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Research review

The emerging importance of the SPX domain-containing proteins in phosphate homeostasis

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Summary

Plant growth and development are strongly influenced by the availability of nutrients in the soil solution. Among them, phosphorus (P) is one of the most essential and most limiting macro-elements for plants. In the environment, plants are often confronted with P starvation as a result of extremely low concentrations of soluble inorganic phosphate (Pi) in the soil. To cope with these conditions, plants have developed a wide spectrum of mechanisms aimed at increasing P use efficiency. At the molecular level, recent studies have shown that several proteins carrying the SPX domain are essential for maintaining Pi homeostasis in plants. The SPX domain is found in numerous eukaryotic proteins, including several proteins from the yeast PHO regulon, involved in maintaining Pi homeostasis. In plants, proteins harboring the SPX domain are classified into four families based on the presence of additional domains in their structure, namely the SPX, SPX-EXS, SPX-MFS and SPX-RING families. In this review, we highlight the recent findings regarding the key roles of the proteins containing the SPX domain in phosphate signaling, as well as providing further research directions in order to improve our knowledge on P nutrition in plants, thus enabling the generation of plants with better P use efficiency.

Involvement of the SPX domain in inorganic phosphate (Pi) signaling

It is well established that plant growth and development are highly dependent on the nutrient availability in soil. Inorganic phosphate (Pi), the main source of phosphorus (P) for plants, is present in soluble form at very low concentrations in most soils, as it is often bound to organic and inorganic compounds, thus creating insoluble complexes (Poirier & Bucher, 2002). As a result, Pi deficiency has become a major problem in many agricultural ecosystems, limiting plant growth and yield. For a long time, the application of fertilizer was chosen to overcome these problems. However, the side effects associated with heavy fertilization, such as the eutrophication of lakes, concomitant with the expected phosphate rock shortage in the coming decades, indicate that, in the long term, this solution is neither economically nor ecologically sustainable. Therefore, several scientific programs aimed at improving nutrient use efficiency have been carried out in recent years in order to maximize plant growth on soils with low nutrient availability. To date, our knowledge of the molecular response of plants to Pi starvation has greatly improved with the identification of several key players involved in Pi signaling, and has been well reviewed in recent years (Nilsson *et al.*, 2010; Rouached *et al.*, 2010; Chiou & Lin, 2011; Peret *et al.*, 2011). Among the many and diverse proteins involved in the plant response to Pi starvation, proteins containing the SPX domain are key players controlling a set of processes involved in the maintenance of an internal steady state of phosphate ions at the level of the cell, defined as Pi homeostasis (Hamburger *et al.*, 2002; Duan *et al.*, 2008; C. Wang *et al.*, 2009; Lin *et al.*, 2010; Secco

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et al., 2010; Kant *et al.*, 2011). The SPX domain (Pfam PF03105) (Fig. 1a) is named after the Suppressor of Yeast *gpa1* (Syg1), the yeast Phosphatase 81 (Pho81) and the human Xenotropic and Polytropic Retrovirus receptor 1 (Xpr1). This hydrophilic domain is found at the N-termini of various proteins in all major eukaryotes, from *Caenorhabditis elegans* and Drosophila to mammals (Stefanovic *et al.*, 2011). Although the organization of the SPX domain is variable, it can be subdivided into three well-conserved sub-domains of 30–40 amino acids each (Fig. 1a–c). In addition, despite having an average length of ~165 amino acids, the SPX domain is found in stretches of 135–380 amino acids, with the three sub-domains being separated from each other by regions of low similarity. In yeast, the working model for nutrient homeostasis in eukaryotes, several proteins belonging to the PHO regulon, involved in the maintenance of Pi homeostasis, possess the SPX domain. Despite the lack of such a regulon in plants, many plant proteins harboring the SPX domain have been shown to be involved in Pi signaling (Table 1; Figs 2, 3). Recent studies in yeast and Arabidopsis have also suggested that the SPX domain itself could be involved in the fine tuning of Pi transport and signaling through mechanisms such as physical interactions with other proteins (Duan *et al.*, 2008; Hurlimann *et al.*, 2009; Zhou & Ni, 2010). In most plants, proteins harboring the SPX domain can be divided into four groups depending on the presence of extra domains: proteins that exclusively contain the SPX domain (Class 1), and proteins that, in addition,

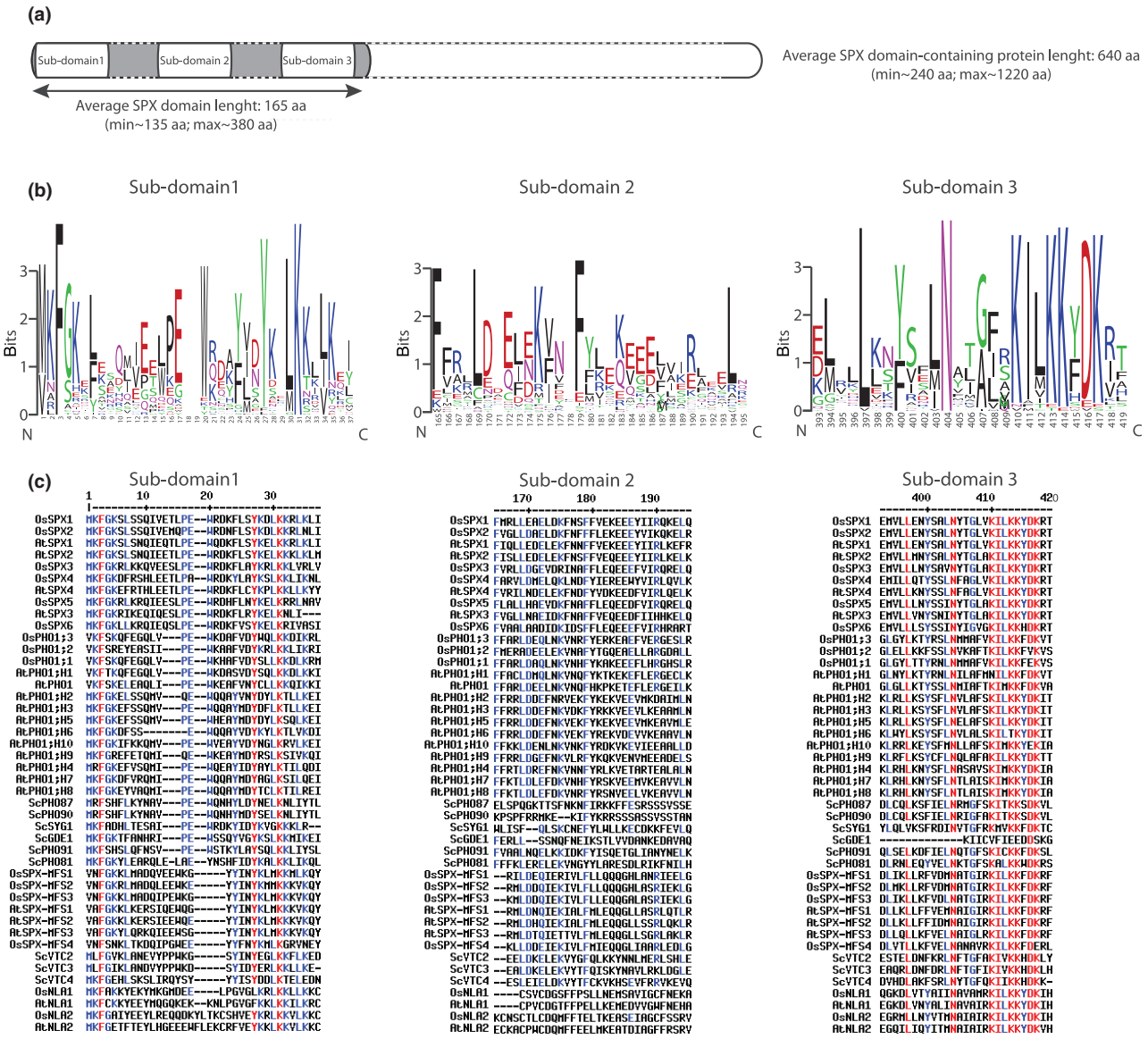

Fig. 1 Characteristics of the SPX domain in plants and yeast. (a) Schematic representation of the SPX domain-containing proteins. The N-terminal SPX domain (gray) is subdivided into three well-conserved sub-domains (sub-domains 1–3), separated from each other by low similarity regions. The dotted line symbolizes the variation in amino acid (aa) length between sub-domains as well as the variation in total protein length. (b) Graphical sequence logo representation of the amino acid conservation of the three SPX sub-domains. (c) Alignment of the three SPX sub-domains of the rice, Arabidopsis and yeast SPX domain-containing proteins.

Table 1 List of the Arabidopsis and rice SPX domain-containing proteins and their characteristics

Class	Organism	Gene name	Locus identifier	Function	Mutant phenotype	Molecular regulators	Induction by Pi starvation	Subcellular localization	References
Class 1 (SPX)	Arabidopsis	*AtSPX1*	At5g20150	Positive regulator of plant adaptation to Pi starvation	KO – Unaltered; OE – Increased expression of PSI genes	*AtPHR1* (+); *AtSIZ1* (+)	+	Nucleus	Duan *et al.* (2008)
		AtSPX2	At2g26660		KO – Unaltered	*AtPHR1* (+); *AtSIZ1* (+)	+	Nucleus	Duan *et al.* (2008)
		AtSPX3	At2g45130	Positive regulator of plant adaptation to Pi starvation. Negative regulator of some PSI genes	KD – High shoot Pi content and increased PSI gene expression levels	*AtPHR1* (+); *AtSIZ1* (+)	+	Cytoplasm speckles	Duan *et al.* (2008)
		AtSPX4	At5g15330		KO – Unaltered	*AtPHR1* (+); *AtSIZ1* (+)	=	Cytoplasmic membrane	Duan *et al.* (2008)
	Rice	*OsSPX1*	Os06g40120	Optimize growth under Pi-deficient conditions. Negative regulator of *OsPHR2*. Involved in resistance to cold stress	KD – Reduced growth. High shoot Pi content, increased PSI gene expression levels; OE – Reduced growth. Suppress the induction of PSI genes under –Pi	*OsPHR2* (+); *OsPHO2* (–)	+	Nucleus	C. Wang *et al.* (2009); Zhao *et al.* (2009); Liu *et al.* (2010)
		OsSPX2	Os02g10780			*OsSPX1* (+)	+	Nucleus	Z. Wang *et al.* (2009)
		OsSPX3	Os10g25310	Negative regulator of some PSI genes, involved in tolerance to Pi starvation, but not in Pi accumulation	OE – Reduced growth under +Pi and –Pi conditions	*OsSPX1* (+ in shoot; – in root)	+	Cytoplasm speckles	Z. Wang *et al.* (2009)
		OsSPX4	Os03g61200				=	Cytoplasmic membrane	Z. Wang *et al.* (2009)
		OsSPX5	Os03g29250			*OsSPX1* (– in root)	+		Z. Wang *et al.* (2009)
		OsSPX6	Os07g42330				+		Z. Wang *et al.* (2009)
Class 2 (SPX-EXS)	Arabidopsis	*AtPHO1*	At3g23430	Involved in Pi transfer form root to shoot, via export of Pi out of the cells of the stele and into the xylem vessel apoplast. Involved in Pi signaling	KO – Reduced shoot growth, low shoot Pi content; KD – Normal growth but low shoot Pi content; OE – Reduced shoot growth, high shoot Pi content	*AtWRKY6* (–); *AtWRKY42* (–)	+		Poirier *et al.* (1991); Hamburger *et al.* (2002); Wang *et al.* (2004); Stefanovic *et al.* (2007); Ribot *et al.* (2008); Chen *et al.* (2009); Rouached *et al.* (2010); Stefanovic *et al.* (2011); Rouached *et al.* (2011)
		AtPHO1;H1	At1g68740	Involved in Pi transfer from root to shoot	KO – Unaltered	*AtPHR1* (+)	+		Wang *et al.* (2004); Stefanovic *et al.* (2007)
		AtPHO1;H2	At2g03260				=		Wang *et al.* (2004)
		AtPHO1;H3	At1g14040				=		Wang *et al.* (2004)
		AtPHO1;H4/ AtSHB1	At4g25350	Involved in cryptochrome signaling, seed development, photoperiodic and autonomous flowering	KO – Short hypocotyl under blue light. Delayed flowering; OE – Long hypocotyl under red, far-red and blue light		=	Nucleus	Wang *et al.* (2004); Kang & Ni (2006); Zhou *et al.* (2009); Zhou & Ni (2009, 2010)
		AtPHO1;H5	At2g03240				–		Wang *et al.* (2004)
		AtPHO1;H6	At2g03250				=		Wang *et al.* (2004)
		AtPHO1;H7	At1g26730				–		Wang *et al.* (2004)
		AtPHO1;H8	At1g35350				=		Wang *et al.* (2004)
		AtPHO1;H9	At3g29060				=		Wang *et al.* (2004)
		AtPHO1;H10	At1g69480	Involved in different biotic and abiotic stress signaling pathways		AtCOI1 (+); AtABI5 (+)	=		Wang *et al.*, 2004 Ribot *et al.*, 2008
	Rice	*OsPHO1;1*	Os01g02000		KO – Unaltered		=		Secco *et al.* (2010)
		OsPHO1;2	Os02g56510	Involved in Pi transfer form root to shoot	KO – Reduced shoot growth, low shoot Pi content		=		Secco *et al.* (2010)
		OsPHO1;3	Os06g29790				+		Secco *et al.* (2010)

Table 1 (Continued)

Class	Organism	Gene name	Locus identifier	Function	Mutant phenotype	Molecular regulators	Induction by Pi starvation	Subcellular localization	References
Class 3 (SPX-MFS)	Arabidopsis	*AtSPX-MFS1*	At4g22990				–		
		AtSPX-MFS2	At4g11810				+		
		AtSPX-MFS3	At1g63010				=	Tonoplast	Dunkley *et al.* (2006)
	Rice	*OsSPX-MFS1*	Os04g48390	Pi transport and remobilization in leaves	KD – Increased Pi content in the leaves and altered Pi re-mobilization from old to young leaves	*osa-miR827* (–); *OsPHR2* (–)	–		Lin *et al.* (2010)
		OsSPX-MFS2	Os02g45520			*osa-miR827* (–); *OsPHR2* (–)	+		Lin *et al.* (2010)
		OsSPX-MFS3	Os06g03860				–		
		OsSPX-MFS4[a]	Os09g34990				=		
Class 4 (SPX-RING)	Arabidopsis	*AtNLA/AtBAH1*	At1g02860	Regulates Pi homeostasis under N limitation condition. Involved in regulation of anthocyanin synthesis	KO – High levels of Pi in the shoot, lack of anthocyanin accumulation in leaves under low N	*ath-miR827* (–)	–	Nuclear speckles	Peng *et al.* (2007, 2008); Yaeno & Iba (2008); Hsieh *et al.* (2009); Kant *et al.* (2011)
		AtNLA2	At2g38920						
	Rice	*OsNLA1*	Os07g47590						
		OsNLA2	Os03g44810						

The column 'Induction by Pi starvation' represents transcript regulation under Pi starvation.
'+', '–', '=', induction, suppression or unaltered transcript expression, respectively.
OsSPX-MFS4[a] shows that *OsSPX-MFS4* is considered as a pseudogene.
KD, knock-down; KO, knock-out; OE, overexpression; PSI genes, Phosphate Starvation Inducible genes.

harbor at the C-terminus an EXS domain (Class 2), an MFS domain (Class 3) or a RING domain (Class 4) (Fig. 2; Table 1) (Chiou & Lin, 2011). Here, we summarize and highlight recent advances and future challenges in understanding the important roles of the different SPX domain-containing protein families in the regulation of phosphate homeostasis in yeast, Arabidopsis and rice.

Function of the SPX domain-containing proteins in yeast

The first characterized proteins possessing the SPX domain were discovered in the budding yeast *Saccharomyces cerevisiae*, with the identification of Pho81 and Syg1 (Schneider *et al.*, 1994; Spain *et al.*, 1995). Since then, strong links between the presence of the SPX domain in proteins and Pi homeostasis have been demonstrated (Fisher *et al.*, 2005; Hurlimann *et al.*, 2007, 2009; Lee *et al.*, 2007; Hothorn *et al.*, 2009; Ghillebert *et al.*, 2011). Among the 10 proteins harboring the SPX domain in yeast, eight are closely linked to phosphate metabolism, namely the three low-affinity Pi transporters, Pho87, Pho90 and Pho91, three of the four yeast polyphosphate synthase subunits, Vacuolar Transporter Chaperone 2 (Vtc2), Vtc3 and Vtc4, the Cyclin-Dependent Kinase inhibitor Pho81 and the glycerophosphocholine phosphodiesterase 1 (Gde1) (Fisher *et al.*, 2005; Lee *et al.*, 2007; Hothorn *et al.*, 2009; Hurlimann *et al.*, 2009; Ghillebert *et al.*, 2011).

In addition to mediating phosphate uptake, the three low-affinity transporters are involved in the sensing of external Pi and the regulation of Pi signaling (Ghillebert *et al.*, 2011). Although Pho87 and Pho90 are localized to the plasma membrane, Pho91 is localized to the vacuolar membrane and is involved in the export of Pi from the vacuole to the cytosol (Hurlimann *et al.*, 2007). Under low-Pi conditions, Pho87 and Pho90 are repressed by Spl2, a negative regulator, which interacts directly with both transporters via the SPX domain. Therefore, the SPX domain of Pho87 and Pho90 can act as an auto-inhibitory domain involved in the regulation of phosphate accumulation in yeast cells (Hurlimann *et al.*, 2009). In addition, on phosphate, nitrogen and carbon source deficiencies, it has been shown that Pho87 and Pho90 can be inactivated via vacuolar targeting, and that this mechanism requires the SPX domain (Ghillebert *et al.*, 2011).

On Pi starvation, Pho81, in association with inositol hepta*kis*phosphate, inactivates the main regulator of the PHO pathway, thus resulting in increased levels of expression of the phosphate starvation genes (Lee *et al.*, 2007).

The vacuolar localized Vtc proteins have recently been shown to synthesize polyphosphate, using ATP as a substrate, before translocating the phosphate polymers to the vacuolar lumen (Hothorn *et al.*, 2009). A truncated form of Vtc4, devoid of the SPX domain, is still active in polyphosphate synthesis, indicating that the SPX domain is not essential for catalytic activity (Hothorn *et al.*, 2009).

Gde1, encoding the only characterized glycerophosphodiester phosphodiesterase in yeast, is responsible for the hydrolysis of glycerophosphocholine in the cell, and thus the scavenging of phosphate from glycerophosphodiesters under low-Pi conditions (Fisher *et al.*, 2005).

The SPX family in plants

In plants, proteins exclusively harboring the SPX domain are referred to as SPX proteins. In Arabidopsis and rice, the SPX family consists of four and six members, respectively (Fig. 2; Table 1) (Duan *et al.*, 2008; C. Wang *et al.*, 2009; Z. Wang *et al.*, 2009). These relatively small proteins (~280 amino acids) were named as AtSPX1–AtSPX4 in Arabidopsis and OsSPX1–OsSPX6 in rice. Localization studies revealed a broad range of expression for the members of the SPX family, ranging from roots, leaves, cotyledons, stems and pollen grains. Transcript and histochemical analyses showed that all the *SPX* genes, with the exception of *AtSPX4* and *OsSPX4*, were highly induced on Pi starvation in roots and/or in shoots (Supporting Information Fig. S1) (Duan *et al.*, 2008; C. Wang *et al.*, 2009; Z. Wang *et al.*, 2009). In addition, studies in Arabidopsis showed that these responses were under the control of *AtPHR1* and its closest family member *AtPHL1* (Fig. S1).

Individual mutant analysis of *AtSPX1*, *AtSPX2* and *AtSPX4* knock-outs did not show any obvious phenotypes, under either Pi-sufficient or Pi-deficient conditions (Duan *et al.*, 2008). However, the overexpression of *AtSPX1* increased the expression levels of some of the Phosphate Starvation Inducible (PSI) genes, such as *ACP5*, *PAP2* and *RNS1*, independent of Pi status, suggesting a potential transcriptional regulation role of *AtSPX1* on Pi starvation (Fig. 3). In addition, repression of *AtSPX3* by RNAi altered the response to Pi starvation at both the phenotypic and gene expression level, resulting in plants with increased Pi concentration in the shoots, and decreased Pi concentration in the roots, suggesting that *AtSPX3* can act as a negative regulator of Pi starvation signaling (Duan *et al.*, 2008).

In rice, *OsSPX1* has been shown to be specifically induced by Pi starvation and to be preferentially expressed in the roots (C. Wang *et al.*, 2009). Suppression of *OsSPX1* by RNAi reduced plant growth and increased Pi accumulation in the shoots, as observed for well-characterized plants overaccumulating Pi, such as *Ospho2* mutants or *OsPHR2*-overexpressing plants (C. Wang *et al.*, 2009). Detailed analysis showed that the increase in shoot Pi concentration in the *OsSPX1* RNAi lines correlated with increased expression of some PSI genes, such as the Pi transporters *OsPT2* and *OsPT8* (C. Wang *et al.*, 2009). By contrast, although growth was still impaired, overexpression of *OsSPX1* suppressed the induction of the PSI genes, suggesting that *OsSPX1*, similar to *AtSPX3*, is involved in a negative feedback loop to adjust the expression of several PSI genes under Pi-limited conditions (C. Wang *et al.*, 2009). Recently, Liu *et al.* (2010), using plants simultaneously overexpressing *OsSPX1* and *OsPHR2*, demonstrated that *OsSPX1* could counteract the function of *OsPHR2* in inducing the expression of *OsPT2*, which plays a major role in Pi translocation and accumulation, thus

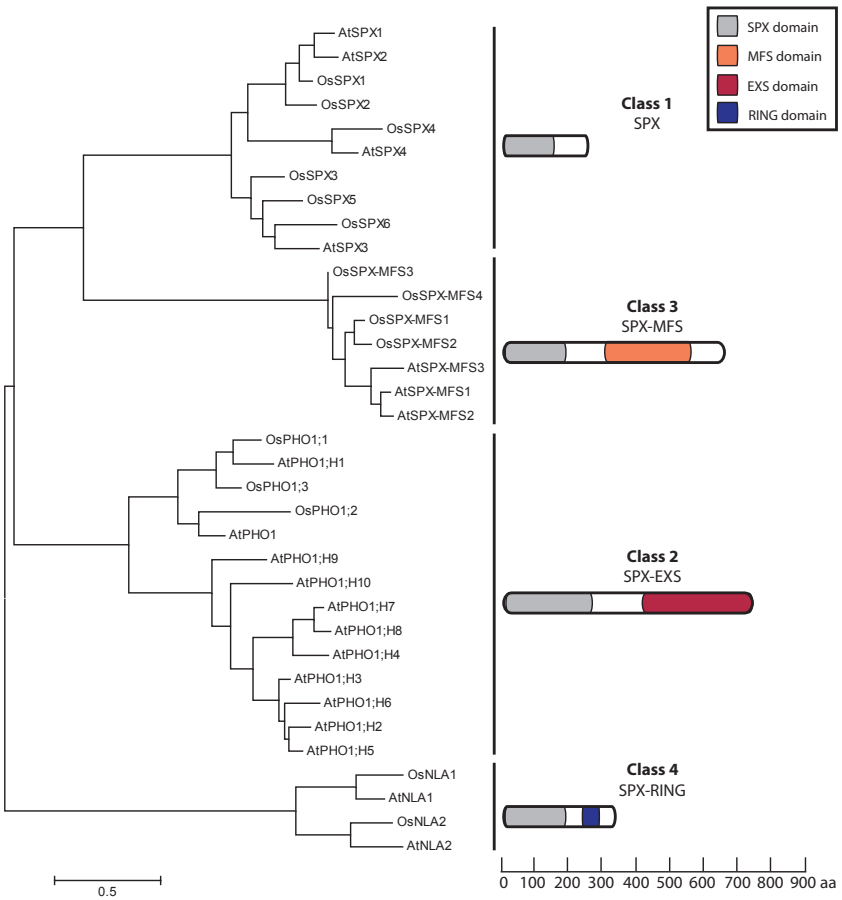

Fig. 2 Phylogenetic relationships and structural characteristics of the Arabidopsis and rice SPX domain-containing proteins. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. At, *Arabidopsis thaliana*; Os, *Oryza sativa*. Right panel shows the schematic representation of the structure of the different classes of SPX domain-containing proteins. For each class of SPX domain-containing protein, the respective domains and the average protein length are shown. aa, amino acid.

demonstrating that *OsSPX1* acts as a negative regulator of *OsPHR2* (Fig. 3) (Liu *et al.*, 2010).

The SPX proteins have a broad range of subcellular localization. AtSPX1, AtSPX2, OsSPX1 and OsSPX2 are exclusively localized to the nucleus, AtSPX3 and OsSPX4 are localized to some unidentified cytoplasmic speckles, and AtSPX4 and OsSPX4 are localized to the plasma membrane (Duan *et al.*, 2008; Z. Wang *et al.*, 2009). Yet, the subcellular localization of the rice SPX proteins was only monitored in a heterologous system, using onion epidermal cells (Z. Wang *et al.*, 2009). Recent studies have shown that *OsSPX1* could regulate the transcription of *OsSPX2*, *3* and *5* (Z. Wang *et al.*, 2009). However, in this study, the authors did not rule out the possibility that the changes observed in gene expression for *OsSPX2*, *3* and *5* could be a result of increased Pi concentration in the *OsSPX1*-overexpressing plants, instead of direct regulation via *OsSPX1*.

Another feature of the rice SPX family has been demonstrated in the response to cold stress. Constitutive overexpression of *OsSPX1* in tobacco plants resulted in decreased total leaf Pi concentration and the accumulation of free proline and sucrose, providing improved cold tolerance compared with the wild-type (WT) (Zhao *et al.*, 2009). It is noteworthy that both cold stress and Pi starvation induce sugar accumulation in plants. A link between low Pi, cold acclimatization and freezing tolerance has also been noted using the Arabidopsis *pho1* and *pho2* mutants (Hurry *et al.*, 2000). The importance of sugars and, more specifically, sucrose in Pi homeostasis is well documented (Hammond & White, 2011; Lei *et al.*, 2011). Therefore, the deciphering of the cross-talk between phosphate starvation and sugar signaling, as well as the involvement of SPX domain-containing proteins in these pathways, requires further investigation.

The SPX-EXS family in plants

The PHO1 family members are the only proteins in eukaryotes that contain both the SPX and EXS domains (Fig. 2) (Wang *et al.*, 2004). The EXS domain is embedded in a hydrophobic region and has an unknown function. It was named after the yeast ERD1, involved in the localization of endogenous endoplasmic reticulum proteins, the human XPR1 and the yeast SYG1 (Pfam entry PF03124). Unlike other SPX-containing protein families, the SPX domain in the *PHO1* gene family is found as a tripartite domain, interspersed with large insertions. Homologs of PHO1 are found in a wide spectrum of eukaryotes, from yeast, *C. elegans*, Drosophila, mammals and plants (Secco *et al.*, 2010). Interestingly, no PHO1 homologs are found in prokaryotes or in the unicellular alga *Chlamydomonas reinhardtii*, but PHO1 homologs are found in bryophytes, monocotyledons and dicotyledons (Wang *et al.*, 2008; Secco *et al.*, 2010).

The *pho1* mutant was first isolated in Arabidopsis as a Pi-deficient mutant with low leaf Pi concentration, reduced shoot growth and increased Pi concentration in the roots (Table 1) (Poirier *et al.*, 1991). Consistent with its function in Pi loading into the xylem, *AtPHO1* is expressed in the cells of the root vascular system and the lower part of the hypocotyl (Hamburger *et al.*, 2002). Recent studies have demonstrated that AtPHO1 is capable of mediating phosphate efflux out of the cell, rendering it the first identified protein in plants and animals involved in Pi export (Stefanovic *et al.*, 2011). It has also been shown that *AtPHO1* plays a key role in the long-distance Pi-deficiency signaling network. Indeed, reducing the level of *AtPHO1* transcripts in Arabidopsis results in a decreased rate of Pi transfer from the root to the shoot, consequently leading to Pi-deficient shoots. Surprisingly, all the usual hallmarks associated with Pi deficiency, such as the induction of PSI genes and reduced growth, are absent in *AtPHO1*-underexpressing lines, demonstrating a clear role of *PHO1* in Pi signaling (Rouached *et al.*, 2011). In Arabidopsis, the *PHO1* family consists of 10 additional members (*AtPHO1;H1–AtPHO1;H10*) with high homology to AtPHO1 (Fig. 2; Table 1). Although the majority of these genes are predominantly expressed in the vascular cylinder of the root and/or the shoot, some genes are more broadly expressed, such as in the trichomes, pollen grains and in response to hormones, implying a broader role for AtPHO1 proteins than simply the long-distance transfer of Pi (Wang *et al.*, 2004; Rouached *et al.*, 2010). To date, four members of the family, *AtPHO1*, *AtPHO1;H1*, *AtPHO1;H4* and *AtPHO1;H10*, have been studied and characterized, with only *AtPHO1* and *AtPHO1;H1* being involved in Pi homeostasis. Genetic complementation studies aimed at rescuing the growth defect phenotype as well as the low shoot Pi concentration of the *Atpho1* mutant, with all the members of the *AtPHO1* gene family, with the exception of *AtPHO1;H10*, showed that only *AtPHO1* and *AtPHO1;H1* were involved in long-distance Pi transfer (Stefanovic *et al.*, 2007). Despite having a similar biological role, and being induced by Pi starvation, *AtPHO1* and *AtPHO1;H1* are differentially regulated (Fig. 3; Table 1). Although the induction of *AtPHO1;H1* on Pi starvation is controlled by *AtPHR1*, the main transcription factor controlling Pi homeostasis, and can be suppressed by the nonmetabolizable phosphate analog, phosphite, the increase in *AtPHO1* expression is independent of *AtPHR1* and is not influenced by phosphite (Stefanovic *et al.*, 2007). Analysis of the expression profile of *AtPHO1* and *AtPHO1;H1* on sucrose and phytohormone treatments revealed further differences in their mode of regulation (Ribot *et al.*, 2008). Recent studies have demonstrated that *AtPHO1* is regulated by two WRKY transcription factors, WRKY6 and WRKY 42 (Chen *et al.*, 2009). Both transcription factors negatively regulate the expression of *PHO1* by binding to two W-box motifs present in the *AtPHO1* promoter, in a Pi-dependent manner (Chen *et al.*, 2009). Taken together, it appears that *AtPHO1* and *AtPHO1;H1* are regulated by distinct signal transduction pathways.

AtPHO1;H4, also named Short Hypocotyl under Blue Light (*SHB1*), and *AtPHO1;H10* have, to date, no direct links with phosphate homeostasis, being involved in the control of hypocotyl elongation under blue light and in response to numerous biotic and abiotic stresses, respectively (Table 1) (Ribot *et al.*, 2008; Zhou & Ni, 2010).

In rice, the *PHO1* family consists of only three genes, namely *OsPHO1;1–OsPHO1;3*. Interestingly, all the rice PHO1 proteins clustered with AtPHO1 and AtPHO1;H1, the only two Arabidopsis members involved in long-distance Pi transfer (Secco *et al.*,

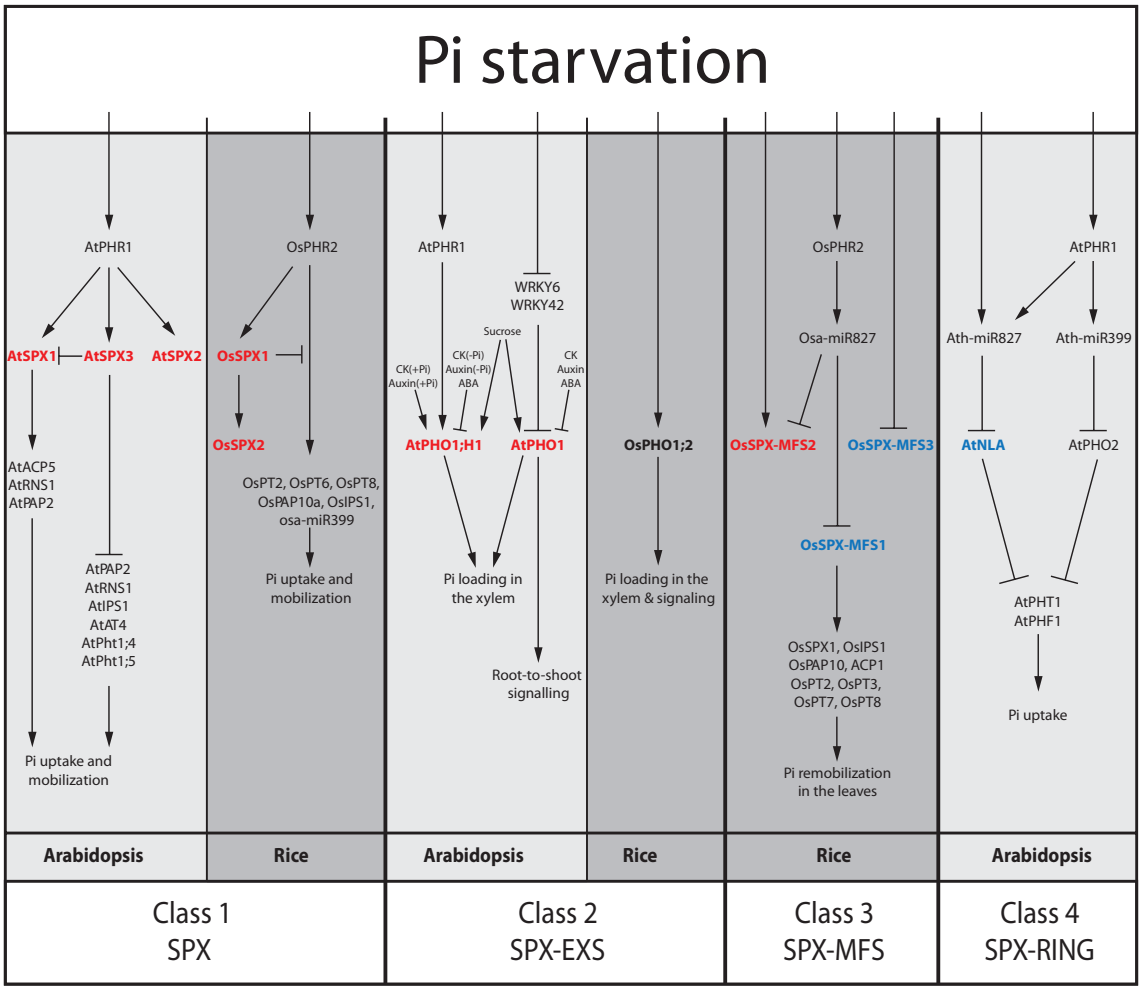

Fig. 3 Overview of the involvement of the rice and Arabidopsis SPX domain-containing proteins in the phosphate starvation signaling pathways. For each of the four class of SPX domain-containing proteins, members with known function are represented with regard to the inorganic phosphate (Pi) starvation signaling pathway, in Arabidopsis (light gray shading) and rice (dark gray shading). Arrowheads show direct or indirect positive regulation; flat-ended lines show negative regulation. SPX domain-containing transcripts in red indicate an increase in transcript abundance under Pi starvation, whereas those in blue indicate a decrease in transcript abundance. ABA, abscisic acid; At, *Arabidopsis thaliana*; CK, cytokinin; Os, *Oryza sativa*.

2010). Mutant analysis revealed that *OsPHO1;2*, the closest homolog of *AtPHO1*, was required to transfer Pi from the roots to the shoots (Fig. 3). A key difference between the Arabidopsis and rice *PHO1* families is the presence of *cis*-Natural Antisense Transcripts (NATs) for all three members of the rice *PHO1* family (Secco *et al.*, 2010). Despite the unknown function of these NATs, their expression pattern, as well as data from the literature, suggest that they could be implicated in regulating the expression of the sense transcript. The function of the other members of the family is still unknown, but could be involved in the maintenance of Pi homeostasis in other tissues, such as flowers, as suggested by their expression profiles, or be functionally redundant with *OsPHO1;2*. Moreover, phylogenetic analyses of PHO1 homologs of different mono- and dicotyledonous plants revealed the emergence of a divergent clade of PHO1 proteins in dicotyledons, which include members that have not yet been involved in Pi homeostasis, such as AtPHO1;H4 (SHB1) (Secco *et al.*, 2010). However, the functionality of this PHO1 dicotyledon-specific clade is still unclear.

The SPX-MFS family in plants

The Major Facilitator Superfamily (MFS) represents the largest group of transport carriers in all organisms, which are often coupled to the movement of another ion. Proteins of this family can function as uniporters, symporters or antiporters, and have a diverse range of substrates, such as ions, sugars, nucleosides, amino acids and peptides. Based on the properties of the SPX and MFS domains, it has been hypothesized that proteins harboring these two domains could be involved in both transport and signaling (Lin *et al.*, 2010).

In rice, although there are four putative genes for this family, namely *SPX-MFS1–SPX-MFS4*, the latter has no reported full-length cDNA or expressed sequence tag (EST) sequence, suggesting that it may be a pseudogene (Fig. 2; Table 1). Transcript analysis of the rice *SPX-MFS* genes showed that they were preferentially expressed in the shoots, and that both *OsSPX-MFS1* and *OsSPX-MFS3* were suppressed by Pi starvation, whereas *OsSPX-MFS2* was induced by Pi deficiency (Fig. 3) (Lin *et al.*, 2010).

OsSPX-MFS1 and *OsSPX-MFS2* have been shown to be specifically regulated by a Pi starvation-induced microRNA, osa-miR827 (Lin *et al.*, 2010). *In situ* hybridization revealed that *OsSPX-MFS1* and *OsSPX-MFS2* were preferentially expressed in the leaf mesophyll and parenchyma cells surrounding the xylem, similar to osa-miR827 (Lin *et al.*, 2010). Analysis of the knock-out T-DNA lines of *OsSPX-MFS1* and *OsSPX-MFS2* and overexpressing plants of osa-miR827 revealed that both *OsSPX-MFS1* and *OsSPX-MFS2* were negatively regulated by osa-miR827 abundance, despite their different responses to external Pi status. Although Lin *et al.* (2010) did not find any obvious phenotype in osa-miR827-suppressing or -overexpressing lines, a recent study has shown that overexpression of osa-miR827 or reduced expression of *OsSPX-MFS1* increases Pi concentration in the leaves, and reduces Pi re-mobilization from old to young leaves (C. Wang *et al.*, unpublished). Moreover, using *OsPHR2*-overexpressing plants, it has also been shown that the osa-miR827/OsSPX-MFS1/2 pathway is under the control of *OsPHR2* (Lin *et al.*, 2010).

Interestingly, in most of the monocotyledons, the preferential cell type for leaf Pi storage is the mesophyll cell, in contrast with Arabidopsis, where Pi is mainly stored in the epidermal and bundle sheath cells (Conn & Gilliham, 2010; Conn *et al.*, 2011). The compartmentalization of nutrient storage in specific cell types aims at reducing the creation of insoluble complexes. Thus, in rice, specific phosphate transporters should be required to concentrate Pi in mesophyll cells. Hence, it is tempting to hypothesize that some members of the SPX-MFS family, as a result of their localization and function, could perform such a function.

The SPX-RING family in plants

The Really Interesting New Gene (RING) finger domain, a specialized type of zinc finger domain, is involved in the mediation of protein–protein interactions. The presence of a RING finger domain is a characteristic of RING-class E3 ubiquitin protein ligases, which are capable of transferring ubiquitin from an E2 enzyme to a substrate protein. In Arabidopsis, despite the RING domain being present in more than 450 proteins, only two proteins in both rice and Arabidopsis possess the RING and SPX domains (Fig. 2; Table 1).

To date, the only characterized member of the SPX-RING family is the Arabidopsis Nitrogen Limitation Adaptation (*NLA*) gene (Peng *et al.*, 2007), also called benzoic acid hypersensitive 1 (*BAH1*), for its role in the immune response (Yaeno & Iba, 2008). The *Atnla* mutant was first identified for its altered growth response on nitrogen (N) starvation, being unable to accumulate anthocyanins, resulting in an early senescence phenotype (Peng *et al.*, 2007, 2008). A recent study has demonstrated the involvement of *AtNLA* in phosphate homeostasis (Kant *et al.*, 2011). Phosphate analysis revealed that the *Atnla* mutant showed increased Pi uptake and Pi content, especially under low-nitrate and high-phosphate availability, relative to WT plants. The phosphate uptake capacity and Pi content of the *Atnla* mutant were similar to those of the well-characterized Pi overaccumulator *Atpho2* mutant. The early senescence phenotype detected in the *Atnla* mutant under low nitrate appeared to be a consequence of shoot Pi toxicity, as observed in *Atpho2* mutants. Phosphate overaccumulation was drastically increased under low-nitrate conditions, for both *Atnla* and *Atpho2* mutants, suggesting that nitrate and phosphate levels have an antagonistic interaction (Kant *et al.*, 2011). The Arabidopsis and rice *PHO2*, encoding an E2 conjugase, are modulated by the Pi starvation-induced miR399 (Rouached *et al.*, 2010; Chiou & Lin, 2011). *PHO2* is a key player in Pi homeostasis, regulating a subset of the PSI genes, such as some Pi transporters. Surprisingly, *AtNLA* has also been shown to be regulated by an miRNA, miR827. Under Pi starvation, miR827, similar to miR399, is specifically up-regulated and targets the degradation of *NLA* mRNA, thus activating Pi uptake and root to shoot translocation (Fig. 3). Consequently, both *AtPHO2* and *AtNLA* act as negative regulators of Pi uptake, and are regulated by miRNAs, in order to avoid Pi overaccumulation and thus leaf Pi toxicity. Moreover, *AtPHO2*, an E2 conjugase, and *AtNLA*, an E3 ligase, are both part of the ubiquitination pathway, targeting proteins for degradation via the ubiquitin-26S proteasome, and could thus interact together. A yeast two-hybrid screen has already demonstrated that *AtNLA* could interact with the ubiquitin E2 conjugase *UBC8* via the RING domain (Peng *et al.*, 2007).

Additional evidence of the involvement of the Arabidopsis *AtNLA* in Pi homeostasis was provided by the identification of two suppressors of the *Atnla* mutation recovering the WT phenotype, namely the Phosphate Transporter Traffic Facilitator1 (*AtPHF1*) and Phosphate Transporter 1.1 (*AtPHT1;1*) (Kant *et al.*, 2011). In addition, *AtPHF1* and *AtPHT1;1* are probably direct or indirect targets of both *AtNLA* and *AtPHO2*, as mutation of *Atphf1* and *Atpht1;1* in the *Atnla* and *Atpho2* mutant backgrounds restored the Pi concentration to WT levels. Thus, in the future, it will be interesting to gain further details of the cross-talk between *AtNLA* and *AtPHO2*, and their role in controlling nitrate-dependent phosphate homeostasis.

Conclusion and perspectives

In recent years, the role and importance of the SPX domain-containing proteins in the regulation of Pi homeostasis have become increasingly clear. The recent discovery of the involvement of NLA and some members of the SPX-MSF family in the control of Pi homeostasis shows that all families of proteins harboring the SPX domain have some members involved in Pi signaling and/or transport in plants. The function of these families is well conserved between Arabidopsis and rice, with the exception of the SPX-RING and SPX-MFS families that 'overlap'. In the future, a key challenge to improve our knowledge on the function of the plant SPX domain-containing protein, and thus Pi homeostasis, will be to determine whether the SPX domain is involved in protein interaction, as observed in yeast, and, if so, to identify the different molecular players. Indeed, several SPX domain-containing proteins have been shown to localize to the nucleus, and thus may interact with transcription factors. It is also interesting to note that several yeast and plant proteins possessing the SPX domain have been shown to negatively regulate Pi

signaling and/or transport. Whether such a negative feedback regulatory function can be generalized to other proteins harboring the SPX domain has yet to be determined. Gaining further information on the localization of the SPX domain-containing proteins will be a key process in the identification of their function. Ultimately, the identification of the role of the SPX domain, and SPX domain-containing proteins, will greatly improve our knowledge of Pi signaling and transport in plants, assisting efforts to breed plants with better P use efficiency.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Overview of the regulation of the rice and Arabidopsis SPX domain-containing transcripts on inorganic phosphate (Pi) starvation.

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