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Phosphate Uptake and Transport in Plants: An Elaborate Regulatory System

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Phosphorus (P) is an essential macronutrient for plant growth and development. Low inorganic phosphate (Pi) availability is a limiting factor for plant growth and yield. To cope with a complex and changing environment, plants have evolved elaborate mechanisms for regulating Pi uptake and use. Recently, the molecular mechanisms of plant Pi signaling have become clearer. Plants absorb Pi from the soil through their roots and transfer Pi to various organs or tissues through phosphate transporters, which are precisely controlled at the transcript and protein levels. Here, we summarize recent progress on the molecular regulatory mechanism of phosphate transporters in Arabidopsis and rice, including the characterization of functional transporters, regulation of transcript levels, protein localization and turnover of phosphate transporters. A more in-depth understanding of plant adaptation to a changing Pi environment will facilitate the genetic improvement of plant P efficiency.

Keywords: Molecular regulation • Phosphate homeostasis • Phosphate transporters • Protein degradation • Protein trafficking.

Introduction

Phosphorus (P) is one of the few macronutrients essential for the growth and development of all organisms. It serves as an integral building block of crucial compounds, including DNA, RNA, sugar-phosphate intermediates for glycolysis, respiration and photosynthesis, the phospholipids that compose membranes and various phosphorylated compounds in many different reactions (Raghothama 1999). Plants take up inorganic phosphate (Pi) as their main source of P, although it is usually complexed with metal ions in the soil, making it difficult for plants to absorb. Therefore, plants have evolved elaborate mechanisms to deal with Pi deficiency, including biochemical and metabolic adaptations to increase the availability of external and internal Pi (Puga et al. 2017). In plants, Pi is taken up into root cells, loaded into the xylem through symplastic or apoplastic pathways, transported to the shoot via xylem flow driven by transpiration and distributed among different shoot tissues through the phloem (Poirier and Bucher 2002). These steps involve specific Pi transporters.

Pi transporters are responsible for Pi uptake, translocation and remobilization. Recent studies indicate that their abundance and activity are exquisitely regulated (Chen et al. 2015, Wang et al. 2018, Yang et al. 2020b). In this review, we summarize recent findings on the complex regulation of Pi transporters, which includes regulation of the transcription rate of their genes, the abundance and stability of their transcripts, the targeting of the proteins to membranes and the abundance of the proteins at the posttranslational level.

The Variety of Functional Phosphate Transporters in Plants

Phosphate transporters belong to different protein families, e.g. the phosphate transporter (PHT) family, the SYG1/Pho81/XPR1 (SPX) domain-containing protein family and the SULFATE TRANSPORTER (SULTR)-like family. Based on their subcellular localization and sequence identity, PHT family members are classified into five types: PHT1 (mainly plasma membrane), PHT2 (chloroplasts), PHT3 (mitochondria), PHT4 (chloroplasts, the Golgi apparatus and non-photosynthetic plastids) and PHT5 (vacuoles) (Liu et al. 2016, Versaw and Garcia 2017). Individual group members are designated by the family type (PHTx, with $x = 1–5$) followed by a semi-colon and a number. SPX domain-containing proteins are divided into four subfamilies depending on the presence of a second domain: SPX, SPX-EXS (ERD1/XPR1/SYG1), SPX-MFS (Major Facilitator Superfamily) and SPX-RING (Really Interesting New Gene) (Secco et al. 2012). Within the SPX domain-containing protein family, SPX-MFS domain proteins (also grouped into the PHT5 family) and SPX-EXS domain proteins have been reported to act as phosphate transporters (Wang et al. 2012, Liu et al. 2015, Wang et al. 2015a, Liu et al. 2016). In addition, the SULTR-like family member OsSULTR3;4 was recently shown to be a Pi transporter that is involved in Pi distribution to rice grain (Yamaji et al. 2017). In this review, we mainly focus on recently identified plant phosphate transporters.

PHT1 family transporters

PHT1-family transporters localize to the plasma membrane and use a Pi/H^+ symport mechanism to take up Pi from the soil. This family consists of 9 members in Arabidopsis (*Arabidopsis thaliana*) and 13 in rice (*Oryza sativa*). PHT1-family transporters have been shown to mediate Pi acquisition from the soil environment and/or Pi translocation between cells or tissues. The functions of PHT1-family phosphate transporters have been extensively reviewed (Zhang et al. 2014, Gu et al. 2016, Wang et al. 2018, He et al. 2019). Recently, OsPHT1;3 was also identified which plays a crucial role in Pi absorption, root-to-shoot translocation and remobilization within the plant under extremely low Pi conditions (Chang et al. 2019). Furthermore, OsPHT1;3 was reported to physically interact with OsPHT1;2 (Chang et al. 2019); Similarly, the Arabidopsis PHT1;1 and PHT1;4 proteins can form homomeric and heteromeric complexes (Fontenot et al. 2015). These results indicate that PHT1 proteins may form oligomeric structures in both monocots and dicots, although the exact biological significance of this interaction is largely unknown.

PHT2/3/4 family transporters

The characterized members of the PHT2/3/4 families are intracellular Pi transporters that are involved in transporting Pi into or out of subcellular organelles (e.g. mitochondria, plastids or Golgi), and they localize to mitochondria, plastids or the Golgi, where they participate in energy metabolism and stress responses (Versaw and Garcia 2017). The functions of Arabidopsis PHT2;1, PHT3, PHT4;1 and PHT4;4 have been the subject of several reviews (Zhang et al. 2014, Gu et al. 2016, Versaw and Garcia 2017, Wang et al. 2018). In brief, Arabidopsis PHT2;1, PHT4;1 and PHT4;4 mediate Pi homeostasis in chloroplasts (Versaw and Harrison 2002, Karlsson et al. 2015, Miyaji et al. 2015), while PHT3 carries out a similar role in mitochondria (Zhu et al. 2012).

Like its Arabidopsis ortholog, OsPHT2;1 was also demonstrated to be a chloroplast Pi influx transporter in rice. Overexpression of *OsPHT2;1* increases Pi content in the shoot under low Pi conditions. Conversely, the loss of OsPHT2;1 function reduces Pi accumulation under Pi-deficient conditions, in addition to altering sucrose and starch content and reducing the accumulation of flavonoids and thus UV tolerance (Liu et al. 2020). Although PHT2/3/4-family proteins play crucial roles in intracellular Pi homeostasis, how Pi homeostasis is established among various subcellular organelles is not clear. It will be of significance to uncover the mechanisms underlying their regulation by Pi levels.

Vacuolar transporters (PHT5 family proteins)

The vacuole is the largest organelle in plant cells and stores over 90% of intracellular Pi under Pi-sufficient conditions. The movement of Pi into and out of vacuoles is very important to buffer cytoplasmic Pi concentrations against fluctuations in external Pi. OsSPX-MFS1 was suggested to be a vacuolar Pi influx transporter that plays a key role in preserving Pi homeostasis in leaves (Wang et al. 2012). Arabidopsis PHT5;1/VPT1, a homolog of OsSPX-MFS1, was also identified as a Pi influx transporter. Mutations in *AtPHT5;1/VPT1* result in lower vacuolar Pi, while plants overexpressing *AtPHT5;1/VPT1* exhibit higher vacuolar Pi and total Pi content (Liu et al. 2015, Liu et al. 2016). OsSPX-MFS3 also operates as a vacuolar Pi transporter, based on electrophysiological and phenotypic analyses (Wang et al. 2015a). The characterization of the *Osspx-mfs3* mutant corroborates these observations, as it shows significantly reduced vacuolar Pi content, a phenotype opposite to that of *OsSPX-MFS3* overexpression lines (Xu et al. 2019). Recently, rice VACUOLAR PHOSPHATE EFFLUX 1 (VPE1) and VPE2, two plasma membrane-localized glycerol-3-phosphate transporter family proteins, were reported as vacuolar Pi efflux transporters (Xu et al. 2019). OsVPE1/2 can transport Pi into yeast (*Saccharomyces cerevisiae*) cells and frog (*Xenopus laevis*) oocytes. Furthermore, the *osvpe1 osvpe2* double mutant displays a higher vacuolar Pi level, while lines overexpressing *OsVPE1* or *OsVPE2* accumulate less Pi in their vacuoles than the wild type. However, how vacuolar Pi transporters achieve coordination with each other and whether there are other Pi transporters involved in vacuole Pi homeostasis need further investigation.

Other phosphate transporters

Arabidopsis PHOSPHATE 1 (PHO1;H1, hereafter PHO1) belongs to the SPX-EXS subfamily and localizes to the Golgi and trans-Golgi networks, which is potentially a phosphate transporter, although there is currently no direct measurement of its Pi transport activity. PHO1 was reported to mediate Pi efflux out of cells and loading of Pi into the xylem (Stefanovic et al. 2011, Arpat et al. 2012, Wang et al. 2020a). Arabidopsis PHO1 and its closest homolog PHO1 HOMOLOG 1 (PHOH1) are also involved in transferring Pi from the seed coat to the embryo in developing seeds (Vogiatzaki et al. 2017). A similar function has been assigned to rice OsPHO1;1 and OsPHO1;2, as they deliver Pi from maternal to embryo tissues during seed development. *OsPHO1;2* is expressed in node I, the uppermost node connecting the panicle to the main stem in rice. OsPHO1;2 is responsible for unloading Pi from the xylem of enlarged vascular bundles, while OsPHO1;1 reloads Pi into the phloem of diffuse vascular bundles for distributing Pi to seeds (Che et al. 2020). Notably, this study also demonstrated that OsPHO1;1 and OsPHO1;2 localize to the plasma membrane, which differs from previous reports that had determined PHO1 localized to the endomembrane system (Stefanovic et al. 2011, Liu et al. 2012, Vogiatzaki et al. 2017). Regardless, it would be interesting to understand how PHO1 exports Pi out of the cell in cases where it is localized inside the cell.

The SULTR family members OsSULTR3;3 and OsSULTR3;4 are likely involved in P accumulation in rice grains (Zhao et al. 2016, Yamaji et al. 2017). *OsSULTR3;3* was identified from a classical genetics screen for low phytic acid (PA) mutants (Zhao et al. 2016). PA and total P content are significantly lower in the *ossutr3;3* mutant. However, OsSULTR3;3 had no detectable transporter activity for phosphate, sulfate, inositol or

inositol 1,4,5 triphosphate when tested heterologously in yeast and Xenopus oocytes (Zhao et al. 2016). OsSULTR3;4, also called SULTR-like phosphorus distribution transporter (OsSPDT), is a plasma membrane-localized phosphate transporter that distributes P from node I to the grain. An insertional allele of *OsSPDT* caused increased P content in leaves and decreased P content in grains but no effect on yield, seed germination or seedling growth (Yamaji et al. 2017). The presumed Arabidopsis ortholog of OsSPDT, AtSPDT, mainly localizes to the vascular cambium of most organs and preferentially allocates Pi to developing tissues (Ding et al. 2020). These studies indicate that some SULTR-like transporters can function as Pi transporters.

Transcriptional and Posttranscriptional Regulation of Phosphate Transporter Abundance

The accumulation of PHT1-family transporters is regulated on at least three distinct levels: *PHT1* transcript levels, the correct targeting of PHT1 transporters to the plasma membrane, and PHT1 protein levels. Transcriptional control of phosphate transporter levels was the first such control mechanism that was experimentally established.

Transcription factors regulate the transcription of phosphate transporter genes

Most phosphate transporters are induced by low Pi conditions at the transcriptional level, a response that is modulated by transcription factors in the nucleus. Chlamydomonas (*Chlamydomonas reinhardtii*) PHOSPHORUS STARVATION RESPONSE 1 (PSR1) and related transcription factors Arabidopsis PHOSPHATE STARVATION RESPONSE 1 (PHR1) and rice PHR2 are involved in the transduction of phosphate starvation signals. They bind to the PHR1-binding site (P1BS element) in the promoter of phosphate transporter genes and other Pi starvation-responsive genes (Rubio et al. 2001, Zhou et al. 2008, Puga et al. 2014, Wang et al. 2014, Zhong et al. 2018). Other transcription factors from the WRKY and MYB domain-containing families also participate in the regulation of *PHT1* gene transcription [for details, see recent reviews (Gu et al. 2016, Wang et al. 2018, He et al. 2019)]. For example, in rice, WRKY21 and WRKY108 activate the expression of *OsPHT1;1* under Pi-sufficient conditions to promote Pi accumulation (Zhang et al. 2020a). Likewise, the GARP-type transcription factors NITRATE-INDUCIBLE GARP-TYPE TRANSCRIPTIONAL REPRESSOR 1 (NIGT1.1-NIGT1.4), also known as HYPERSENSITIVITY TO LOW PI-ELICITED PRIMARY ROOT SHORTENING 1 (HRS) and HRS1-HOMOLOG (HHO), regulate nitrate signaling and transduce Pi starvation signals in Arabidopsis and Maize (Medici et al. 2015, Maeda et al. 2018, Ueda et al. 2020, Wang et al. 2020b). For instance, NIGT1.2 directly upregulates the transcript levels of *PHT1;1* and *PHT1;4* in Arabidopsis to increase Pi uptake under Pi starvation conditions (Wang et al. 2020b). *NIGT1* transcription is also regulated by PHR1, while NIGT1 regulates the expression of *SPX 1–3* genes, which encode negative regulators of PHR1 function (Maeda et al. 2018, Ueda et al. 2020).

Proteins regulate the transcription of phosphate transporter genes by affecting the function of related transcription factor(s)

Just as a series of transcription factors regulate the transcription of phosphate transporter genes, a number of proteins modulate the activity of these transcription factors.

AtPHR1/OsPHR2 transcript levels are almost unaffected by Pi status, as are the protein levels of their translation products (Rubio et al. 2001, Zhou et al. 2008, Lv et al. 2014, Wang et al. 2014), suggesting that regulation of AtPHR1/OsPHR2 activity by Pi status may employ posttranslational modifications. Arabidopsis SAP AND MIZ1 DOMAIN-CONTAINING LIGASE 1 (SIZ1) likely activates PHR1 through sumoylation in vivo, although modification of PHR1 by SIZ1 has only been demonstrated in vitro (Miura et al. 2005). In rice, Pi and total P contents significantly increase in the shoots and roots of *ossiz1* mutants, and the transcript levels of a suite of Pi starvation genes are also altered in *ossiz1* mutants (Wang et al. 2015b). These results indicate that OsSIZ1 may act as a regulator of Pi responses in rice.

SPX proteins (proteins harboring the SPX domain alone) act as negative regulators of Pi starvation signaling by inhibiting the activity of AtPHR1/OsPHR2 in a Pi-dependent manner (Lv et al. 2014, Puga et al. 2014, Wang et al. 2014, Zhong et al. 2018). The SPX-domain functions as an intracellular Pi sensor that binds inositol polyphosphate (InsPs). Two recent studies found that inactivating *VIP1 HOMOLOG 1* (*VIH1*) and *VIH2*, two redundant genes encoding diphosphoinositol pentakisphosphate kinase (PPIP5K), blocks the biosynthesis of InsP8 and causes the upregulation of *PHT1* genes and excessive Pi accumulation (Dong et al. 2019, ZHu et al. 2019). It is suggested that InsP8 acts as an intracellular phosphate signaling molecule, which binds to the SPX domain and affects the interaction between SPX1 and PHR1 (Wild et al. 2016, Dong et al. 2019).

The Arabidopsis genome encodes four SPX proteins and the rice genome six SPX proteins. Their transcription, with the exception of *SPX4*, is induced by Pi starvation (Secco et al. 2012). Under Pi-replete but not Pi-deficient conditions, Arabidopsis and rice SPX1 and SPX2 interact with AtPHR1 and OsPHR2, respectively, through the SPX domain, thus preventing AtPHR1/OsPHR2 from promoting the transcription of *PHT1* genes (Puga et al. 2014, Wang et al. 2014). Rice SPX3 and SPX5 are also repressors of OsPHR2, based on genetic interaction analysis (Shi et al. 2014). In particular, OsSPX4 and OsSPX6 negatively regulate the transcription of phosphate transporters by preventing the nuclear accumulation of OsPHR2 under Pi-sufficient conditions. However, under Pi-deficient conditions, OsSPX4 and OsSPX6 are degraded, resulting in the release of OsPHR2 from the cytoplasm, which allows its translocation to the nucleus to upregulate *PHT1* transcription (Lv et al. 2014, Zhong et al. 2018).

Three E3 ubiquitin ligases have been identified that mediate the degradation of OsSPX4 under phosphate-deficient conditions and

nitrate-replete conditions (Hu et al. 2019, Ruan et al. 2019). Under Pi starvation conditions, SPX4 DEGRADATION E3 LIGASE 1 (SDEL1) and SDEL2 ubiquitinate the Lys-213 and Lys-299 residues of OsSPX4, targeting it for degradation (Ruan et al. 2019). Similarly, the nitrate sensor NRT1.1B enhances its interaction with OsSPX4 in the presence of nitrate and recruits the E3 ubiquitin ligase NRT1.1B-interacting protein 1 (NBIP1). NBIP1 then mediates OsSPX4 degradation and OsPHR2 is activated to upregulate the expression of *PHT1*-family genes and promote Pi uptake (Hu et al. 2019). Therefore, the NRT1.1B–OsSPX4–NBIP1 cascade links phosphate signaling and nitrate signaling. In addition, a recent study indicated that NIGT1 can transcriptionally suppress the *SPX4* gene in Arabidopsis and modulate phosphate uptake and signaling (Ueda et al. 2020).

Most recently, we identified a new Pi starvation-responsive transcription factor OsbHLH6, which interacts with and antagonizes OsSPX4 to release PHR2 and then regulate the expression of PHT1-family genes and homeostasis (He et al. 2020). In addition, Zhang et al. (2020b) reported that RLI1/HINGE1 is involved in modulating nitrate-induced phosphate response. RLI1 also interacts with SPX proteins to release the OsPHR2 function and regulate the expression of PHT1-family genes (Zhang et al. 2020b). Posttranscriptional regulation of phosphate transporter gene expression has also been reported in plants. The vacuolar Pi transporters *OsSPX-MFS1* and *OsSPX-MFS2* are direct targets of microRNA 827 (miR827), which is strongly induced by Pi starvation, resulting in transcript cleavage (Lin et al. 2010). In Arabidopsis, miR827 targets the transcripts of *NITROGEN LIMITATION ADAPTATION* (*NLA*) gene, leading to the higher protein accumulation of AtPHT1, the target of NLA, under Pi-deficient conditions (Kant et al. 2011, Lin et al. 2013). miR827 also targets *AtSPX-MFS* genes, although its cleavage efficiency is not as good as *NLA* (Lin et al. 2018).

Trafficking of Phosphate Transporters from the Endoplasmic Reticulum to the Plasma Membrane

PHT1-family phosphate transporters are integral membrane proteins that are translated by ribosomes attached to the endoplasmic reticulum (ER) surface and folded and modified in the ER lumen, before a final trafficking step to the plasma membrane through membrane vesicles (Rodriguez-Furlan et al. 2019). Trafficking from the ER to the plasma membrane is necessary for PHT1 to execute its function in phosphate uptake.

PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR 1 (PHF1), a protein related to yeast SECRETORY 12 (SEC12), regulates the ER exit of all PHT1 phosphate transporters in Arabidopsis (Gonzalez et al. 2005). In rice, OsPHF1 appears to regulate the trafficking of both low- and high-affinity phosphate transporters to the plasma membrane (Chen et al. 2011). PHF1 acts as an accessory protein during the ER exit of PHT1 proteins (Bayle et al. 2011). However, some PHT1 still localizes to the plasma membrane in the absence of PHF1 (Bayle et al. 2011, Chen et al. 2011), indicating that there may be additional protein(s) that assist with PHT1 localization to the plasma membrane.

Protein phosphorylation is one of the most well-known and significant posttranslational modifications. The phosphorylation status of Arabidopsis PHT1;1 at Ser-514 modulates its exit from the ER and subsequent trafficking to the plasma membrane (Bayle et al. 2011). Similarly, phosphorylation of OsPHT1;2 (also named OsPT2 for PHOSPHATE TRANSPORTER 2) and OsPHT1;8 (OsPT8) affects their ER exit and trafficking to the plasma membrane (Chen et al. 2015). Rice Casein Kinase 2 (OsCK2) phosphorylates OsPHT1;2 and OsPHT1;8 at Ser-517 (equivalent to Ser-514 in Arabidopsis PHT1;1) in response to phosphate levels, which then affects the trafficking of OsPHT1;2 and OsPHT1;8 to the plasma membrane. Further study showed that OsPHT1;8 cannot interact with OsPHF1 when phosphorylated at Ser-517, resulting in the ER retention of OsPHT1;8 and thus the lower accumulation of OsPHT1;8 at the plasma membrane. The phosphorylation of OsPHT1 transporters is reversible, as we recently demonstrated (Yang et al. 2020b). Rice PROTEIN PHOSPHATASE 95 (OsPP95) dephosphorylates OsPHT1;2 and OsPHT1;8, acting antagonistically with OsCK2 to modulate the phosphorylation status of OsPHT1;2 and OsPHT1;8 and thus affecting their trafficking from the ER to the plasma membrane to regulate Pi homeostasis (Yang et al. 2020b). Overexpression of *OsPP95* reduces the phosphorylation state of both OsPHT1;8 and OsPHT1;2, in turn promoting their relocation to the plasma membrane to promote Pi acquisition. In agreement with these observations, the *ospp95* mutation impairs Pi distribution from old leaves to new leaves under Pi-sufficient conditions. Notably, OsPHO2 mediates the degradation of OsPP95 under Pi-sufficient conditions (Yang et al. 2020b). In addition, OsCK2 affects the stability of OsPHO2 through phosphorylation of OsPHO2 by OsCK2α3. Indeed, OsCK2α3 interacts with and phosphorylates OsPHO2 at Ser-841, which makes OsPHO2 more prone to degradation and thus affects Pi homeostasis through the OsPHO2 target protein OsPHO1 (Wang et al. 2020a). These results demonstrate that the phosphorylation status of OsPHO2 regulates phosphate homeostasis.

In addition, OsPHO2 probably mediates the degradation of OsPHF1, based on the physical interaction of these two proteins in a yeast two-hybrid assay and in rice seedlings (Ying et al. 2017). Although direct evidence for OsPHO2-dependent ubiquitination and degradation of OsPHF1 has yet to be reported, OsPHO2 encodes an E2 ubiquitin-conjugating enzyme, which could regulate OsPHF1 protein levels through the 26S proteasome (Ying et al. 2017). Furthermore, Arabidopsis PHF1 protein levels are higher in the *pho2* mutant background (Liu et al. 2012, Huang et al. 2013, Lin et al. 2013). In addition, both PHO2 and PHF1 localize to the ER (Gonzalez et al. 2005, Huang et al. 2013), suggesting the intriguing possibility that PHO2 may target PHF1 for degradation.

Phosphate Transporter Degradation through the 26S Proteasome and Endosomal Sorting

Proteins have a finite life span, ranging from several minutes to a few hours, after they exit translation. Protein homeostasis is achieved by a balance between protein biosynthesis and

degradation. Plant phosphate transporters also undergo dynamic changes in their rates of translation, recycling and degradation to maintain phosphate homeostasis (Bayle et al. 2011, Liu et al. 2012, Huang et al. 2013, Lin et al. 2013, Park et al. 2014, Cardona-López et al. 2015, Wang et al. 2020a).

PHT1-family transporters are degraded in response to changes in physiological and environmental Pi. In Arabidopsis, PHT1;1 is targeted to sorting endosomes for recycling in Pi-deficient conditions, or to the vacuole for degradation in Pi-sufficient conditions (Bayle et al. 2011). Furthermore, PHT1;1 is rapidly degraded especially in Pi-replete conditions, and this degradation may be independent of the proteasome pathway (Bayle et al. 2011).

NLA, mentioned earlier in the context of miR827, is an RING-type E3 ubiquitin ligase that directs the ubiquitination of PHT1;1 and PHT1;4 at the plasma membrane and triggers clathrin-dependent endocytosis and subsequent sorting to the vacuole for degradation (Lin et al. 2013). OsNLA1, the rice ortholog of Arabidopsis NLA, has been reported to direct the degradation of several OsPHT1 proteins, such as OsPHT1;1, OsPHT1;2, OsPHT1;4, OsPHT1;7, OsPHT1;8 and OsPHT1;12 (Yue et al. 2017, Yang et al. 2020a). Furthermore, the *OsNLA1* genomic locus bears an upstream open reading frame that is necessary for normal transcriptional regulation of *OsNLA1* in response to Pi levels (Yang et al. 2020a).

PHO2 also negatively regulates the uptake and translocation of Pi by mediating the direct degradation of phosphate transporters, and its expression is negatively regulated by miR399 (Bari et al. 2006) In Arabidopsis, PHO2 mediates PHO1 degradation in endomembranes and is involved in multi-vesicular body (MVB)-mediated vacuole proteolysis (Liu et al. 2012). Likewise in rice, we found that OsPHO2 interacts with OsPHO1 to target it for degradation through an MVB-mediated pathway similar to that seen in Arabidopsis (Wang et al. 2020a), indicating the conservation of the PHO2–PHO1 module in plants. In Arabidopsis, PHO2 also physically interacts with PHT1;1 at the ER membrane and mediates its ubiquitination and degradation (Huang et al. 2013). NLA and PHO2 work together to target AtPHT1;4 for degradation through the 26S proteasome (Park et al. 2014). In rice, however, OsPHO2 does not appear to interact with OsPHT1;2 or OsPHT1;8. Whether OsPHO2 targets PHT1 proteins for degradation in rice remains to be determined.

In addition, Cardona-López et al. (2015) reported that ALG-2 INTERACTING PROTEIN-X (ALIX), one of the components of the ENDOSOMAL SORTING COMPLEXES REQUIRED FOR TRANSPORT (ESCRT)-III complex, mediates PHT1;1 sorting and vacuolar degradation via binding to the ESCRT-III component Sucrose NonFermenting 7 in vivo. ESCRT-III protein complexes mediate the selective sorting of protein cargoes into MVB intraluminal vesicles. This study further confirms that the degradation of PHT1 protein is dependent on sorting to the vacuole.

Improving P Use Efficiency via Phosphate Transporters

The ultimate goal of research pertaining to phosphate is to improve P use efficiency and reduce fertilizer use while maintaining or even increasing crop yields in agricultural production. Attempts to achieve this have included the overexpression of phosphate transporters to increase Pi uptake, but such strategies usually also cause Pi toxicity and growth retardation (Gu et al. 2016, Wang et al. 2018, He et al. 2019). This fact suggests that simply increasing the expression level of phosphate transporters will not improve crop yield and P use efficiency. However, transgenic rice plants overexpressing *OsPHF1* show improved Pi uptake and yield, possibly as a result of increased ER exit and plasma membrane targeting of PHT1-family transporters. Similarly, transgenic rice plants expressing a non-phosphorylatable version of OsPHT1;8 (S517A) exhibit improved phosphate uptake and retain high yield by promoting the plasma membrane localization of OsPHT1;8 (Wu et al. 2013, Chen et al. 2015). This result suggests that we can improve P use efficiency and crop yield under Pi-limited conditions by modulating the plasma membrane localization of Pi transporters.

Concluding Remarks

From gene transcription in the nucleus, protein synthesis and processing in the ER, trafficking to the plasma membrane to carry out their function, to their sorting to vacuoles for degradation, phosphate transporters are regulated by a complex array of mechanisms. The current state of knowledge on phosphate uptake and translocation in plants is beginning to take shape into a precise regulatory system (**Fig. 1**). Under Pi-sufficient conditions, SPX4/6 preventing the nuclear accumulation of PHR proteins, SPX1/2/6 further interact with PHR in nucleus, preventing PHR from promoting the transcription of PHT1-family genes. The OsCK2α3/β3 holoenzyme phosphorylates PHT1s (e.g. OsPHT1;8), preventing their interaction with PHF1 and their trafficking from the ER to the plasma membrane. Furthermore, some plasma membrane-localized PHT1s are degraded via ESCRT-III/ALIX-mediated pathway. In parallel, PHO2 mediates the degradation of PP95 to prevent it from dephosphorylating PHT1 proteins. PHO2 may also mediates the degradation of PHF1 to block PHT1 trafficking. These regulatory proteins work coordinately to prevent the trafficking of phosphate transporters from the ER to the plasma membrane and thus maintain low levels of Pi uptake. At the same time, excess Pi is moved into vacuole by SPX-MFS, and less Pi translocated to shoot as PHO1 was degraded by PHO2. By contrast, under Pi-deficient conditions, SPX4/6 are degraded, and SPX1/2 do not interact with PHR, resulting in the release of PHR function; therefore, most phosphate transporters are transcriptionally upregulated by PHRs or WRKY transcription factor. The CK2β3 subunit is also degraded, leaving a larger fraction of PHT1 proteins non-phosphorylated. Furthermore, PHO2 protein levels decrease, as miR399 is upregulated and targets *PHO2* transcripts for cleavage. The PHO2 target protein PP95 accumulates, resulting in the increased dephosphorylation of PHT1 proteins. At the same time, PHF1 accumulates, facilitating the trafficking of non-phosphorylated phosphate transporters from the ER to the plasma membrane for enhanced Pi acquisition from the outside environment (of the cell or the plant). In addition, the other PHO2 target, PHO1, also accumulates

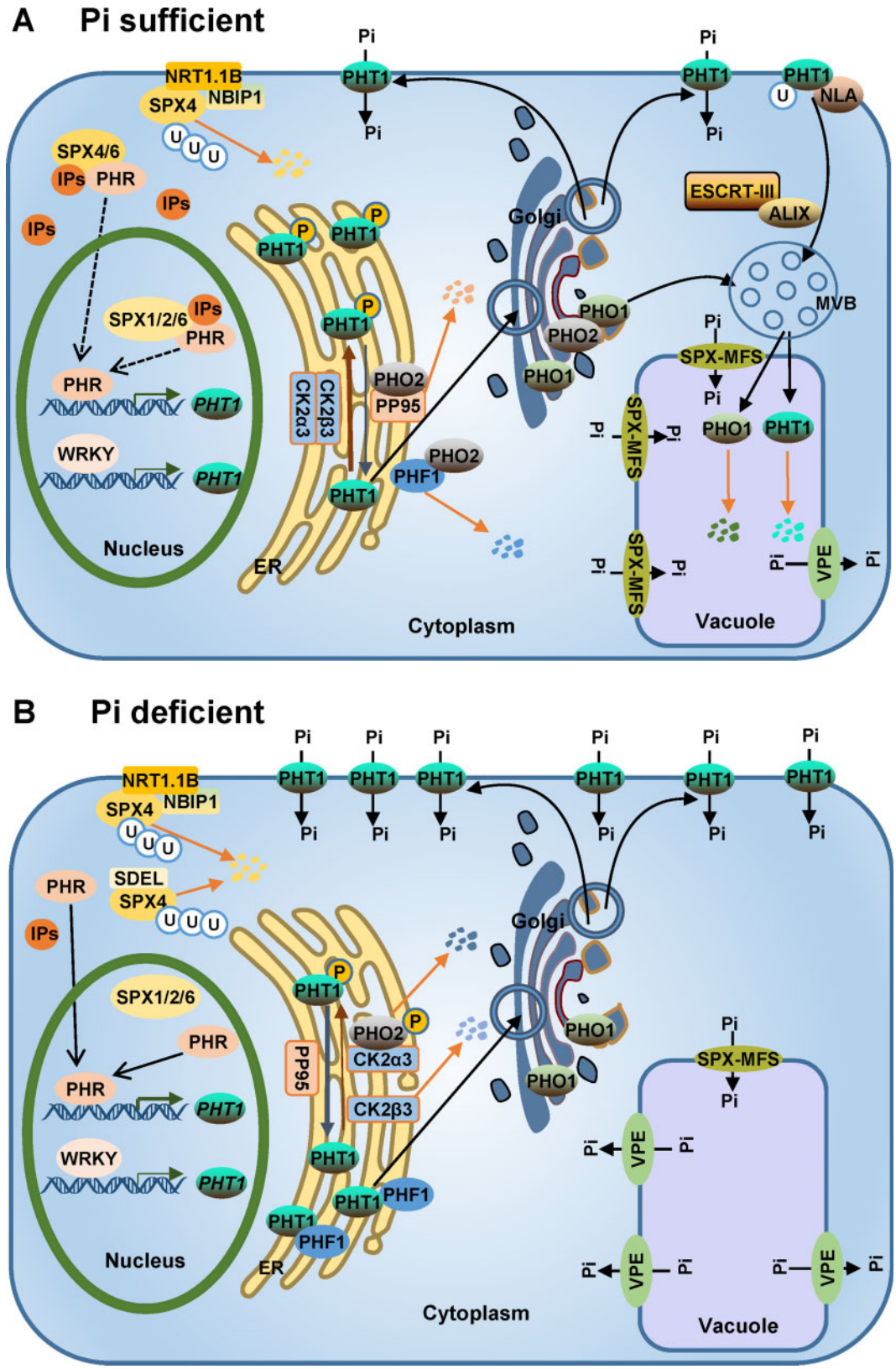

Fig. 1. Model of the biochemical and molecular regulation of phosphate transporters under Pi-sufficient and -deficient conditions based on the model systems Arabidopsis and rice. Solid black arrows represent protein movement, trafficking or protein activity; dotted black arrows represent the inhibition of protein activity; orange solid arrows represent protein degradation; blue rings refer to coatomer (COPII)-coated vesicles; P in yellow circles represents phosphorylation; and U in blue circles stands for ubiquitination. The thickness of the green arrows in the nucleus corresponds to transcription levels. inositol polyphosphates (IPs) refer to inositol polyphosphates.

to promote Pi translocation from roots to shoots. Furthermore, more Pi move out of vacuole by VPE. The combined effect of these strategies is to increase the Pi acquisition by cells to maintain plant phosphate homeostasis under Pi-limited soil conditions.

However, the regulatory mechanisms also raise several questions: are there additional proteins involved in the correct localization of PHT1? What is the detailed mechanism of PHT1 protein trafficking? In addition, the phosphate transporters that are involved in Pi remobilization and reuse and their regulation are largely unknown. Integrating those phosphate transporters responsible for absorption with those phosphate transporters responsible for remobilization and distribution may improve P use efficiency more effectively.

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