Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain

Ji-Yul Jung[1], Martina K Ried[2], Michael Hothorn[2] and Yves Poirier[1]

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Proteins containing a SPX domain are involved in phosphate (Pi) homeostasis, including Pi transport and adaptation to Pi deficiency. The SPX domain harbors a basic surface binding Pi at low affinity and inositol pyrophosphates (PP-InsPs) at high affinity. Genetic and biochemical studies revealed that PP-InsPs serve as ligands for the SPX domain. Residues in the PHO1 SPX domain involved in PP-InsPs binding are critical for its Pi export activity, and the interaction between SPX proteins and the PHR1 transcription factor, which results in PHR1 inactivation, is promoted by PP-InsPs. Changes in PP-InsPs levels in response to Pi deficiency may thus contribute to the adaptation of plants to stress via the modulation of the activity of SPX-containing proteins and their interactors. Modulating PP-InsP levels or the affinity/specificity of the SPX domain for PP-InsP could potentially be used to engineer crops to maintain high yield under reduced Pi fertilizer input.

Addresses
[1] Department of Plant Molecular Biology, University of Lausanne, Lausanne, Switzerland
[2] Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland

Corresponding authors: Hothorn, Michael (Michael.hothorn@unige.ch), Poirier, Yves (yves.poirier@unil.ch)

Introduction

The availability of phosphorus (P) limits plant growth in natural and agricultural ecosystems. Plants essentially acquire P as water-soluble inorganic orthophosphate (Pi, $H_2PO_4^-$). While P may be quite abundant in most soils, the level of Pi accessible to plants is very low, principally because it readily forms insoluble complexes with calcium as well as with oxides and hydroxides of aluminum and iron. While application of Pi fertilizers to optimize crop yield is an essential practice in modern agriculture, its sustainability has been put into question [1]. P is essentially mined from a limited number of rock phosphate deposits and high quality rock phosphate is a finite resource [1,2]. This fact, combined with the expected increased demand for Pi fertilizers to sustain not only crops but also grassland productivity, raised concerns about its availability for future generations [3•]. Furthermore, overuse of fertilizers leads to P run-off in streams and lakes, resulting in eutrophication. In this perspective, one important goal of plant biotechnology is to develop plants that can maintain maximal productivity under reduced fertilizer input. This will require new approaches, as past breeding programs typically focused on improving yield and pest resistance of plants grown under well-fertilized conditions [4].
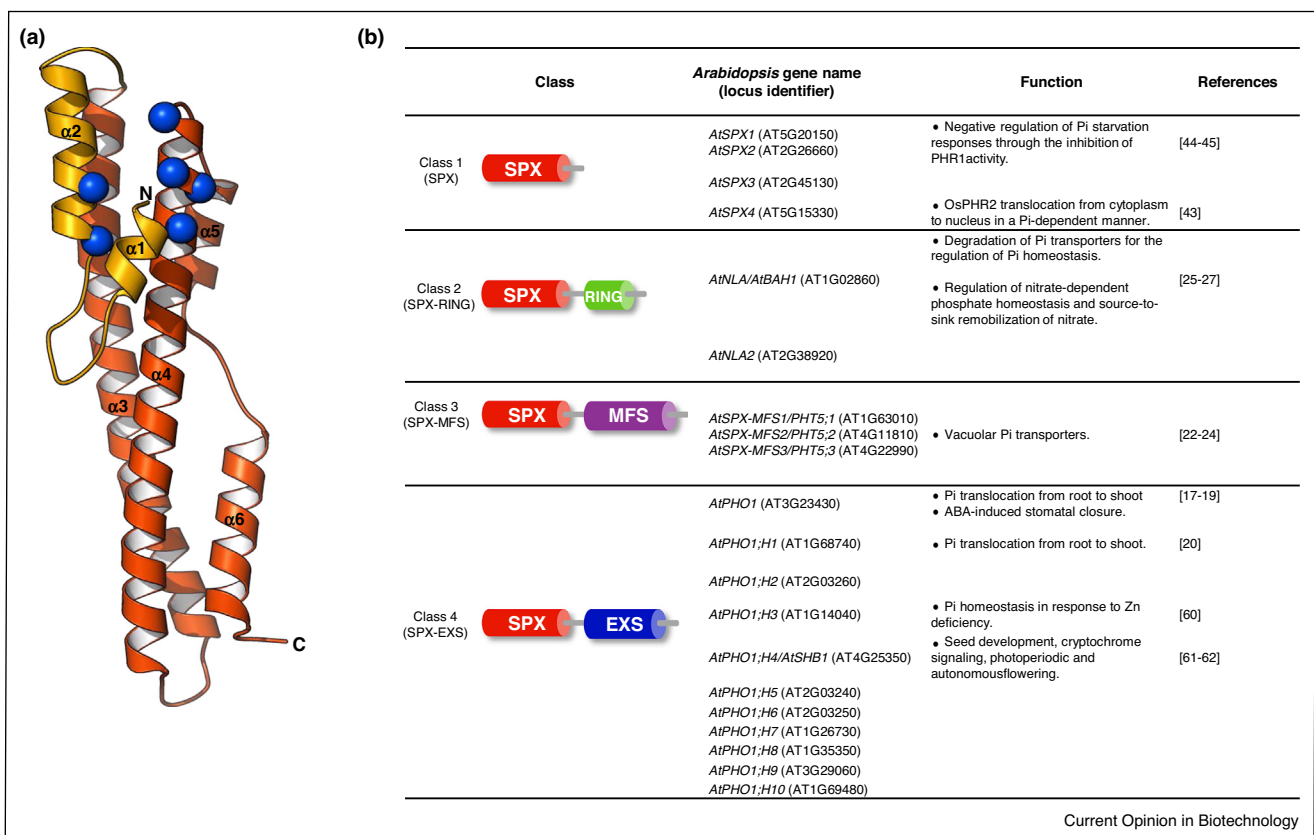
Plants have evolved complex adaptation mechanisms to grow and survive in Pi-poor soils. Root development is profoundly modified under Pi deficiency: root hair length and density are increased, primary root length is reduced and root branching is enhanced [5]. Pi-deficient roots also secrete more protons, citrate as well as various esterases, and enhance association with mycorrhizae [6]. Collectively, these modifications are aimed at increasing the solubility of soil P, and enhancing the ability of the root system to explore and mine the soil for this precious resource. Plants also adjust their metabolic pathways to optimize internal Pi use, such as shifting phospholipids toward galactolipids, glucuronolipids and sulfolipids [7,8]. At the genetic level, these adaptations are accompanied by the coordinated up-regulation of hundreds of genes (named phosphate starvation induced, PSI) [9,10]. In the last decade, several components have been identified that are involved in sensing and coordinating the complex Pi-deficiency response. One key player is PHR1, a member of the MYB transcription factor family [11]. PHR1 and its closest homologue PHL1 control the expression of the majority of PSI genes and influence numerous metabolic and developmental adaptations to Pi deficiency [10–13]. PHR1 has also recently been shown to integrate the PSI response with the plant immune response and to influence the root microbiota [14••]. Since the PHR1 mRNA level is not modulated by Pi deficiency, questions as to how PHR1 activity is regulated by Pi deficiency remained unanswered. Recently, a role for inositol pyrophosphates and SPX-containing proteins has been uncovered in the PHR1-mediated Pi-deficiency response [15••].

SPX-containing proteins in Pi homeostasis

The hydrophilic SPX domain (Pfam PF03105, named after the *Saccharomyces cerevisiae* Syg1 and Pho81proteins, and the mammalian Xpr1) comprises 160–350 amino acids and contains a set of invariant lysine residues forming a conserved sequence fingerprint (Figure 1). SPX-containing proteins are found throughout the eukaryotic tree of life, including fungi, plants, and metazoans. Plant SPX-containing proteins are divided into four subfamilies, SPX-EXS (Pfam PF03124, named after the *S. cerevisiae* Erd1, mammalian Xpr1 and *S. cerevisiae* Syg1), SPX-MFS (Pfam Clan CL0015, Major Facilitator Superfamily), SPX-RING (Pfam 13920, Really Interesting New Gene), and SPX, depending on the presence of additional domains, which are always fused to the C-terminus of the SPX domain (Figure 1) [16]. PHO1 is the prototypical member of the SPX-EXS subfamily and encodes a Pi exporter involved in loading Pi into the root vascular cylinder [17]. The EXS domain contains two trans-membrane helices and is implicated in proper localization and activity of PHO1 as a Pi exporter and in participating in the Pi-deficiency responses [18]. The diversity of PHO1-related genes has expanded in dicots compared to monocots. The *Arabidopsis thaliana* genome contains 10 PHO1 homologues (PHO1;H1-H10), of which only PHO1;H1 has been directly associated with Pi homeostasis [19–21]. Proteins belonging to the SPX-MFS subfamily contain numerous transmembrane helices and encode the long sought-after tonoplast Pi transporters, named PHT5 or VPT, moving Pi in and out of the vacuole [22••,23••,24••]. The subfamily SPX-RING includes NLA, an E3 ubiquitin ligase that targets the Pi transporter PHT1 for ubiquitination and degradation [25–27]. The fourth subfamily consists of stand-alone SPX proteins (SPX1 to SPX4), all of which are differentially regulated under Pi deficiency [28]. Modulation of the expression of some

Figure 1

Class		*Arabidopsis* gene name (locus identifier)	Function	References
Class 1 (SPX)	SPX	*AtSPX1* (AT5G20150) *AtSPX2* (AT2G26660)	• Negative regulation of Pi starvation responses through the inhibition of PHR1activity.	[44-45]
		AtSPX3 (AT2G45130)		
		AtSPX4 (AT5G15330)	• OsPHR2 translocation from cytoplasm to nucleus in a Pi-dependent manner.	[43]
Class 2 (SPX-RING)	SPX — RING	*AtNLA/AtBAH1* (AT1G02860)	• Degradation of Pi transporters for the regulation of Pi homeostasis. • Regulation of nitrate-dependent phosphate homeostasis and source-to-sink remobilization of nitrate.	[25-27]
		AtNLA2 (AT2G38920)		
Class 3 (SPX-MFS)	SPX — MFS	*AtSPX-MFS1/PHT5;1* (AT1G63010) *AtSPX-MFS2/PHT5;2* (AT4G11810) *AtSPX-MFS3/PHT5;3* (AT4G22990)	• Vacuolar Pi transporters.	[22-24]
Class 4 (SPX-EXS)	SPX — EXS	*AtPHO1* (AT3G23430)	• Pi translocation from root to shoot. • ABA-induced stomatal closure.	[17-19]
		AtPHO1;H1 (AT1G68740)	• Pi translocation from root to shoot.	[20]
		AtPHO1;H2 (AT2G03260)		
		AtPHO1;H3 (AT1G14040)	• Pi homeostasis in response to Zn deficiency.	[60]
		AtPHO1;H4/AtSHB1 (AT4G25350)	• Seed development, cryptochrome signaling, photoperiodic and autonomousflowering.	[61-62]
		AtPHO1;H5 (AT2G03240) *AtPHO1;H6* (AT2G03250) *AtPHO1;H7* (AT1G26730) *AtPHO1;H8* (AT1G35350) *AtPHO1;H9* (AT3G29060) *AtPHO1;H10* (AT1G69480)		

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Structure of the SPX domain and function of SPX domain-containing proteins in plants. **(a)** Ribbon diagram of the ScVtc4 SPX domain (http://rcsb. org, PDB-ID 5IIG). The 3-helix bundle, consisting of two long core helices and two smaller C-terminal helices, is shown in orange. The N-terminal α-helical hairpin motif is highlighted in yellow. The position of the conserved lysine residues, which form sequence fingerprints for the SPX domain, are depicted by blue spheres. The C-terminal α6 helix can be connected to extra domains such as RING, MFS, and EXS. **(b)** In *Arabidopsis thaliana*, there are 20 SPX proteins that are classified into four different sub-families depending on the presence of extra domains: four proteins contain only the SPX domain (SPX1 to SPX4) [43••,44••,45••], two proteins combine SPX and a RING domain (NLA and NLA2) [25–27], three combine SPX and a MFS domain (PHT5 or VPT) [22••,23••,24••], and eleven proteins combine SPX and an EXS domain (the PHO1 family) [17–20, 60–62]. The functions of these proteins are indicated.