2011). In fact, LCOs have a striking structural similarity to rhizobial Nod factors (Dénarié et al. 1996).

Myc-COs have been isolated from distantly related AM fungi, *Rhizophagus irregularis* and *Gigaspora rosea* (Genre et al. 2013). They are the most effective elicitors of **nuclear Ca^{2+} spiking** patterns that resemble the irregular spiking triggered by GSE. Myc-COs are active in both legumes and non-legumes at very low concentration, down to 10^{-8} M (Genre et al. 2013; Sun et al. 2015), and can be considered as universal AM-specific elicitors.

1. The Common Symbiotic Signaling Pathway

The study of Myc factor signaling mechanisms in legumes such as *Medicago truncatula* and *Lotus japonicus* has mostly come as a follow-up of analogous research on **symbiotic nitrogen fixation** (SNF; Dénarié and Cullimore 1993; Maillet et al. 2011). Since the latter evolved almost 400 million years later than the oldest known AM-related interactions, it is currently acknowledged that legumes and rhizobia have adapted the pre-existing AM signaling pathway to control the new interaction. Indeed, several legume mutants that cannot transduce Nod factor signals are equally impaired in the early development of SNF and AM (Sagan et al. 1995; Catoira et al. 2000; Oldroyd and Downie 2006; Kosuta et al. 2008; Parniske 2008). Once characterized, the corresponding genes have been associated in the so-called common symbiotic signaling pathway (CSSP), a signal transduction pathway that mediates AM and – in legumes – SNF establishment (Oldroyd 2013; Gobbato 2015). Phylogenetic studies have demonstrated that key CSSP genes are present throughout eudicots, monocots, basal land plants, and charophytes (Banba et al. 2008; Gutjahr et al. 2008; Chen et al. 2009; Wang et al. 2010; Delaux et al. 2013, 2015).

The CSSP mediates **fungal and bacterial signal transduction** from plasma membrane-

bound receptors into the nucleus, where gene expression is modulated. The LRR receptor-like kinase *Lj*SYMRK is believed to interact with so far unidentified Myc factor receptor(s) (Gobbato 2015). In its cytoplasmic domain, the *M. truncatula* homolog of *Lj*SYMRK, *Mt*DMI2, interacts with a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR1) involved in mevalonate synthesis (Kevei et al. 2007). Mevalonate is therefore believed to be produced in the vicinity of the cytoplasmic face of the plasma membrane (Venkateshwaran et al. 2015) and acts as an intermediate messenger. The following group of known CSSP proteins are localized on the nuclear envelope. They include three nucleoporins (NUP85, NUP133, and NENA; Kanamori et al. 2006; Saito et al. 2007; Groth et al. 2010), the ATP-powered Ca^{2+} pump (*Mt*MCA8; Capoen et al. 2011), a cyclic nucleotide-gated channel (*Mt*CNGC15s; Charpentier et al. 2016), and the cationic channels *Lj*CASTOR (*Mt*DMI1) and *Lj*POLLUX (Ané et al. 2004; Charpentier et al. 2008). Nucleoporins were proposed to be involved in the targeting of CSSP channels and pumps to the inner nuclear membrane (Kanamori et al. 2006; Saito et al. 2007; Groth et al. 2010), whereas the latter are directly involved in generating nuclear Ca^{2+} spiking: a series of repeated oscillations in Ca^{2+} concentration (Ané et al. 2004; Imaizumi-Anraku et al. 2005; Capoen et al. 2011; Venkateshwaran et al. 2012). In more detail, CNGC15 channels are predicted to release Ca^{2+} from the nuclear envelope lumen, a release compensated by the opposite flow of K^+ ions through CASTOR (Parniske 2008; Venkateshwaran et al. 2012). MCA8 activity contributes to the restoration of Ca^{2+} concentration at the end of each peak.

The last group of CSSP proteins resides in the nucleoplasm: Ca^{2+} spiking is supposed to activate a Ca^{2+}- and calmodulin-dependent protein kinase (*Lj*CCaMK; Miller et al. 2013), which in turn phosphorylates its interacting partner, CYCLOPS (Yano et al. 2008). Activated CYCLOPS regulates gene expression either directly or through the action of other transcription factors like NSP1, NSP2, and RAM1 (Oldroyd 2013). Importantly, the identified CSSP actors only constitute subsets of the whole transduction pathway, leaving gaps that still hamper the definition of a complete picture of the signaling process (Genre and Russo 2016).

Beside canonical CSSP members, recent evidence showed a role for additional players: the Nod factor receptor *Mt*LYK3 (*Lj*NFR1) – but not *Mt*NFP (*Lj*NFR5) – is required for AM-specific activation of the CSSP and subsequent colonization (Miyata et al. 2014; Zhang et al. 2015). Furthermore, Myc-LCO-induced responses were shown to be NFP-dependent, indicating the involvement of this receptor in both Nod- and Myc-LCO perception (Op den Camp et al. 2011). Although *nfp* mutants exhibit normal Ca^{2+} spiking and AM colonization (Maillet et al. 2011; Genre et al. 2013; Zhang et al. 2015), such mutants do not display nuclear Ca^{2+} spiking in response to Myc-LCOs (Sun et al. 2015). Taken together, these findings point to possible **partial overlaps and crosstalks between Nod and Myc signaling in legumes**.

By contrast, studies in non-legumes are opening new perspectives in the characterization of AM-specific CSSP activation. A **dual role** has been demonstrated for the rice receptor-like kinase CERK1. This gene is required for both chitin-triggered immunity and AM colonization (Miya et al. 2007; Shimizu et al. 2010). In defense-related chitin perception, OsCERK1 acts with its LysM co-receptor *Os*CEBiP (Kaku et al. 2006; Shimizu et al. 2010), but *Os*CEBiP is not required for AM symbiosis (Miyata et al. 2014), suggesting the involvement of additional players in Myc factor perception. In short, legumes and non-legumes appear to differ in their perception of Myc-LCO and Myc-CO signals, outlining a more complex scenario, where different plant species respond to different components in the mix of signals produced by AM fungi (Sun et al. 2015).

Since CSSP activation is required for the transcription of genes involved in both AM and SNF, it is not fully clear how this pathway can **discriminate between the two signals** and induce different developmental programs. Evidence points to a differential regulation of CCaMK by Ca^{2+} and calmodulin. In fact, the CaM-binding domain is redundant for AM but

essential for SNF establishment (Shimoda et al. 2012). Moreover, different calcium signatures have been proposed to act in the two symbioses, with specific spiking patterns and differential responding cell types (Russo et al. 2013).

2. Symbiosis Establishment, Functioning, and Senescence

IPD3/CYCLOPS targets a number of genes through a cis-element called AM-CYC box (Favre et al. 2014; Pimprikar et al. 2016). Interestingly, CYCLOPS also interacts with the gibberellin signaling repressor DELLA (Pimprikar et al. 2016), and *Medicago truncatula della* mutants are impaired in AM and SNF colonization (Floss et al. 2013; Jin et al. 2016). DELLA proteins are repressors of gibberellic acid (GA) signaling (Alvey and Harberd 2005), and GA was shown to accumulate in *Lotus japonicus* roots during mycorrhization (Takeda et al. 2015). Furthermore, DELLA proteins also control key developmental processes such as the cell cycle (Gallego-Bartolome et al. 2012; Daviere and Achard 2013). In *M. truncatula*, *DELLA1* and *DELLA2* act redundantly to promote arbuscule development (Floss et al. 2013; Foo et al. 2013; Yu et al. 2014). In short, the CCaMK-CYCLOPS-DELLA pathway may act in the adjustment of AM establishment based on plant developmental and environmental stimuli (Carbonnel and Gutjahr 2014; Breuillin-Sessoms et al. 2015; Nagata et al. 2015; Konvalinkova and Jansa 2016).

Among the CCaMK/CYCLOPS-regulated transcription factors, the GRAS-domain proteins NSP1 (Nodulation Signalling Pathway 1) and NSP2 play an essential role in Nod factor signaling (Catoira et al. 2000; Kaló et al. 2005; Smit et al. 2005). Evidence suggests direct roles of NSP1 and NSP2 also in Myc factor signaling: with NSP2 being involved in NS-LCO-induced lateral root growth (Maillet et al. 2011) and NSP1 being required for the induction of three mycorrhizal genes in response to NS-LCO (Delaux et al. 2013). NSP1 and NSP2 interaction is required for the induction of nodulation-specific promoters (Hirsch et al. 2009; Jin et al. 2016) but not crucial for AM symbiosis, suggesting that different GRAS transcription factor complexes regulate distinct groups of genes (Pimprikar and Gutjahr 2018).

RAM1 is an AM-specific transcription factor regulated by the CYCLOPS/DELLA complex (Gobbato et al. 2012). RAM1 expression restores arbuscule formation in *cyclops* mutants (Pimprikar et al. 2016), and both RAM1 expression and arbuscule formation are rescued in *cyclops* mutants by either overexpression of DELLA1 (Floss et al. 2013; Park et al. 2015) or GA treatment (Pimprikar et al. 2016). RAM1 forms a complex with NSP2 that was proposed to regulate the expression of AM-specific genes, similarly to the NSP1/NSP2 complex in SNF (Gobbato et al. 2012).

Additional GRAS proteins were shown to have a role in fungal colonization and arbuscule development, such as RAD1 (Required for Arbuscule Development 1), an interactor of RAM1 and NSP2 (Xue et al. 2015), or DIP1 (Della Interacting Protein 1), a rice GRAS family interactor of the DELLA protein SLR1 (Yu et al. 2014). Furthermore, two CAAT-box transcription factors, *MtCbf3* and *MtCbf4*, were involved in the pre-symbiotic stage (Hogekamp et al. 2011; Czaja et al. 2012; Hogekamp and Kuster 2013).

Lastly, recent studies revealed that the CSSP also activates several other effectors, including miRNA and small interfering RNA (siRNA) with a critical role in transcriptional regulation (Lelandais-Briére et al. 2016; Bazin et al. 2017), mRNA splicing, RNA-directed DNA methylation, and epigenetic functions (Ariel et al. 2015; Chekanova 2015).

Following this chemical courtship, the **presymbiotic phase of AM development** culminates in physical contact between symbionts, with a hyphal tip touching the root surface (Bonfante and Genre 2010). This crucial step in AM symbiosis consists in the formation of a large, swollen, and often branched **hyphopodium**, attached to the root epidermal surface. Epidermal cell wall was proposed to stimulate hyphopodium differentiation (Nagahashi and Douds 1997) through specific physicochemical signals. This idea arose from observations in rice – where hyphopodia form on large lateral

roots but never on fine lateral roots (Gutjahr et al. 2009) – and seminal studies of Giovannetti et al. (1993) and Nagahashi and Douds (1997) pointing at the presence of **wall-associated hyphopodium-stimulating signals**. More recently, one such signal has been identified in monomeric cutin. This deduction came from the observation of mutants in *ram2* (Gobbato et al. 2012), a glycerol-3-phosphate acyltransferase that is highly induced by RAM1 during AM symbiosis (Harrison 2012) and is involved in the biosynthesis of cutin precursors (Wang et al. 2012; Vijayakumar et al. 2015). In fact, root-bound cuticle monomers (Wang et al. 2012) that have also been reported to stimulate AM hyphal branching (Nagahashi and Douds 2011) are less abundant in *ram2* mutants.

Hyphopodium development is followed by hyphal penetration in the sub-hyphopodial epidermal cell (Genre et al. 2005; Bonfante and Genre 2010). **Intracellular fungal accommodation** is the central feature of AM symbiosis, and plant cells have to change their architecture and molecular composition in a process referred to as host cell reprogramming (Dörmann et al. 2014). Epidermal cells reorganize to accommodate the fungal symbiont with precise **nuclear movements, cytoplasm aggregation, and cytoskeleton remodeling**. This cellular reorganization allows the assembling of a subcellular column-shaped structure, the so-called **prepenetration apparatus** (PPA), that the plant cell forms in anticipation of fungal infection (Genre et al. 2005). PPA assembly may require 4–6 h and starts with the movement of the epidermal cell nucleus toward the hyphopodium. The nucleus then moves away from the contact site and traverses the plant cell vacuole inside a broad cytoplasmic bridge. The resulting columnar **cytoplasmic aggregation** includes numerous Golgi stacks, extensive trans-Golgi network, endoplasmic reticulum, cytoskeleton, and secretory vesicles (Genre et al. 2005, 2008, 2012). Only at this stage a hyphopodium-derived hypha starts penetrating the epidermal cell.

Endoplasmic reticulum and Golgi membranes that surround the penetrating hypha are ideally positioned for the synthesis of the **perifungal membrane**, which is believed to be the main function of the PPA. In fact, intense exocytic activity and the accumulation of SNARE and exocyst proteins have been observed around the penetrating hyphal tip (Genre et al. 2008, 2012; Ivanov et al. 2012), alongside the upregulation of the corresponding genes (Ivanov et al. 2012; Zhang et al. 2015).

PPA formation was shown to be CSSP-dependent (Genre et al. 2005; Gutjahr and Parniske 2013), and several GRAS and CAAT-box transcription factors are active during this stage (Hogekamp et al. 2011; Hogekamp and Kuster 2013), regulating a large number of genes (Diédhiou and Diouf 2018). *Mt*ENOD11 is an atypical cell wall-associated protein presumed to limit cross-linking between other wall components (Journet et al. 2001). As such, *Mt*ENOD11 expression during AM colonization may contribute to **cell wall plasticity**, especially considering the lack of cell wall-degrading enzymes in glomeromycotan genomes (Tisserant et al. 2012, 2013). Additional cell wall remodeling enzymes are expressed in roots during AM colonization: a xyloglucan endotransglycosidase (van Buuren et al. 1999) and cellulose synthase-like and expansin-like proteins (Balestrini and Bonfante 2005; Siciliano et al. 2007). Vapyrin, a VAMP-associated protein, is also expressed during early AM and SNF establishment (Pumplin et al. 2010; Murray et al. 2011).

Penetrating hyphae cross the epidermal cell lumen strictly following the route traced by the PPA and reach the root cortex. Overall, prepenetration responses in outer cortical cells resemble those observed in epidermal cells (Genre et al. 2008). By contrast, as AM hyphae reach the inner cortex, a substantial change is observed in both fungal growth pattern and host cell responses: the fungus switches from **radial to longitudinal growth,** and inner cortical cells develop broad PPA-like structures in preparation of **arbuscule accommodation** (Genre et al. 2008).

Arbuscule accommodation in cortical cells involves the biogenesis of an extensive apoplastic compartment, the **symbiotic interface** (Bonfante 2001; Balestrini and Bonfante 2014), which consists of the periarbuscular space, containing plant cell wall material and directly out-

lining the fungal cell wall (Balestrini and Bonfante 2014), and **periarbuscular membrane** (PAM; Harrison 2012), continuous with the host cell plasmalemma.

The process of arbuscule accommodation in cortical cells is the most striking feature of AM development and requires a broad reorganization of the host cells in strict coordination with fungal development: hyphal penetration associates with **nuclear movement** at the center of the cell (Bonfante 2001), engulfed by a broad PPA (Genre et al. 2008). This anticipates the formation of the arbuscule trunk and the PAM trunk domain (Pumplin and Harrison 2009), characterized by a set of proteins that is analogous to that of the plasma membrane. Later on, smaller PPA-like aggregates organize in the areas where the **arbuscule branches** and their associated PAM branch domain develop (Genre et al. 2008), harboring a **specific set of proteins** devoted to nutrient exchange (Pumplin and Harrison 2009), whose genes are only expressed during this phase of arbuscule development (McLean et al. 2017).

The extensive, repeated branching of arbuscule hyphae requires a very intense membrane synthesis (Pumplin and Harrison 2009) and a **polarized exocytic process** that dwarfs the analogous mechanisms described in outer cell layers and positions AM-specific membrane proteins in the PAM. An important transcriptional response drives such cellular changes, and the roles of individual genes are gradually being revealed (McLean et al. 2017; Hogekamp et al. 2011; Gaude et al. 2012; Hogekamp and Kuster 2013).

In response to CSSP activation, several transcription factors are expressed during either early or later stages of arbuscule formation (Bucher et al. 2014; Luginbuehl and Oldroyd 2017; Diédhiou and Diouf 2018; Pimprikar and Gutjahr 2018), in turn regulating the expression of genes involved in nutrient transfer, primary and specialized metabolism, membrane and cell wall modifications, secretion, and signal transduction (Hohnjec et al. 2005; Gaude et al. 2012; Hogekamp and Kuster 2013; Handa et al. 2015). The BLUE COPPER-BINDING PROTEIN 1 localizes to the peripheral plasma membrane and PAM trunk domain (Pumplin and Harrison 2009; Pumplin et al. 2012; Ivanov and Harrison 2014). By contrast, the GRAS-domain transcription factor, RAM1, is regulated by DELLA proteins and required for arbuscule branch development (Gobbato et al. 2013; Park et al. 2015; Rich et al. 2015; Pimprikar et al. 2016). RAM1 regulates the expression of exocytic markers such as the EXO70I subunit of the exocyst complex (Zhang et al. 2015b). Indeed, several proteins involved in membrane dynamics are expressed during the PAM branch domain development, such as the symbiosis-specific t-SNARE SYP132A (Huisman et al. 2016; Pan et al. 2016), VAPYRIN, involved in membrane fusion processes (Feddermann et al. 2010; Pumplin et al. 2010; Murray et al. 2011), and two symbiosis-specific v-SNARES of the VAMP721 group (Ivanov et al. 2012).

Other GRAS-domain proteins, such as RAD1 (Xue et al. 2015) and DIP1 (Yu et al. 2014), play a role in arbuscule development by interacting with RAM1 and DELLAs, suggesting the existence of a large transcription factor complex (Floss et al. 2016). Furthermore, another GRAS protein, MIG1 (MYCORRHIZA-INDUCED GRAS 1), was proposed to control radial expansion of cortical cells during arbuscule formation and interact with DELLA1 to regulate AM root development (Heck et al. 2016; Luginbuehl and Oldroyd 2017). AM-upregulated transcription factors also include AP2 (APETALA2)-EREBP, an ethylene-responsive element binding protein with a role in arbuscule development (Devers et al. 2013), and *Mt*ERF1, specifically expressed in arbusculated cells and required for arbuscule maturation (Devers et al. 2013).

AM fungi provide the host plant with a **more efficient access to soil mineral nutrients**, in particular **phosphate** and **ammonium**. After absorption, phosphorus (P) and nitrogen (N) are translocated along the extraradical and intraradical mycelium in the form of polyphosphate and arginine. From arbuscules, they are released into the periarbuscular space as phosphate (Javot et al. 2007) and ammonium (Tanaka and Yano 2005), respectively.

AM fungal **exporters** for nutrients bound to the periarbuscular space have not yet been

identified. By contrast, symbiosis-specific phosphate **importers** – PT4 in *Medicago truncatula* and PT11 in rice (Javot et al. 2007; Pumplin and Harrison 2009; Kobae et al. 2010) – have been localized to the PAM. *M. truncatula pt4* deletion induces a premature arbuscule collapse and symbiosis abortion (Javot et al. 2007), suggesting that host cells monitor phosphate delivery from arbuscules and induce arbuscule degeneration if it is not sufficient. Similarly, nitrogen has also proven to act as a signal supporting arbuscule survival (Javot et al. 2011). Ammonium transporters are transcriptionally induced in mycorrhized roots and localize in the periarbuscular membrane (Kobae et al. 2010; Koegel et al. 2013). In fact, an ammonium transporter 2 family protein AMT2-3 has been identified in *M. truncatula*, whose mutation induces premature arbuscule degeneration (Yang et al. 2012; Breuillin-Sessoms et al. 2015). In the same context, PAM-associated H^+-ATPases of the MtHA1 family (Krajinski et al. 2014; Hogekamp et al. 2011; Gaude et al. 2012) are believed to generate the proton gradient required for the import of different nutrients from the periarbuscular space.

A few studies have analyzed the molecular bases of **carbon transfer from the host plant to the AM fungus**. The sugar transporter *Mt*ST1 was found to be expressed in *Medicago truncatula* root tissues colonized by AM fungi (Harrison 1996). Radiolabelling studies suggest that glucose can be absorbed by intraradical but not extraradical hyphae (Bago et al. 2000; Douds et al. 2000). Glucose transfer requires the expression of invertases and sucrose synthases in mycorrhizal roots, suggesting sucrose as a possible source of the hexoses delivered to the fungus. In parallel, a high-affinity monosaccharide transporter (MST2) has been identified in arbuscules and intraradical hyphae of *Rhizophagus irregularis* (Helber et al. 2011). Once the assimilated **sugars** reach the fungal cytoplasm, they are converted into glycogen and trehalose and exported to the extraradical mycelium (Pfeffer et al. 1999).

Even if the main form of carbon storage in AM fungi is represented by triacylglycerols (TAGs), extraradical hyphae and fungal spores are not capable of de novo fatty acid synthesis (Pfeffer et al. 1999; Gobbato et al. 2013). Indeed, studies revealed that **host plants also provide fatty acids to AM fungi**, likely in the form of palmitic acid (Wewer et al. 2014). This scenario is supported by the upregulation of several genes involved in lipid biosynthesis and secretion in arbusculated cells. They include the acyl-ACP (acyl carrier protein) thioesterase FatM and the glycerol-3-phosphate acyltransferase RAM2, both required for the establishment of a functional AM symbiosis (Gobbato et al. 2013; Luginbuehl et al. 2017). FatM has been suggested to produce palmitic acid from palmitoyl-ACP in the chloroplast (Bravo et al. 2017), whereas RAM2 has been shown to preferentially use palmitoyl-coenzyme A as a substrate to produce 2-monopalmitin (Luginbuehl et al. 2017). Interestingly, RAM1-dependent expression has been reported for the ABCG lipid exporters STR and STR2, which localize to the branch domain of the PAM (Gutjahr et al. 2012; Bravo et al. 2017; Luginbuehl et al. 2017).

The functional **lifetime** of arbuscules has been estimated a few days (Kobae and Hata 2010) after which senescence processes are initiated. Coupled with their non-synchronous formation, this relatively short time of activity leads to the **coexistence of symbiotic structures at different stages** within the same area of the root. Arbuscule collapse and degeneration involves the rapid shrinkage of arbuscule branches and PAM, including the dismantling of its associated proteins (Kobae and Hata 2010). Abundant vesicles and endoplasmic reticulum cisternae persist around the collapsing PAM, alongside peroxisomes, possibly assisting lipid breakdown or protecting the host cell from oxidative damage (Pumplin and Harrison 2009). As senescence proceeds, progressively larger branches and the arbuscule trunk become septate and collapse, eventually leading to the disappearance of the arbuscule from its host cell (Luginbuehl and Oldroyd 2017). The MYB1 transcription factor is required for the expression of several *M. truncatula* genes associated with arbuscule collapse. MYB1 was shown to interact with DELLAs and NSP1 to form a transcription factor complex

that is believed to trigger arbuscule degeneration by inducing hydrolase genes (Floss et al. 2017). In fact, arbuscule senescence is a regulated process where the host cell remains active during and after arbuscule collapse and maintains the ability to be colonized again by a new arbuscule.

B. Mycorrhizal Omics: From Local to Systemic Responses

The AM symbiosis develops in roots where extensive cellular reorganizations and specific metabolic changes occur, which are mirrored by local changes in the transcript profiles as it has been demonstrated by **transcriptomic** analyses carried out on several plant species. The root metabolome is also reprogrammed upon mycorrhization. Laparre et al. (2014) detected 71 compounds exclusively present or more abundant in *M. truncatula* roots colonized by *R. irregularis*, including propionyl- and butyryl-carnitines. Remarkably, the accumulation of carnitine, which is known to be involved in lipid metabolism, could reflect changes in the fungal metabolism and can be linked to the described fatty acid auxotrophy of AM fungi.

An untargeted **metabolomic** analysis was also recently performed on tomato mycorrhizal roots with the aim to identify key metabolites involved in the mycorrhiza-induced protection against osmotic stresses (Rivero et al. 2018). AM-colonized roots accumulated some amino acids, lignans, oxylipins, and carotenoids, among which some compounds are known to be involved in plant stress adaptation (Havaux 2014). Interestingly, the protective effect of the mycorrhizal symbiosis was higher than that observed upon exogenous application of purified compounds highlighting that the AM symbiosis could be considered a more versatile strategy for plant protection.

Even if AM colonization is physically confined to root tissues, epigeous portions of the plants also experience physiological and metabolic changes. Indeed, transcriptomic analyses revealed significant gene modulation in shoots (Liu et al. 2007; Fiorilli et al. 2009; Cervantes-Gámez et al. 2016) and even fruits (Zouari et al. 2014) of mycorrhizal plants, indicating the occurrence of a long-distance systemic response. Reorganization of the metabolic profiles was also observed in leaves of mycorrhizal plants. A comparative multi-species metabolomic approach carried out on plants inoculated with the same AM fungus revealed that, although a core metabolome could be identified, leaves metabolic responses to arbuscular mycorrhiza showed strong species specificity (Schweiger et al. 2014).

It has been envisaged that these **AM-induced changes at systemic level may have an impact on the outcome of biotic and abiotic interactions.** Martinez-Medina et al. (2016) have described how mycorrhizal fungi as beneficial microbes induce a priming status (i.e., the induction of a physiological state in which a plant is conditioned for the activation of defenses against environmental challenges). Interestingly, such priming status is raised also by native microbiota which are associated to tomato, including also AM fungi (Chialva et al. 2018). As a consequence, mycorrhizal plants acquire the so-called **mycorrhiza-induced resistance** (MIR; Jung et al. 2012; Cameron et al. 2013), thanks to which they have been shown to alleviate the damage caused by pathogen attacks. The dissection of the tripartite interaction among wheat, the AM fungus *Funneliformis mosseae*, and the bacterial pathogen *Xanthomonas translucens* by using a combined transcriptomic-proteomic-metabolomic approach revealed that AM symbiosis does exert a positive effect on wheat growth and productivity but also does provide protection against *X. translucens* (Fiorilli et al. 2018). Indeed, induction of genes involved in a general defense line (e.g., coding for pathogenesis-related proteins, or leading to ROS formation) was the result of the AM fungus presence at local and systemic level, while specific defense genes (encoding, e.g., a cytochrome P450 enzyme, involved in iron binding and with oxidoreductase activity) were detected exclusively after the pathogen attack (Fiorilli et al. 2018).

It is worth to mention that studies on leaf metabolome have also been instrumental for the identification of blumenol-derived compounds which were detected in leaves of several

dicot and monocotyledonous plants so that they have been proposed as foliar markers of the AM association (Wang et al. 2018). **Blumenols** therefore represent an extremely powerful tool for high-throughput screening for a functional AM symbiosis and can be used instead of laborious measurements of AM-induced transcripts or microscopic analyses.

Small RNA (sRNA) molecules are also attractive candidates for long-distance signaling and have been targeted by -omics analyses in arbuscular mycorrhizas. So far investigations focused on roots revealed a large-scale reprograming of microRNAs (miRNAs) upon AM colonization (Devers et al. 2011; Wu et al. 2016; Pandey et al. 2018). In a recent work, the analysis of putative targets of selected miRNAs revealed an involvement in P starvation, phytohormone signaling, and defense (Pandey et al. 2018).

In conclusion, all these studies convincingly demonstrate that AM fungi have a local and systemic influence on their host plant, since they lead to a deep reorganization of the plant biology acting on multiple transcriptomic, regulatory, and metabolomic pathways.

V. The AM Symbiosis in the Light of Natural Variation

Despite the low morphological variation and their large host range, **AM fungal species and isolates show different efficiency in promoting plant performance**; on the other hand, the plant genotype has an important role in determining the extent of plant responsiveness to the AM symbiosis (Smith et al. 2004). AM fungi can present high functional diversity: even isolates belonging to the same species can exert on a specific host plant different growth effects, which can vary in amplitude and direction (promotion or inhibition) (Hart and Reader 2002; Munkvold et al. 2004; Feddermann et al. 2008; Antunes et al. 2011; Hong et al. 2012). An extensive comparative study, considering 56 AM isolates belonging to 6 different families and 17 genera inoculated on 3 different host plants, revealed that the plant growth response could not be predicted from AM species identity or morphological traits, such as extra- and intraradical fungal volumes (Koch et al. 2017). It can be hypothesized that the functional variability of the fungal symbionts may rely on other factors such as qualitative and/or quantitative differences in the production of signaling molecules, such as chitin oligomers, and/or effectors, which are crucial for triggering the symbiotic program and dampening the plant immune response, and in the expression and functioning of transporters and/or metabolic enzymes which control nutrient exchanges and may guarantee, in the end, a highly compatible and efficient mutualistic symbiosis. Since the mycorrhizal growth effect is also dependent on the host plant, it is likely that the host plant contributes, to some extent, to the regulation of these fungal genetic determinants. The differential expression of some secreted proteins, candidate effectors, on different host plants (Kamel et al. 2017; Zeng et al. 2018) already provides a support to this hypothesis (Fig. 3).

Mateus et al. (2019) analyzed transcriptomes from five genetically different cultivars of another important crop worldwide, cassava (*Manihot esculenta*), each inoculated with two different *R. irregularis* isolates to explore how plant and fungal gene expression profiles are affected by the intra-specific variability of the partner organism. Interestingly, expression of most plant genes responded in a different direction or magnitude depending on the plant genotype. The abundance of several fungal transcripts was also strongly influenced by the genotype of the plant.

Comparative genomics and functional genomics studies on several AM strains and species will be instrumental to determine the genetic, and possibly epigenetic, polymorphisms controlling the impact of specific AM inocula on host plant performance. Hopefully, evaluation of plant performance should not be limited to the growth effect but also to other traits provided by AM fungi, such as enhanced tolerance to abiotic and abiotic stresses to cover all the potential benefits of the symbiosis.

Exploring the genomic variations of AM fungi is already giving important insights on

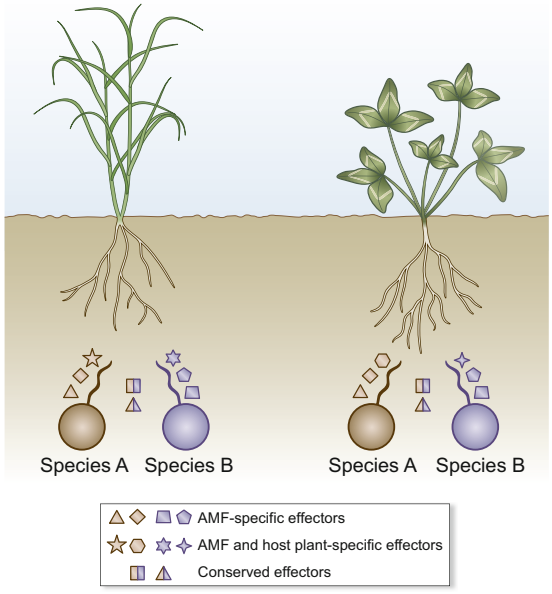

Fig. 3 Scheme of the variety of symbiotic effectors produced by AM fungi during the interaction with host plants. For a single fungal species, some effectors are expressed in association with all plant species, whereas others are expressed in a host plant-specific manner. Some effectors are conserved among AM fungi and may play core symbiotic functions. From Lanfranco et al. (2018) with permission

the nature of their genome organization: the discovery of homokaryotic and dikaryotic strains of *R. irregularis* (Ropars et al. 2016; Corradi and Brachmann 2017) and the finding that distinct nuclear genotypes can undergo recombination events (possibly through a meiotic process) in dikaryotic mycelia (Chen et al. 2018b) highlight the **potential of AM fungi for sexual reproduction and** offer **perspectives for genetic strain improvement.** Together with functional analyses, this knowledge will be fundamental to allow a selection of AM fungi with specific impact on plant performance.

On the plant side, susceptibility to AM fungi, evaluated by the measurement of colonized root length, depends on several environmental factors, among which soil nutrient availability, in particular that of P, is a crucial parameter, with high fertilization having generally a negative effect (Sawers et al. 2010; Chu et al. 2013). In addition, not only susceptibility to AM fungi but also the mycorrhizal growth response depends on the plant genotype. It is worth to note that current literature data, however, could not highlight a clear correlation between the amount of colonization and plant performance (Koch et al. 2017; Sawers et al. 2017; Lekberg and Koide 2005).

There is increasing interest in exploring the variations in AM susceptibility and responsiveness in cultivated accessions and, through genome-wide association analyses, in identifying the genetic determinants associated to those traits. One of the first large-scale studies considering **genetic variation in AM fungi susceptibility was carried out on 94 wheat (*Triticum aestivum*) genotypes** inoculated by a mixed inoculum of 3 AM species (Lehnert et al. 2017). Interestingly, six quantitative trait loci (QTLs) associated with colonization level could be identified: they contained genes related to cell wall metabolism and defense, suggesting that they may be involved in controlling root colonization.

A recent work analyzed a large collection of wild, domesticated, and cultivated lines of *Triticum turgidum* ssp. *durum* colonized by two AM fungi (*Funneliformis mosseae* and *Rhizoglomus irregulare*). Seven QTLs were linked to mycorrhizal susceptibility, and candidate proteins with roles in host-parasite interactions, degradation of cellular proteins, homeostasis regulation, plant growth, and disease/defense were identified (De Vita et al. 2018).

Concerning the **responsiveness to AM fungi**, Sawers et al. (2017) analyzed the growth response of 30 maize lines upon colonization by *F. mosseae*; variations in shoot dry weight and shoot Pi content were observed, and, interestingly, these correlated with the amount of extraradical mycelium, suggesting a plant-fungus reciprocal effect on growth performances. The molecular bases for this effect are completely unknown and may rely on differential regulation of genes involved in nutrient transport in both partners. In addition, the concentration of 19 elements was also determined in roots and leaves of the same maize lines (Ramirez-Flores et al. 2017): a number of

other elements, besides P, responded significantly to inoculation, and the impact of AM symbiosis on the concentration of these ions was genotype specific, indicating again the relevance of plant genetics.

In the attempt to map the genetic bases of AM symbiotic variations, also wild relatives and old varieties are often analyzed since they represent important genetic resources for breeding (Singh et al. 2012; Lehmann et al. 2012). It has been hypothesized that the selection of modern varieties, which was likely carried out under highly fertilized conditions, may have decreased the susceptibility/responsiveness to the AM symbiosis (Zhu et al. 2001; Lehmann et al. 2012). Depending on the crop species diverse mycorrhizal response patterns were observed (Kapulnik & Kushnir 1991; Koltai & Kapulnik 2010; Steinkellner et al. 2012; Xing et al. 2012; Turrini et al. 2016). A recent work, through a comparative investigation on 27 crop species and their wild progenitors, showed that **the growth benefits exerted by the AM symbiosis were dependent on P availability**; while wild progenitors positively responded to the AM symbiosis irrespective of P availability, in domesticated plants the growth effect observed at low P became negligible when P availability increased (Martín-Robles et al. 2018). In addition, domesticated plants reduced AM fungal colonization more strongly than did wild progenitors in response to increased P availability.

On the whole these studies indicate **a strong fungal genotype X plant genotype interaction in the mycorrhizal symbiosis.** This variation may have profound impact in natural populations and has to be considered in agricultural practices where AM fungi are exploited to improve plant health and productivity.

VI. Conclusions

Genetics and genomics have recently provided crucial novel information on the biology of arbuscular mycorrhizas. The genome sequencing of a number of AM fungal species is allowing to identify common features such as the fatty acid auxotrophy but also dispensable species-specific components. The detailed characterization of several isolates of *R. irregularis* at the level of single nuclei has even opened a window on the potentials to genetically manipulate AM fungi (Chen et al. 2018).

On the plant perspective, phylogenomics analyses based on genomes from host and non-host species are emerging as powerful tools to identify conserved genes required for the AM symbiosis (Bravo et al. 2016) and to trace the evolution of the underlying genetic network from basal plants to angiosperms (Delaux et al. 2015). We can also envisage that the CRISPR/Cas-based genome editing technique will offer an efficient strategy for producing plant genotypes with mutations in genes of interest. These genes could be selected among those responsible of the molecular dialogue between partners (also considering the pre-symbiotic steps) and among those which regulate AM functionality. In the frame of a more friendly agriculture, these plant genes could be the targets for the development of new crop varieties more susceptible and responsive to the beneficial AM fungi.

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