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Opinion Article

Micronutrient transport in mycorrhizal symbiosis; zinc steals the show

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ABSTRACT

Mycorrhizas are mutually beneficial associations between soil-borne fungi and plant roots. Mycorrhizal fungi provide their host plant with essential nutrients in exchange for sugars and/or lipids. Traditionally, transport and translocation of macronutrients, including nitrogen and phosphorus, throughout the fungal mycelium and towards the host plant are well studied. However, the regulation of nutrient exchange and their contribution in the morphogenesis and development of mycorrhizas remains unclear. In this Opinion, we argue that adding micronutrients in the current models of symbiotic transport is essential to fully understand the establishment, maintenance, and functioning of mycorrhizal associations. Homeostatic mechanisms at the cellular level and the first transport proteins involved have been recently documented for zinc (Zn) in arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizal fungi. Mycorrhizal plants benefit from an improved Zn status in control conditions and are better protected when environmental Zn availability fluctuates. These recent progresses are paving the way for a better understanding of micronutrient allocation in mycorrhizas. Revising our vision on the role of micronutrients, particularly of Zn, in these interactions will allow a better use of mycorrhizal fungi in sustainable agriculture and forestry, and will increase management practices in waste land, as well as in agricultural and natural ecosystems.

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1. Introduction

The conquest of land by plants, around 450 million years ago, was facilitated by the recruitment of fungal root symbionts, forming the premise of mycorrhizal symbiosis (Field et al., 2015; Strullu-Derrien et al., 2018). Today, the vast majority of land plants still associate with mycorrhizal fungi and rely on them for their nutrient supply (Brundrett and Tedersoo, 2018). Different types of mycorrhiza can be distinguished. The most widespread and studied types are arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) symbioses. Less common types are restricted to particular plant hosts and include ericoid mycorrhizal (ErM) symbiosis. Regardless of their striking morphological divergence and evolutionary independence, all types ensure reciprocal exchanges between host plant and fungus. Plants allocate photosynthetic carbon (C) to their fungal symbionts in exchange for water and essential nutrients (Garcia et al., 2016; Martin et al., 2016; Rich et al., 2017). Regulation of these exchanges and the mechanisms by which nutrients impact the establishment and maintenance of mycorrhiza remain largely unclear (Carbonnel and Gutjahr, 2014; Garcia et al., 2015).

Recent evidence indicates that reciprocity in C/phosphorus (P) or C/nitrogen (N) exchanges determines sustainability in the symbiosis (Hammer et al., 2011; Kiers et al., 2011; Fellbaum et al., 2012; Bogar et al., 2019; Kafle et al., 2019). In mycorrhizal fungi, glucose availability triggers the degradation of soil organic N sources, induces N assimilation pathways, and alters the transcription of various N transporters (Fellbaum et al., 2012; Rineau et al., 2013). However, N provision towards the host plant does not always explain the amount of C that a particular fungal partner receives (Corr ea et al., 2015). Also, reduction of N supply by EcM fungi results in host defense response rather than in restricted C allocation (Hortal et al., 2017). This suggests that the regulation of nutrient exchange and its impact on maintenance of the symbiosis must be more complex than originally thought and might involve multiple elements. Experiments with *Oryza sativa* colonized by the AM fungus *Rhizophagus irregularis* revealed changes in C/N exchange ratio depending on Zn conditions (Corr ea et al., 2014). This indicates the synergy of N and Zn to be important for mycorrhizal plant responses. Therefore, the bioavailability and plant demand in micronutrients, particularly in Zn, seem to play a more important role in the maintenance of the AM symbiosis and C allocation than previously thought.

Micronutrients, such as iron (Fe), manganese (Mn), copper (Cu), and Zn, are essential for fungal and plant growth but become toxic when present in excess. Their homeostatic concentration ranges in most plants are narrow (Pilon et al., 2009; Sinclair and Kr amer, 2012; Connorton et al., 2017). Unlike macronutrients, such as N, P, potassium (K), magnesium (Mg) and sulfur (S), micronutrients are needed in low amounts but fulfill indispensable roles in cellular metabolism. They act as structural or catalytic compounds for many proteins thanks to their specific chemical properties (H ansch and Mendel, 2009). In particular, Zn homeostasis is of great interest since its non-optimal soil bioavailability is estimated to impact on agricultural production of 40% of cultivated lands (Alloway, 2008).

Low Zn bioavailability results in compromised growth and inferior grain quality for major crops (e.g. Johnson-Beebout et al., 2016), whereas its excess inhibits plant growth and results in waste lands (e.g. Chowdhary et al., 2018; He et al., 2019). Gathering additional knowledge on fungal Zn homeostasis, its regulation, and its impact on mycorrhizal symbiosis has the potential to lead to meaningful applications in agriculture and waste land management.

2. The dual effect of mycorrhizal fungi on plant micronutrient status

To balance micronutrient concentration in their tissues, plants develop various strategies, including modification of root architecture, change in chemistry of root exudates, or interaction with soil microbes like AM and EcM fungi (Rasouli-Sadaghiani et al., 2011; Ferrol et al., 2016; Nanda and Wissuwa, 2016). These fungi play a dual role on plant micronutrient status, either by improving their acquisition under limiting conditions, or by preventing their accumulation in plant tissues in contaminated soils. Moreover, mycorrhizal symbiosis alleviates plant stress induced by environmental micronutrient excess, through the activation of detoxification mechanisms (Bui and Franken, 2018; Cicatelli et al., 2010; Merlos et al., 2016). This results in considerable plant growth, fitness, and crop quality improvement (Watts-Williams et al., 2013), making possible the use of these fungi in phytoremediation/mycoremediation, as well as in biofortification practices (e.g. Abu-Elsaoud et al., 2017).

Both EcM and AM fungi contribute to Zn, Cu, Fe, and Mn acquisition under deficient conditions and alleviate their accumulation under potentially toxic conditions (e.g. Adriaensen et al., 2005; Canton et al., 2016; Liu et al., 2000, 2018). However, the involvement of mycorrhizal fungi in these responses greatly depends on the plant species or even cultivars. The AM fungus *R. irregularis* increases Cu tolerance of particular maize cultivars whereas it has no effect on growth in other cultivars (Merlos et al., 2016). Similar results were obtained when comparing plants growing on soils from Mexican Mn-mining sites, with AM fungi decreasing Mn uptake in native vegetation (*Ambrosia psilostachya*) but not in maize (Rivera-Becerril et al., 2013). This differential Mn response might ultimately have supported environmental adaptation of the particular native plant species. Facilitation of adaptation towards high environmental Cu by AM symbiosis was also described for *Elsholzia splendens* (Li et al., 2017), a Cu hyper-accumulating plant and indicator species of Chinese Cu mining sites (Lou et al., 2004).

Many crops colonized by AM fungi, including barley (Watts-Williams and Cavagnaro, 2018), tomato (Cavagnaro et al., 2006; Watts-Williams et al., 2015), maize (Jansa et al., 2003; Ortas, 2012), pepper (Ortas, 2012), or soybean (Ibiang et al., 2017) display higher Zn concentrations than non-mycorrhizal plants in control or Zn-limiting conditions. Interestingly, plant Zn acquisition via these symbiotic fungi depends on soil P concentrations (Watts-Williams and Cavagnaro, 2012; Watts-Williams et al., 2014; 2019), highlighting the synergy between nutrients in mycorrhizal

associations. In northern hemisphere forests, tree Zn nutrition is also greatly modulated by the presence of root colonizing EcM fungi (Adriaensen et al., 2004), as described for example for *Pinus radiata* and *Araucaria cunninghamii* (Bowen et al., 1974). The efficiency of mycorrhizal fungi to regulate plant Zn supply is not well understood, but we can reasonably assume that this might be related to their ability to tolerate a wide range of external Zn concentrations. Indeed, Zn tolerant *Suillus* isolates (EcM) protect their pine host more effectively from Zn toxicity compared to their non-tolerant relatives by reducing Zn translocation (Adriaensen et al., 2004). However, further studies are needed to characterize mechanisms of Zn homeostasis and transport and to investigate their natural diversity among mycorrhizal fungi.

3. Micronutrient transport and homeostasis in mycorrhizal symbiosis

Gathering knowledge on the transport of nutrients towards and throughout the fungal symbiont at the molecular level is essential for a good understanding of mycorrhizal symbioses. Undoubtedly, the most complete view on micronutrient acquisition, transport and translocation within mycorrhizal symbiosis is available from the AM fungus *R. irregularis* and its plant partners (Casieri et al., 2013). Different compounds of the high-affinity reductive Fe uptake system, including a ferric reductase RiFRE1 and two Fe permeases RiFTR1-2, were identified and functionally characterized by heterologous expression in yeast (Tamayo et al., 2018). Glutaredoxins, RiGRX4 and RiGRX5, are highly regulated by the presence of excess Fe. These oxidoreductases most likely impact on Fe homeostasis by influencing cellular redox state (Tamayo et al., 2016). Cu uptake in *R. irregularis* is mediated by RiCTR1, a plasma membrane localized CTR-family transporter, and regulated by environmental Cu availability. RiCTR2 is involved in the mobilization of Cu stored in the vacuoles under severe Cu deficient conditions. Interestingly, a particular splicing variant RiCTR3A of a CTR-like protein (RiCTR3) might function as a Cu receptor in sensing external Cu availability (Gómez-Gallego et al., 2019). Also, a metallothionein, RiMT1 (originally GintMT1), complexes excess cytosolic Cu (González-Guerrero et al., 2007). Moreover, fungal transporters supposed to be involved in micronutrient uptake are tightly regulated at the fungal–plant interface despite growth in control conditions (Tamayo et al., 2018; Gomez-Gallego et al., 2019). These observations highlight the limited knowledge still available on the importance of micronutrients in mycorrhizal symbiosis.

In other AM fungal species and EcM fungi, knowledge on the molecular mechanisms involved in micronutrient transport and homeostasis is rather scarce and limited to the characterization of several metallothioneins (Bellion et al., 2007; Lanfranco et al., 2002; Nguyen et al., 2017; Reddy et al., 2016) and a couple of Cu transporters in *Amanita strobiliformis* (Benes et al., 2018, 2016). Concerning Zn however, physiological and molecular mechanisms of transport and homeostasis are well explored in AM and EcM fungi and some data are also available for ErM fungi. Extending current knowledge on Zn transport, homeostasis and their regulation in different environmental and developmental conditions for several species

could shed light on the role of this particular element in mycorrhizal symbiosis and serve as a model to uncover the role of micronutrients in general.

Cellular zinc homeostasis in the mycelium

To ensure growth and overcome toxicity, mycorrhizal fungi need to control cytoplasmic Zn concentrations tightly by transport and compartmentalization into organelles (Fig. 1). Membrane transporters contribute to cellular Zn homeostasis by regulating Zn uptake, efflux, and redistribution. Vacuoles and ER-derived vesicles (so-called zincosomes; Blaudez and Chalot, 2011) are cellular Zn accumulation sites (Fig. 1a,g). Different metal transporter families that mediate Zn uptake or redistribution have been found in all organisms and are particularly well described in yeast (Gaither and Eide, 2001; Eide, 2006). The main transporter family for Zn release into the cytosol is the ZIP family (zinc-iron permease or ZRT-IRT-like Protein). Redistribution of Zn out of the cytosol towards intracellular organelles or the environment is mediated by members of the CDF family (Cation Diffusion Facilitator). However, ZIP and CDF families are not restricted to Zn transport and can also transport other metals, including Fe, Mn and Cu (Guerinot, 2000; Montanini et al., 2007). Members of both families harbor histidine-rich regions which are assumed to mediate metal selectivity. Yet, in certain conditions most members have the potential to transport multiple metals to a lower extent.

Members of the CDF and ZIP families are present in mycorrhizal fungi (Table 1; Tisserant et al., 2012; Kohler et al., 2015) but only few have been functionally characterized. As predicted, CDF transporters from mycorrhizal fungi are involved in Zn storage and exclusion (Fig. 1a,b,g). Zn homeostasis was modified in yeast by expression of the CDF member from the AM fungus *R. irregularis* GintZnT1 (ID: 70407; González-Guerrero et al., 2005). The CDF-type transporter HcZnT1 from the EcM fungus *Hebeloma cylindrosporum* is localized at the ER membrane and mediates specifically Zn tolerance upon functional expression in yeast, presumably by vesicle mediated efflux (Blaudez and Chalot, 2011). The homologue OmZnT1 has been identified in the ErM fungus *Oidiodendron maius* and attributed the same function based on heterologous expression experiments in yeast (Khouja et al., 2013). Two members of the CDF family were identified from the EcM fungus *Suillus luteus*, SlZnT1 (ID: 807028) and SlZnT2 (ID: 814105), and for SlZnT1 its contribution to transport Zn in excess towards the vacuole was suggested (Ruytinx et al., 2017). In *Russula atropurpurea*, RaCDF1 and RaCDF2 were identified. They both rescue the Zn sensitive phenotype of yeast mutants and localize to the tonoplast and plasma membrane, respectively (Sácký et al., 2016). ZIP family transporters were characterized in the EcM fungi *S. luteus* and *R. atropurpurea*. Respectively, SlZRT1 (ID: 22926) and RaZIP1 were predicted as plasma membrane transporters and were shown to mediate Zn uptake upon expression in yeast (Coninx et al., 2017; Leonhardt et al., 2018). In the ErM fungus *O. maius*, the Fe permease OmFET4 is suggested to import Zn and Fe in deficient conditions whereas it counteracts Zn toxicity by importing Mg in environmental Zn excess (Khouja et al., 2013).

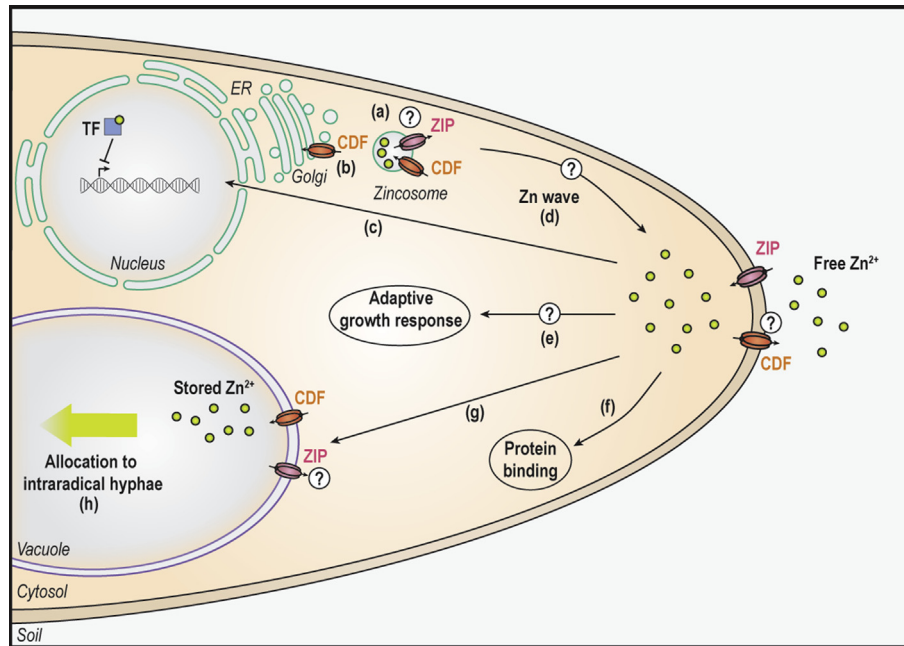


Fig. 1 – Working model of Zn homeostasis in mycorrhizal fungi, including potential cross-links with growth and development. Zn enters the cytosol mainly via ZIP transporters, and excess Zn is redistributed by CDF transporters towards cellular compartments or the environment. Thus, it may be imported into endoplasmic reticulum (ER) derived zincosomes (a) or directly into the ER (b) eventually followed by vesicle-mediated efflux. The cytosolic Zn pool supplies proteins, including transcription factors (c) and metallothionein-like zinc binding proteins (f), to support their structure and function. Although it should be demonstrated for mycorrhizal fungi, Zn is released in the cytoplasm from zincosomes and can lead to temporary concentration changes (Zn waves) (d). Alterations in cytoplasmic Zn concentrations result in an adaptive growth response mediated via an unknown regulation mechanism (e). Finally, Zn can also be stored in vacuoles (g) and may be allocated to intraradical hyphae to supply colonized plant roots through unknown delivery mechanisms (h).

In addition to tightly controlled membrane transport, Zn sequestration by bio-chelators contributes to the cellular Zn homeostasis. In particular, cysteine-rich metallothionein-like proteins (so-called zinc binding proteins) were identified in the Zn-accumulating EcM fungus *R. atropurpurea* (RaZBP1 and 2). They are described to bind a major part of the cellular Zn pool (Fig. 1f; Leonhardt et al., 2014). In vacuoles and vesicles, Zn is bound to polyphosphate granules (Bücking and Heyser, 1999; Olsson et al., 2011).

Zinc allocation into mycorrhizal roots

Extraradical hyphae of mycorrhizal fungi extend the rhizosphere, allowing the host plant to explore a larger volume of soil. As mentioned above, Zn is acquired by fungi through ZIP transporters (Fig. 1), stored into vacuoles through CDF transporters (Fig. 1g), translocated towards intraradical hyphae along with polyphosphates (Fig. 1h), released to the plant–fungus interface, and taken up by plant cortical cells (reviewed in Becquer et al., 2019 for EcM). The black-box in this cascade of events touches on the plant–fungus interface. Indeed, information about the molecular mechanisms driving Zn delivery from fungal hyphae to the symbiotic interface is sparse. For instance, no transport system, passive diffusion, or vesicle-dependent mechanism, has been identified so far. We can hypothesize that characterized fungal ZIP or CDF

transporters might facilitate Zn unload at the interface. It was shown recently in EcM symbiosis that the same fungal transporter is involved in the uptake of P from the soil and its release to plant cells, following an unknown regulatory mechanism (Becquer et al., 2018). On the plant side, genes encoding for ZIP transporters were found up- or down-regulated in AM roots, compared to non-mycorrhizal ones. More specifically, *HvZIP13* and *MtZIP6* transcripts were up-regulated in barley and *Medicago truncatula* AM roots, respectively, at low Zn (Watts-Williams et al., 2017; Watts-Williams and Cavagnaro, 2018). On the other hand, *MtZIP2* was found down-regulated in *M. truncatula* AM roots upon Zn fertilization, presumably preventing the plant from over-accumulating Zn (Burleigh et al., 2003). In the fungus, Zn excess results in either its immobilization by compartmentalization in vacuoles or its release outside the hyphae, preventing its transfer to the host plant (González-Guerrero et al., 2008; Ruytinx et al., 2013; Leonhardt et al., 2014).

Regulation of zinc transport in mycorrhizal fungi

Sensing the environment is required to support adaptive growth in suboptimal conditions. In response to external Zn availability, mycorrhizal fungi regulate the expression of some Zn transporters along with genes involved in primary metabolism (Fig. 1c, e) (González-Guerrero et al., 2005; Muller

Table 1 – Putative Zn ZIP and CDF transporters identified in selected AM and EcM fungi and the symbiotic regulation of their gene expression. Putative Zn transporters are organized following protein similarity and clusters are named according to their yeast ortholog. Ratio of expression levels (fold change) in symbiotic (ECM) to free-living mycelium (FLM), or intraradical (IRM) to extraradical mycelium (ERM), was calculated from published micro-array (*L. bicolor*; Plett et al., 2015) and RNAseq data (*R. irregularis*, Tisserant et al., 2012; all other species, Kohler et al., 2015). Genes more than two times up- or down-regulated upon symbiosis are highlighted in red and blue, respectively. “-”, non-detected transcript; blank, missing gene copy.

		AM		ECM									
		<i>Rhizophagus irregularis</i> DAOM 181602 v1.0		<i>Amanita muscaria</i> Koide v1.0		<i>Laccaria bicolor</i> S238N v1.0		<i>Paxillus involutus</i> ATCC 200175 v1.0		<i>Piloderma croceum</i> F 1598 v1.0		<i>Suillus luteus</i> UH-Slu-Lm8-n1 v1.0	
		JGI prot ID	Fold change IRM/ERM	JGI prot ID	Fold change ECM/FLM	JGI prot ID	Fold change ECM/FLM	JGI prot ID	Fold change ECM/FLM	JGI prot ID	Fold change ECM/FLM	JGI prot ID	Fold change ECM/FLM
ZIP-cluster	ZRT1/ZRT2	327155	8	783450	1.6 ± 0.13	180140	1.36 ± 0.37	169551	0.46 ± 0.05	187786	2.24 ± 0.11	22926	7.09 ± 0.18
				111574	1.76 ± 0.02	305445	0.18 ± 0.01	125102	0.32 ± 0.03	814564	0.55 ± 0.02	720881	2.33 ± 0.01
	YKE4	337446	-	159552	0.54 ± 0.01	189929	0.36 ± 0.06	117146	0.93 ± 0	407918	0.87 ± 0.04	811220	0.84 ± 0.05
	ZRT3	13899	-										
		336612	14										
CDF-cluster		70407	-	115937	1.18 ± 0.03	144453	0.55 ± 0.05	123727	1.16 ± 0.01	817242	1.64 ± 0.05	807028	0.94 ± 0.04
	ZRC1/COT1	286233	-	774413	13.17 ± 2.85	307944	2.17 ± 0.6			82630	1.53 ± 0.05	814105	0.2 ± 0.01
						305317	0.13 ± 0.02			94955	233.71 ± 29.1		
	MSC2	340453	0.9	515693	1.07 ± 0.05	191080	0.2 ± 0.03	165627	0.86 ± 0.03	827968	0.84 ± 0.02	72657	0.72 ± 0.05
	ZRG17	67256	0.9	11809	0.78 ± 0.01	296580	0.94 ± 0.15	10992	0.85 ± 0.06	819977	0.91 ± 0.08	798077	1.3 ± 0.01

et al., 2007; Coninx et al., 2017). Zn sensor(s) and eventual molecules involved in cellular signal transduction are unknown. Current knowledge of the regulation of homeostasis in response to Zn availability in fungi mainly relies on extensive experimentation in yeasts. Both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* sense Zn availability intracellularly by a transcription factor (Zhao and Eide, 1997; Corkins et al., 2013). Zn stabilizes binding of the particular transcription factor to promoter elements and alters directly the transcription of target genes (Wilson and Bird, 2016, Fig. 1a). Orthologs of the *S. cerevisiae* transcription factor are widely dispersed in fungi, including those with a mycorrhizal lifestyle (Tisserant et al., 2012; Kohler et al., 2015). Consequently, they might orchestrate Zn responses in these important root symbionts (Fig. 1a). Alternatively, Zn is sensed by yeast at the plasma membrane. The ZIP family Zn transceptor ScZRT1 has a dual function as Zn transporter and receptor. Changes in Zn availability trigger ScZRT1-dependent activation (cAMP-independent) of the protein kinase A (PKA) pathway (Schothorst et al., 2017). Zn-dependent activation of a phosphorylation pathway results in a swift adaptation of growth in changing environments (Fig. 1e). In plants, Zn is sensed by a ZIP transporter and was shown to induce phosphorylation pathways to result in local responses to micronutrient availability (Cointry and Vert, 2019). Zn availability alters root morphology and architecture through interaction with auxin metabolism (Sofo et al., 2017) but a direct interaction of ZIP transporter-activated phosphorylation pathways and auxin homeostasis is not yet investigated. Up to now, only two ScZRT1 orthologs in mycorrhizal fungi were functionally characterized (Coninx et al., 2017; Leonhardt et al., 2018). Their potential as activators of phosphorylation pathways is not yet evidenced. However, it might be worth to test this and eventual associated

implications in mycorrhizal symbiosis. A function as nutrient transceptor is proven for the phosphate transporter GigmPT of AM fungus *Gigaspora margarita* (Xie et al., 2016) and suggested for the CTR-like protein RiCTR3A of *R. irregularis* (Gómez-Gallego et al., 2019).

Several putative Zn transporters of the CDF and ZIP family are regulated in mycorrhizal fungi as a consequence of their symbiotic status (Table 1; Tisserant et al., 2012; Kohler et al., 2015). In the AM fungus *R. irregularis*, ZIP transporters localized at the plasma membrane and tonoplast show a 3 to 8-fold change in gene expression when comparing intra- and extra-radicular mycelium (Tisserant et al., 2012; Tamayo et al., 2014). Although it differs among species (Table 1), a stronger alteration of the expression of Zn transporters can be observed in EcM fungi upon symbiosis (e.g. 233-fold for a *Piloderma croceum* CDF transporter). It is still unclear whether Zn at the symbiotic interface is only restricted to a trophic resource that both symbiotic partners may compete for, as suggested in AM symbiosis (Tamayo et al., 2014). Indeed, Zn has the potential to act as a signaling molecule. In animals, Zn release from intracellular compartments or into the apoplasmic space results in Zn waves that facilitates the transduction of many signaling cascades in response to external stimuli (Yamasaki et al., 2007). Although it needs to be experimentally demonstrated, we hypothesize that Zn waves might also exist in fungi (Fig. 1d). Indeed, in the fungus *Candida albicans*, glucose is able to induce mobilization of intracellular Zn through the activation of the cyclic AMP pathway (Kjellerup et al., 2018). It will be worth exploring the influence of glucose, or other plant-derived molecules, on Zn metabolism in mycorrhizal fungi to elucidate the regulation of symbiotic Zn transporters, and the possible regulatory role of this micronutrient at the plant–fungus interface.

4. Conclusion

Despite many efforts in the characterization of transporters (González-Guerrero et al., 2005; Blaudez and Chalot, 2011; Sácáký et al., 2016; Coninx et al., 2017; Ruytinx et al., 2017; Leonhardt et al., 2018), our knowledge on cellular Zn and other micronutrient transport in mycorrhizal fungi remains fragmented. There is no single mycorrhizal fungus for which the complete Zn transportome, including all identified ZIP and CDF encoding genes, is functionally characterized. Observations only point to differences in transporter regulation due to external Zn availability and symbiotic status (Table 1). With around 9% of the proteome interacting with Zn for catalytic or structural traits (Andreini et al., 2006), it is clear that alterations in cytoplasmic Zn availability, even temporary, have far-reaching consequences and result in adaptive growth responses. A role for Zn as secondary messenger in symbiosis establishment and maintenance besides its trophic role deserves to be considered. Therefore, incorporating Zn and other micronutrients in the current models of symbiotic transport is urgently needed to understand better the links between nutrient availability, symbiosis development and functioning, and to provide valuable insights in how mycorrhizal fungi balance the nutritional status of plants.

Conflicts of interest

None.

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