Trends in Plant Science

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Opinion

Phosphorus Transport in Mycorrhiza: How Far Are We?

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Mycorrhizal fungi considerably improve plant nutrition and help them to cope with changing environments. Particularly, these fungi express proteins to transfer inorganic phosphate (P_i) from the soil to colonized roots through symbiotic interfaces. The mechanisms involved in P_i transfer from fungal to plant cells are still largely unknown. Here, we discuss the recent progress made on the description of these mechanisms and we propose the most promising hypotheses and alternative mechanisms for this process. Specifically, we present a phylogenetic survey of candidate P_i transporters of mycorrhizal fungi that might ensure P_i unload into the symbiotic interfaces. Gathering additional knowledge on mycorrhizal P_i transport will improve the P_i-useefficiency in agroecological systems and will guide towards addressing future research challenges.

Highlights

The acquisition of phosphorus by plants is often mediated by soil microbes colonizing the roots, particularly mycorrhizal fungi.

Key molecular mechanisms involved in the transport of phosphorus from the soil to mycorrhizal fungi have been revealed recently. However, the release of phosphorus towards colonized roots is still understudied, even if a recent report highlighted the possible involvement of fungal H^+:P_i transporters.

Based on a survey of fungal transport proteins, others mechanisms possibly ensuring phosphorus efflux in mycorrhizas must coexist besides H^+:P_i transporters. These include the putative involvement of P_i :Na^+, low-affinity inorganic phosphate, and organic phosphate transporters.

Unravelling the fungal phosphorus transportome will allow a better use of plant–fungus symbioses for the improvement of plant nutrition in cropping systems.

Phosphate Efflux in Mycorrhizal Roots: The Missing Step

The roots of most land plants fulfill an important part of their need for phosphorus (P) through the association with soil fungi, called mycorrhizal symbiosis [1,2]. Fungal species belonging to the Mucoromycota, subphylum Glomeromycotina, Ascomycota, and Basidiomycota phyla form different types of mycorrhizas [3]. **Arbuscular mycorrhizal (AM) symbiosis** (see Glossary), characterized by the formation of fungal **arbuscules** and/or **vesicles** within plant cortical cells, is formed by Glomeromycotina species. Other types of endomycorrhizas specific to Ericaceae and Orchidaceae species and called **ericoid** and **orchid mycorrhizas**, respectively, are also described [4,5]. Fungal species belonging to Ascomycota and Basidiomycota phyla can form both endomycorrhizas and more often **ectomycorrhizas**. In ectomycorrhizal (ECM) symbiosis, the fungus develops a **sheath** around short roots and a network of hyphae between plant cells, called the **Hartig net** [6]. Despite the great morphological differences between AM, ECM, and other types of mycorrhizas, the **mycelium** always differentiates in two pseudo-tissues: **extraradical hyphae** exploring the soil to gather water and nutrients and **intraradical hyphae** responsible for resource allocation to the plant. Whatever the type of mycorrhiza, there is no direct contact between fungal and plant cortical cells, which are separated by a common apoplast, also called the **symbiotic interface**. Therefore, water and nutrients transit through the plasma membrane of the 'donor' cell before absorption by the 'receiving' cell. Carbon in the form of sugars and/or lipids originating from the plant also transits by this interface through specialized proteins and is acquired by intraradical hyphae [7–11]. The ability of mycorrhizal fungi to facilitate the plant mineral acquisition depends on influx and efflux mechanisms in extraradical and intraradical hyphae, respectively. In both AM and ECM symbioses, proteins involved in influx transport have been well documented for water, macro-, and micronutrients (reviewed in [12–14]), whereas those enabling efflux at the symbiotic interface remain largely unknown, particularly for P [1]. In this opinion article, we (i) report the recent progress made on the description of fungal proteins involved in P delivery towards mycorrhizal roots, (ii) reveal that other efflux mechanisms must coexist in mycorrhizal fungi, and (iii) propose potential fungal candidates that might ensure this role.

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High-Affinity Transporters Mediate Inorganic Phosphate Efflux in Ectomycorrhizas

The simplest hypothesis to explain the fungal P delivery at the symbiotic interface is the release of free orthophosphate ions (P_i) through phosphate transporters (PT). Fungal P transport has been widely studied in baker's yeast (*Saccharomyces cerevisiae*), in which two **high-affinity P_i symporters** have been characterized, one coupled with H^+ (Pho84) [15] and one with Na^+ (Pho89) [16]. Interestingly, alignment of proteins retrieved from publicly available mycorrhizal species using the sequences of yeast Pho84 and Pho89 revealed the occurrence of two distinct groups: H^+:P_i transporters clustering with Pho84, and P_i:Na^+ transporters clustering with Pho89 [17]. A pioneering study identified a Pho84 ortholog in the AM fungus *Glomus versiforme*, GvPT, whose heterologous expression in yeast demonstrated its function as a high-affinity H^+:P_i transporter [18]. Ever since, further genes putatively encoding PT have been discovered in other AM species, *GiPT* in *Rhizophagus irregularis*, *GmosPT* in *Glomus mosseae*, and *GigmPT* in *Gigaspora margarita* [19,20]. Interestingly, mRNA and/or proteins of *GmosPT* and *GimPT* were found in plant cells containing arbuscules, suggesting their possible involvement in Pi efflux [19,20]. Orthologs were also functionally characterized in some ECM species, including *Hebeloma cylindrosporum* (*HcPT1.1*, *HcPT1.2*, and *HcPT2*), *Boletus edulis* (*BePT*), *Rhizopogon luteolus* (*RlPT*), and *Leucocortinarius bulbiger* (*LbPT*) [21–25]. HcPT1.1 and HcPT2 were both localized in the Hartig net hyphae of ectomycorrhizas [22,26], and the overexpression of *HcPT2* increased the export of P in the central cylinder of colonized maritime pine roots and the amounts of P accumulated in shoots, indicating that HcPT2 might mediate P_i efflux at the symbiotic interface [26]. HcPT2 of the ECM fungus *H. cylindrosporum* is the only candidate involved in fungal P_i efflux within mycorrhizas investigated so far.

Efflux of Inorganic Phosphate through H^+:P_i Symporters Is Tightly Regulated by Undescribed Mechanisms

Determining whether an efflux of P_i is possible through fungal H^+:P_i transporters from a thermodynamic point of view is a key question that was addressed recently in AM symbiosis [27,28]. Indeed, computational modeling was used to simulate *in silico* the conditions required for P_i exchange through fungal and plant H^+:P_i transporters located at the symbiotic interface of AM roots. Briefly, the authors modelized a network based on the activity of fungal and plant membrane ATPases that release protons into the interface. This determines the pH value at the symbiotic interface and the activity of H^+:P_i transporters from both partners. Based on this model, P_i concentrations in both fungal and plant cytosols are the only factors determining P_i movements through the symbiotic interface. Finding and characterizing the molecular players driving this transport through the symbiotic interface is challenging since only a few fungal (reviewed here) and plant members [29–31] have been identified so far. Further studies are indeed needed to evaluate the efflux and influx capacities of both fungal and plant H^+:P_i transport systems expressed at the symbiotic interface and to validate *in vivo* the computational models developed by Schott *et al.* [27] and Dreyer *et al.* [28].

Alternatively, P_i efflux might also result from plant-inducible modifications of fungal H^+:P_i transporters that modify their expression and/or activity. This hypothesis is supported by the measure of differential efflux of ^{32}P in the ECM fungus *H. cylindrosporum* overexpressing HcPT2 after incubation into a solution mimicking the symbiotic interface, with or without the plant [26]. Two days after incubation, the mycelia overexpressing HcPT2 released less than 2% of ^{32}P when incubated without the plant, but significantly more (17%) than the control strains (10.5%) in presence of the roots. These data confirm that P efflux from fungal cells remains low without the plant, and suggest that the host plant somehow controls the HcPT2-dependent efflux of P_i. It is worth hypothesizing that an unknown signal originating from the plant might affect the

Glossary

Arbuscular mycorrhiza: symbiotic association between roots of most land plants and fungi belonging to the Mucoromycota phylum, Glomeromycotina subphylum.
Arbuscule: highly ramified fungal structure developing within plant cortical cells in arbuscular mycorrhizal symbiosis.
Ectomycorrhiza: symbiotic association between roots from trees and shrubs and fungi belonging to the Ascomycota and Basidiomycota phyla.
Ericoid mycorrhiza: symbiotic association between roots from Ericaceae plants and mycorrhizal fungi.
Extraradical hyphae: hyphae exploring the soil in mycorrhizas to gather water and nutrients.
Hartig net: fungal hyphae internally colonizing roots between plant cortical cells in ectomycorrhizal symbiosis. Described for the first time by Robert Hartig.
High-affinity P_i symporters: proteins showing saturable kinetics and mediating the transport of P_i available at low concentrations (μM range) across membranes. In yeast, two high-affinity P_i transporters are known, Pho84 and Pho89, which are both located at the plasma membrane. Pho84 is a symporter (see the definition below) cotransporting protons and P_i, and Pho89 is a symporter cotransporting sodium ions and P_i.
Intraradical hyphae: hyphae internally colonizing plant roots in mycorrhizas.
Low-affinity P_i transporters: proteins showing linear kinetics and mediating the transport of P_i available at high concentrations (mM range) across membranes. In yeast, three low-affinity P_i transporters are known; two of them (Pho87 and Pho90) are symporters (see the definition below) cotransporting Na^+ and P_i and are located at the plasma-membrane. The third one (Pho91) is located at the tonoplast and is supposed to export P_i from the vacuole towards the cytosol.
Mycelium: vegetative part of a fungus, consisting of a network of branching and threadlike hyphae, often underground.
Orchid mycorrhiza: symbiotic association between roots from Orchidaceae plants and mycorrhizal fungi.
Sheath: layers of hyphae encompassing tree roots in ectomycorrhizas.

expression and/or activity of HcPT2. Interestingly, it was recently demonstrated that effector-like proteins from poplar were induced upon ECM symbiosis and even enter into *Laccaria bicolor* hyphae [32]. We can hypothesize that these plant effectors might control nutrient delivery from the fungus through the regulation of transporter expression and/or activity. Fungal effectors were also reported in multiple sequenced mycorrhizal fungi but only a couple of them were described as plant defense modulators, allowing the fungal colonization [33–36]. Consequently, we can also hypothesize that fungal effectors might modulate the activity of plant carbon transporters, regulating the carbon delivery from the host to fungal cells. The investigation of such mutual regulations will certainly provide a deeper understanding of the P transport from fungi to plants and will help to fine-tune and improve the existing *in silico* models.

The Intriguing Case of High-Affinity P_i:Na^+ Symporters

Based on recent data reporting the role of HcPT2 in ECM symbiosis [26], it is tempting to hypothesize that H^+:P_i, and particularly PT2-like transporters, could be the main proteins unloading P_i into the symbiotic interface. Here, we show that all studied mycorrhizal fungi, except Sebacinales, harbor at least one ortholog of H^+:P_i PT1-like transporter, whereas most Ascomycota (truffles and parented species) and Glomeromycotina species do not have a PT2-like member (Figure 1). This indicates that alternative mechanisms for Pi efflux at the symbiotic interface must coexist in mycorrhizal fungi. The only Basidiomycota lacking PT2-like members is *Amanita muscaria*, but its genome harbors a high number of *PT1*-like genes compared with other species (Figure 1). This suggests that one PT1-like protein or other types of transporters might ensure P_i efflux into the interface.

In all other fungi, the lack of PT2-like transporter is correlated with the presence of at least one protein clustering with Pho89 that codes for a high-affinity P_i:Na^+ transporter. Moreover, most Basidiomycota harboring a PT2-like ortholog do not possess any P_i:Na^+ transporter protein. One can argue that the presence and conservation of this protein might be related to the lifestyle of these fungi, including *R. irregularis* (AM) and *Tuber* species (ECM) that can be found in neutral or alkaline pH soils, respectively. In these conditions, the lack of protons in the soil solution would prevent the energization of Pi uptake by the fungal cells through H^+:P_i transporters. The acquisition of Pi would be only possible through P_i:Na^+ transporters. Along the same line, P_i:Na^+ transporters might also mediate P_i efflux at the interface independently of proton gradients occurring between the neutral cytosol and the acidic apoplastic space. For example, it was described that some Ascomycota species can better tolerate high external NaCl concentrations than Basidiomycota, indicating a greater disposition of these fungi to mobilize and utilize sodium ions [37]. However, other Basidiomycota species can also efficiently tolerate, and help host plants to tolerate, salt stress conditions(e.g., through the expression of Na^+:H^+ transporters) [38,39]. Consequently, the presence or absence of fungal P_i:Na^+ transporters is certainly not the only feature for salt stress tolerance in mycorrhizal plants and various mechanisms must coexist (reviewed in [40]). Investigating the role of fungal P_i:Na^+ transporters in mycorrhizas is one logical next step, since we do not have any data yet on their transport activity, *in situ* localization, and symbiotic function.

Low-Affinity Transporters Could Also Mediate Inorganic Phosphate Efflux in Mycorrhiza

So far, the most studied mechanisms for P_i efflux in mycorrhizal symbiosis have focused on H^+:P_i transporters. In yeast, P_i can also be transported through three low-affinity transporters, described as Na^+/dicarboxylate/sulfate/P_i transporter in databases (Pho87, Pho90, Pho91)[41]. When Pho84 and Pho89 are either not expressed in yeast or degraded at high P_i, Pho87 and Pho90 are located at the plasma membrane and ensure P_i acquisition [41–43]. Pho91 is localized at the vacuolar membrane of yeast cells and ensures the export of P_i towards the cytosol [44].

Symbiotic interface: (synonymous, apoplast) the cellular space between plant and fungal membranes, delimiting the site of reciprocal nutrient exchanges between partners.
Symporters: proteins mediating the transport of different types of molecules against their concentration gradient using the transport of another molecule (protons or sodium ions) along their concentration gradient, previously created by the cell (i.e., ATPase activity extruding protons outside the cytosol).
Transportome: repertoire of genes encoding proteins responsible for the transport of molecules across cellular membranes.
Vesicles: fungal storage structure developing within plant cortical cells in arbuscular mycorrhizal symbiosis.

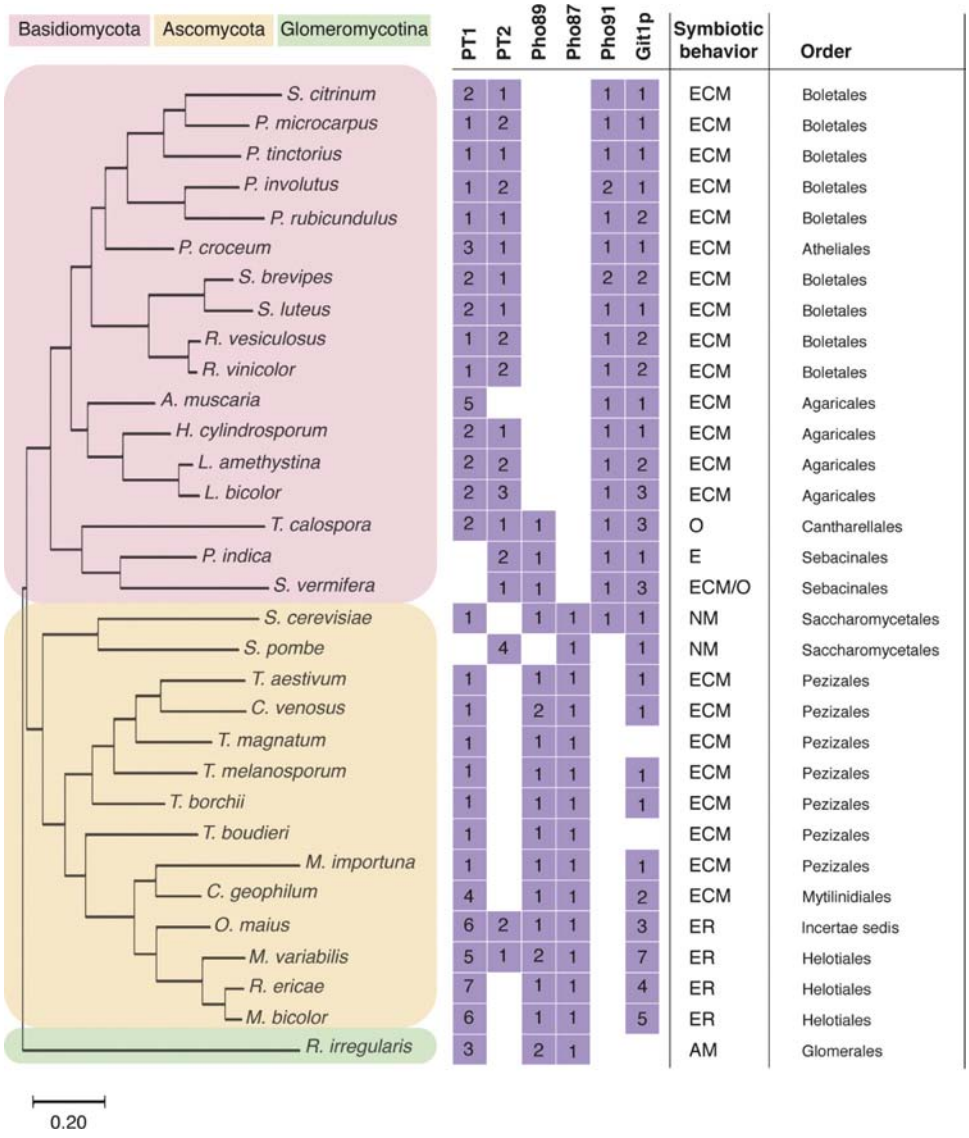

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Figure1. Number of Predicted Phosphorus Transport Proteins in Publicly Available Sequenced Mycorrhizal Fungi. Partial 18S ribosomal RNA gene sequences were retrieved from NCBI database and aligned using multiple sequence comparison by log-expectation alignment (MUSCLE). The phylogenetic tree was constructed in MEGA7 [55] using the Maximum Likelihood method based on the JTT matrix-based model [56]. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Predicted phosphorus transport proteins were retrieved from the Mycocosm database (https://genome.jgi.doe.gov/programs/fungi/index.jsf) and used to construct two phylogenetic trees (data not shown) with the same method as for species evolution, but using 100 replicates for bootstrap. The first tree was generated with predicted proteins retrieved with HcPT1.1 (PT1 [46]) or HcPT2 (PT2 [46]) from *Hebeloma cylindrosporum* as query in BlastP. The retrieved proteins clustered into four distinct groups: high-affinity H^+:Pi transporters orthologous to HcPT1 (PT1) or HcPT2 (PT2), high-affinity Pi:Na^+ transporters orthologous to Scpho89 (Pho89) from *Saccharomyces cerevisiae*, and P-diester transporters orthologous to ScGit1p (Git1p) from *S. cerevisiae*. The second tree was generated with predicted proteins retrieved with ScPho87, ScPho90, and ScPho91 proteins, the **low-affinity Pi transporters** from *S. cerevisiae*. The predicted proteins clustered either with ScPho87 (Pho87) or with ScPho91 (Pho91). The fungal genera used to construct the tree were, by alphabetic order: *Amanita* (*A. muscaria* [57]), *Cenococcum* (*C. geophilum* [58]), *Choiromyces* (*C. venosus* [59]), *Hebeloma* (*H. cylindrosporum* [57]), *Laccaria* (*L.*

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These three proteins are characterized by an SPX domain, which is a 180-residue length domain that interacts with cytosolic 5-inositol-P7,the level of which reflects yeast P status [45,46]. In mycorrhizal fungi, only one putative protein corresponding to yeast Pho87/Pho90/Pho91 can be found, except in *Pisolithus tinctorius* and *Paxillus involutus*, which have two orthologs. Interestingly, the identified protein clusters either with Pho91 in Basisdiomycota species or with Pho87 in Ascomycota and Glomeromycotina fungi (Figure 1). There is no data yet on the regulation, localization, and function of the corresponding proteins in mycorrhizal fungi. Although their role at the vacuolar membrane to release P in the cytosol would make sense based on their yeast orthologs, we cannot exclude that these proteins could be addressed to the plasma membrane and participate in P_i efflux into the symbiotic interface [26,47,48].

Organic Phosphate, an Alternative Phosphorus Source in Mycorrhizas?

We assumed for decades that the form of P released to the symbiotic interface is P_i. However, most mycorrhizal species also harbor at least one ortholog of the yeast organic P transporter, ScGit1p (Figure 1). In yeast, this protein is upregulated at low P and is able to import several phospho-diesters, which are by order of preference: glycerophosphoinositol (GroPIns) ≫ glycerophosphoserine ≫ glycerol-3-phosphate ≫ glycerophosphoethanolamine ≫ glycerophosphocholine [49]. A release of GroPIns has been measured in culture media only when yeasts were grown in presence of inositol [49]. The production of external GroPIns is increased by addition of glucose, along with GroPIns 4-phosphate and GroPIns 4,5-bisphosphate [50]. In addition, the production of GroPIns is due to the deacylation of phosphatidylinositol, a membrane phospholipid, suggesting that the turn-over of fungal membrane phospholipids might represent a pool of organic P. Hence, if phospho-diester compounds are released at the symbiotic interface of mycorrhizas, we hypothesize that these compounds might be an important P source for the host due to high concentrations in glucose originating from plant cells. However, none of these mechanisms have been identified thus far.

Concluding Remarks and Future Perspectives

Although the improvement of plant P nutrition by mycorrhizal fungi was described for the first time more than 50 years ago [51,52], critical steps in the transport of P from the soil to colonized roots are still missing. Specifically, only a handful of candidate proteins possibly involved in the release of P from fungal cells into the symbiotic interface have been reported so far in both AM and ECM fungi [53]. With our present survey of fungal P transporters putatively involved in this crucial step, we have proposed a hypothetical model for P efflux into the symbiotic interface, regrouping all identified fungal candidate proteins (Figure 2). This model clearly highlights differences between fungi forming ectomycorrhizas (Ascomycota and Basidiomycota), arbuscular mycorrhizas, ericoid mycorrhizas, and orchid mycorrhizas, and suggests that P efflux into the symbiotic interface is more complex than originally thought (see Outstanding Questions). However, validating the symbiotic role of these proteins is still challenging since molecular tools and transformable species are still lacking for mycorrhizal fungi. Thus, efforts in this direction are still needed to describe and unravel the mycorrhizal P **transportome** and to improve the use of plant–fungus symbioses in natural and agroecosystems [54].

Outstanding Questions

Are there other alternative mechanisms for fungal inorganic phosphate delivery that are still undescribed?

Is organic phosphate an alternative phosphorus source in mycorrhizas?

What are the plant transporters involved in phosphorus acquisition originating from the fungus in ectomycorrhizas, ericoid mycorrhizas, and orchid mycorrhizas?

What are the plant signals, if any, triggering the release of phosphorus into the symbiotic interface?

What are the molecular players involved in the formation, stabilization, and transport of polyphosphates in mycorrhizal fungi?

Is the release of phosphorus from fungal cells into the symbiotic interface dependent of carbon supply from the plant cortical cells? Reciprocally, is the release of carbon from plant cortical cells into the interface dependent on phosphorus supply from the fungal cells?

amethystina, *L. bicolor* [57]), *Meliniomyces* (*M. variabilis*, *M. bicolor* [60]), *Morchella* (*M. importuna* [59]), *Oidiodendron* (*O. maius* [57]), *Paxillus* (*P. involutus*, *P. rubicundulus* [57]), *Piloderma* (*P. croceum* [57]), *Piriformospora* (*P. indica* [61]), *Pisolithus* (*P. microcarpus*, *P. tinctorius* [57]), *Rhizophagus* (*R. irregularis* [62]), *Rhizopogon* (*R. vesiculosus*, *R. vinicolor* [63]), *Rhizoscyphus* (*R. ericae* [60]), *Saccharomyces* (*S. cerevisiae* [64]), *Schizosaccharomyces* (*S. pombe* [65,66]), *Scleroderma* (*S. citrinum* [57]), *Sebacina* (*S. vermifera* [57]), *Suillus* (*S. brevipes* [38], *S. luteus* [57]), *Terfezia* (*T. boudieri* [59]), *Tuber* (*T. aestivum* [59], *T. magnatum* [59], *T. melanosporum* [67], *T. borchii* [59]), and *Tulasnella* (*T. calospora* [57]). The symbiotic behavior was non-mycorrhizal (NM), endophytic (E), or ectomycorrhiza (ECM), orchid (O), ericoid (ER) or arbuscular (AM) mycorrhiza forming.

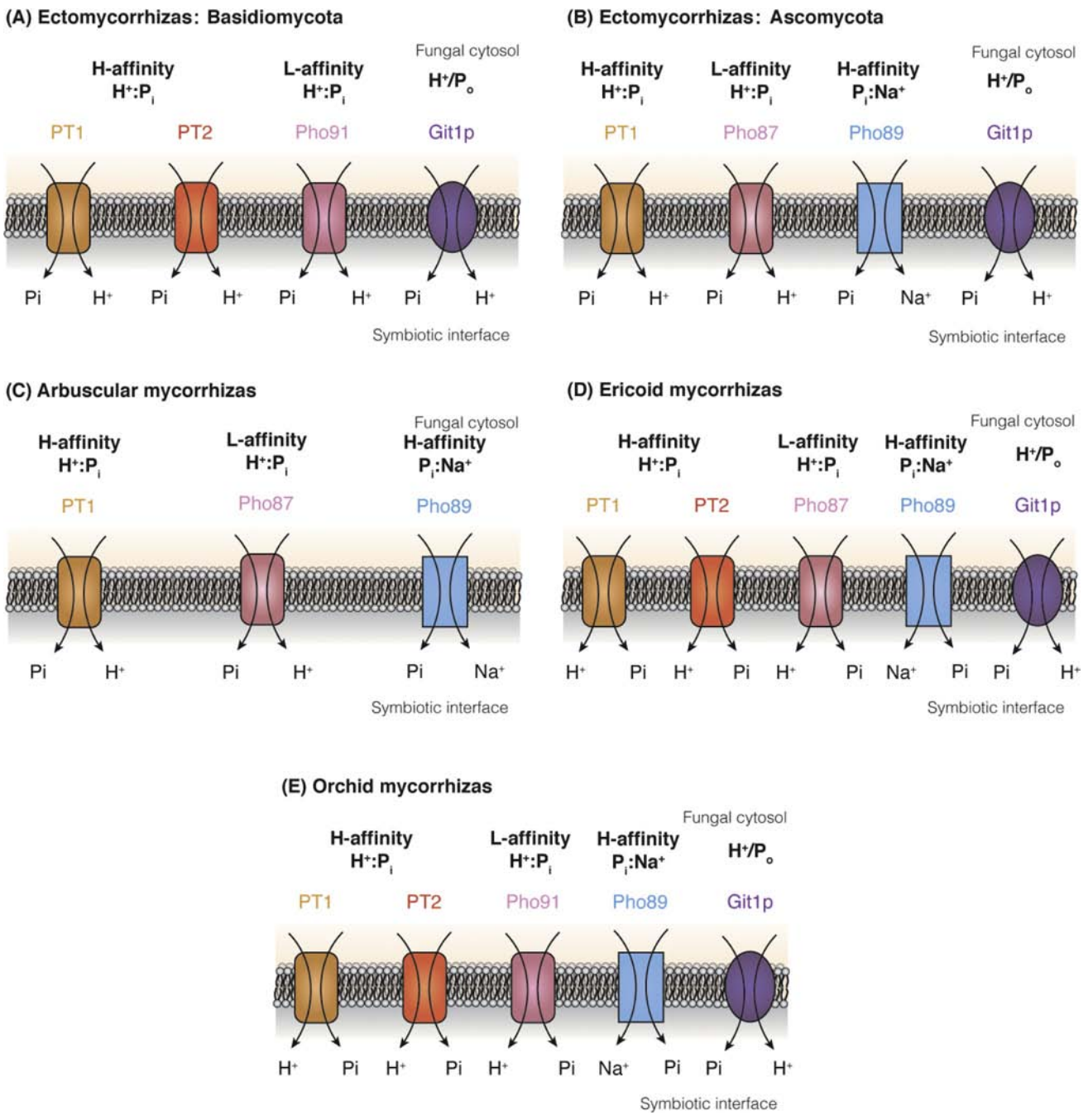

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Figure2. Hypothetical Model of Phosphorus Efflux from Fungal Cells to the Symbiotic Interface in Different Types of Mycorrhizas. Based on our survey of putative transport proteins in mycorrhizal fungi (Figure 1), we propose that the transport of phosphorus from the cytosol of intraradical hyphae to the symbiotic interface might be mediated by PT1-like (A–E) and PT2-like (A,D,E) high-affinity (H-affinity) H^+:P_i transporters; Pho87-like (B–D) or Pho91-like (A,E) low-affinity (L-affinity) H^+:P_i transporters; Pho89-like high-affinity P_i:Na^+ transporters (B–E); or Git1p-like organic phosphate (P_o) transporters (A,B,D,E). (A) Ectomycorrhizal symbiosis formed by Basidiomycota species, (B) ectomycorrhizal symbiosis formed by Ascomycota species, (C) arbuscular mycorrhizal symbiosis, (D) ericoid mycorrhizal symbiosis, and (E) orchid mycorrhizal symbiosis.

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