

**Serveur Académique Lausannois SERVAL [serval.unil.ch](https://serval.unil.ch)**

## **Author Manuscript**

**Faculty of Biology and Medicine Publication**

**This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.**

Published in final edited form as:

**Title:** Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain.

**Authors:** Jung J.Y., Ried M.K., Hothorn M., Poirier Y.

**Journal:** Current Opinion in Biotechnology

**Year:** 2018

**Volume:** 49

**Pages:** 156-162

**DOI:** [10.1016/j.copbio.2017.08.012](https://doi.org/10.1016/j.copbio.2017.08.012)

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

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

Ji-Yul Jung<sup>a</sup>, Martina Reid<sup>b</sup> Michael Hothorn<sup>b</sup> and Yves Poirier<sup>a</sup>

<sup>a</sup> Department of Plant Molecular Biology, University of Lausanne, Lausanne, Switzerland

<sup>b</sup> Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland

Corresponding authors:

Yves Poirier; [yves.poirier@unil.ch](mailto:yves.poirier@unil.ch)

Michael Hothorn; [Michael.hothorn@unige.ch](mailto:Michael.hothorn@unige.ch)

## **Summary**

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.

## Introduction

The availability of phosphorus (P) limits plant growth in natural and agricultural ecosystems. Plants essentially acquire P as water-soluble inorganic orthophosphate (Pi,  $\text{H}_2\text{PO}_4^-$ ). 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, over-use 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 galacto-, glucurono-, and sulfo-lipids [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 Pho81 proteins, 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 sub-families, 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 sub-family 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 ten 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 sub-family 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 [22-24]. The sub-family SPX-RING includes NLA, an E3 ubiquitin ligase that targets the Pi transporter PHT1 for ubiquitination and degradation [25-27]. The fourth sub-family 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 members of the SPX sub-family is associated with changes in the expression of PSI genes and influences the adaptation of plants to Pi deficiency [28-30].

The common element of plant SPX-containing proteins is their implication in Pi homeostasis, which also extends to the ten *Saccharomyces cerevisiae* proteins containing a SPX domain, including Pi transporters (Pho87, Pho90, Pho91), a PHO1-homologue (Syg1), a Pi-recycling

enzyme (Gde) and components of the Pi-signaling cascade (Pho81) or subunits of the inorganic polyphosphate polymerase (Vtc2 to Vtc5) [31]. The connection between SPX and Pi homeostasis raised the possibility that this domain may be involved in the direct binding of Pi or some metabolite derived from it. In this context, it is important to know the concentrations of Pi and related metabolites that are available in the plant cytosol for potential interaction with the SPX domain. Measurements of cytoplasmic (e.g. cytosol and organelles except for the vacuole) Pi concentration by *in vivo* <sup>31</sup>P-NMR typically gives estimates of 5-10 mM [32]. However, by using a NMR method allowing the separation of the organellar (primarily mitochondria and plastids) and cytosolic signals, Pi concentrations of 60-80 μM for the cytosol and 4-7 mM for the organelles were obtained [33]. Such a cytosolic Pi concentration would be in the  $K_m$  range of Pi of several Pi-dependent enzymes but not of others, including SPX proteins ([15] see below). Dynamic changes of cytosolic Pi content in root cells grown on Pi-deficient or Pi-sufficient media have recently been monitored *in vitro* with a Pi-biosensor having a  $K_d$  for Pi of 6.4 mM, thus putting the cytosolic Pi concentration potentially in that range [34].

### **SPX domains are sensors for inositol pyrophosphate signaling molecules**

Crystal structures of fungal and human SPX domains revealed a novel protein fold, with a central three-helix bundle preceded by a conserved N-terminal  $\alpha$ -hairpin motif (Figure 1) [15]. In different SPX domain structures, the conserved lysine cluster forms a highly basic binding surface at the N-terminus of the protein. This surface can interact with Pi itself, albeit with low millimolar affinity ( $K_d \approx 5\text{-}20$  mM) [15]. Furthermore, SPX domains cannot discriminate between Pi and other inorganic anions, such as sulfate (Figure 2A). A combined genetic, biochemical and structural screen identified inositol pyrophosphates (PP-InsPs) as *bona fide* ligands for the SPX domain, which specifically interact with the basic binding surface with nano- to micromolar affinity ( $K_d \approx 50$  nM- 100 μM) [15,35] (Fig. 2B). PP-InsPs consist of a fully phosphorylated inositol ring that carries additional pyrophosphate groups in one or several positions [36]. In yeast, the concentrations of PP-InsPs change in response to Pi starvation and their biosynthesis route has been well-characterized [15,36,37]. Deletion of the PP-InsP kinase Kcs1 leads to an inhibition of the inorganic polyphosphate polymerase activity of the yeast VTC complex via its SPX domain. In this mutant, Pi is only stored as free inorganic phosphate under Pi sufficient growth conditions [15,38,39]. In plants, homologs of diphosphoinositol pentakisphosphate kinase (PPIP5K/Vip1-like) have been identified [40,41]. However, PP-InsP biosynthesis is generally poorly understood and PP-InsPs have thus far not

been implicated in the response of plants to Pi deficiency [41,42]. This may, in part, be due to the challenges in detecting and quantifying different PP-InsP isomers from plant tissues.

### **Inositol pyrophosphate binding renders SPX domains competent to interact with other proteins**

Previous work has established that SPX proteins can associate with the Arabidopsis PHR1 or its rice homologue PHR2 both *in vitro* and *in vivo* [43-45]. Formation of the SPX-PHR1/2 complex in Pi-sufficient plants prevents the binding of PHR1/2 to its target promoters. In contrast, under Pi-deficient conditions, PHR1/2 is free and can act as a transcriptional activator of PSI genes. Initially, Pi itself was proposed as a ligand enabling SPX proteins to interact with PHR1/2. The dissociation constants for Pi in such a complex range from 10 to 20 mM [43-45]. In contrast, PP-InsPs promote the high-affinity interaction between the rice SPX4 and PHR2, with Kds in the low micromolar range (7-50  $\mu$ M) [15]. Thus, PP-InsPs may regulate the activity of PHR1/2 via SPX proteins. Consistently, plants unable to synthesize the PP-InsPs precursor inositol hexakisphosphate (InsP6) show constitutive Pi starvation responses, despite having higher cellular Pi levels compared to wild-type [36,46,47]. Couso and colleagues report levels for different PP-InsPs, but it remains an open question which isomer(s) are involved in the PSI response [48]. It is likely that, besides Pi starvation, PP-InsPs control other signaling pathways such as hormone perception, as purification of plant hormone receptors heterologously expressed in insect cells resulted in co-purification of endogenous InsP6 with the auxin receptor complex TIR1-ASK1 and of inositol pentaphosphate (InsP5) with the jasmonate receptor complex COI1-ASK1 [49,50]. In addition, jasmonate specifically induced the production of the PP-InsP InsP8, and *in silico* docking experiments together with site-directed mutagenesis of COI1 hint at InsP8 being essential for COI1-JAZ1 complex formation [41]. Finally, PP-InsPs have also been implicated in COI1-mediated plant wounding responses [51,52]. Interestingly, modulation of TIR1 activity was implicated in the developmental adaptation of roots to Pi deficiency and low Pi induces increased resistance to herbivory via the JA-COI1 pathway [53,54]. It is thus possible that PP-InsPs coordinate multiple aspects of the Pi-deficiency response via their interaction with proteins involved in different signal transduction pathways.

## Future perspective

While research on the yeast VTC and the plant PHO1, PHR1 and SPX-containing proteins clearly demonstrated the implication of PP-InsPs in Pi homeostasis and signaling, one may wonder if there is still room for a direct role of Pi as a signal molecule. One of the strongest evidence for this comes from classical studies using phosphite (Phi,  $\text{H}_2\text{PO}_3^-$ ), a reduced form of Pi. Application of Phi to Pi-deficient plants suppresses a wide range of typical Pi-deficiency responses, including local responses, such as root hair elongation, and systemic responses, such as lipid remodeling and the expression of numerous PSI genes [55,56]. Since plants cannot convert Phi into Pi and Phi itself is non-metabolizable, it can be concluded that Phi likely mimics Pi as a signal molecule. Like Pi, Phi was also shown to promote the interaction between SPX proteins and PHR1/2 [43-45], but it remains to be investigated if and how SPX can sense Phi. Furthermore,  $^{31}\text{P}$ -NMR experiments have shown that cytosolic Pi concentration can rapidly fluctuate in response to shifts in external Pi supply, indicating that cytosolic Pi levels are more dynamic than previously thought, a characteristic that would also fulfill an important criterion for a signaling molecule [33]. However, as discussed previously, it is currently uncertain whether the concentration of Pi in the cytosol is sufficient to warrant any significant signaling function via SPX considering the relatively high  $K_d$  of Pi for this domain. In this context, it will be important to measure plant cytosolic PP-InsP concentrations under different plant growth conditions. With this information it will be possible to discuss the  $K_d$  of PP-InsPs for SPX domains in respect to physiological conditions on the one hand, and to correlate changes in Pi nutrition and cytosolic Pi concentrations with those of PP-InsPs on the other hand.

There is growing evidence for PP-InsPs being master regulators of Pi signaling in plants. Point mutations in the SPX domain of PHO1 in amino acids involved in coordinating with PP-InsPs but not Pi showed that these residues were essential for the complementation of the *pho1* mutant, thus highlighting the role of PP-InsPs versus Pi for PHO1 as a Pi exporter [15]. Similar genetic dissection experiments on different SPX-containing proteins are key experiments enabling to discern the role of Pi and PP-InsPs in their function. It will be also important to determine if the binding of PP-InsPs to the SPX domain of other SPX-containing proteins, such as PHT5/VPT and NLA, regulate their activity through the promotion of interaction with other effector/regulatory proteins, or via distinct mechanisms, such as protein pyrophosphorylation [57].



The high affinity of the SPX domain for PP-InsPs raises the possibility of using this domain as biosensors for the detection of PP-InsPs in vivo [58]. However, distinguishing between different PP-InsPs isomers may be a limiting factor of such a biosensor. In this respect, it is noteworthy that the SPX domain may require protein-protein interaction partners to discriminate PP-InsP isomers [15].

One major goal of future research will be the dissection and characterization of plant PP-InsPs biosynthetic and catabolic pathways as targets for the efficient manipulation of plant Pi homeostasis and the Pi-deficiency signal transduction pathway. Previous studies with PHO1 have shown that it is possible to uncouple shoot phosphate deficiency from its negative effects on growth [59]. Optimization of the PSI response, e.g. enhancement or attenuation, via the engineering of the SPX domain or the modulation of PP-InsPs content may thus be associated with improved growth under low Pi supply. The transfer of such knowledge to agronomically important plant species, such as rice, would contribute to the engineering of crops that have a reduced demand for valuable Pi-fertilizers but maintain maximum yield.

### **Acknowledgments**

Research projects on SPX-containing proteins, the Pi-deficiency signaling pathway and PP-InsPs are funded in the YP lab by the Swiss National Foundation (grant 31003A-159998) and the Herbette Foundation, and in the MH lab by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement 310856 and the European Molecular Biology Organisation (EMBO) Young Investigator Programme.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Gilbert N: **Environment: The disappearing nutrient**. *Nature* 2009, **461**:716-718.
2. Herrera-Estrella L, Lopez-Arredondo D: **Phosphorus: the underrated element for feeding the world**. *Trends Plant Sci* 2016, **21**:461-463.
3. Sattari SZ, Bouwman AF, Rodriguez RM, Beusen AHW, van Ittersum MK: **Negative global phosphorus budgets challenge sustainable intensification of grasslands**. *Nature Comm* 2016, **7**:10696.
  - Reports the future needs of P-based fertilizers to maintain the high productivity of agricultural systems relying on grasslands. It is estimated that mineral P fertilizer use must double by 2050 to sustain future crop and grassland production.
4. Lopez-Arredondo DL, Leyva-Gonzalez MA, Gonzalez-Morales SI, Lopez-Bucio J, Herrera-Estrella L: **Phosphate nutrition: improving low-phosphate tolerance in crops**. *Ann Plant Rev* 2014, **65**:95-123.
5. Peret B, Clement M, Nussaume L, Desnos T: **Root developmental adaptation to phosphate starvation: better safe than sorry**. *Trends Plant Sci* 2011, **16**:442-450.
6. Poirier Y, Bucher M: **Phosphate transport and homeostasis in Arabidopsis**. In *The Arabidopsis Book*. Edited by Somerville CR, Meyerowitz EM: American Society of Plant Biologists; 2002.
7. Okazaki Y, Otsuki H, Narisawa T, Kobayashi M, Sawai S, Kamide Y, Kusano M, Aoki T, Hirai MY, Saito K: **A new class of plant lipid is essential for protection against phosphorus depletion**. *Nature Comm* 2013, **4**:1510.

8. Shimojima M, Ohta H: **Critical regulation of galactolipid synthesis controls membrane differentiation and remodeling in distinct plant organs and following environmental changes.** *Prog Lipid Res* 2011, **50**:258-266.
  9. Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet P, Creff A, Somerville S, Rolland N, et al.: **A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation.** *Proc Natl Acad Sci USA* 2005, **102**:11934-11939.
  10. Morcuende R, Bari RP, Gibon Y, KZheng W, Datt Pant B, Bläsing O, Usadel B, Czechowski T, Udvardi MK, Stitt M, et al.: **Genome-wide reprogramming of metabolism and regulatory networks of *Arabidopsis* in response to phosphorus.** *Plant Cell Environ* 2007, **30**:85-112.
  11. Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J: **A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae.** *Genes Dev* 2001, **15**:2122-2133.
  12. Bustos R, G. C, Linhares F, Puga MI, Rubio V, Pérez-Pérez J, Solano R, Leyva A, Paz-Ares J: **A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*.** *PLoS Genet* 2010, **6**: e1001102.
  13. Pant BD, Pant P, Erban A, Huhman D, Kopka J, Scheible WR: **Identification of primary and secondary metabolites with phosphorus status-dependent abundance in *Arabidopsis*, and of the transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation.** *Plant Cell Environ* 2015, **38**:172-187.
  14. Castrillo G, Teixeira P, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, et al.: **Root microbiota drive direct integration of phosphate stress and immunity.** *Nature* 2017, **543**:513-518.
- This work examined the interplay between the phosphate starvation response and the root

microbiome. It demonstrates a key role for the PHR1 transcription factor in integrating the phosphate starvation response with the plant immune output.

15. Wild R, Gerasimaite R, Jung J-Y, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Poirier Y, Hothorn M, et al.: **Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains.** *Science* 2016, **352**:986-990.

•• This work identified SPX domains as *bona fide* sensors for PP-InsPs that act as signaling molecules for cytosolic Pi levels. Under Pi-deficiency, PP-InsPs bind to SPX domains, enabling interaction with several proteins involved in the regulation of Pi signaling and the phosphate starvation response.

16. Secco D, Wang C, Arpat BA, Wang Z, Poirier Y, Tyerman SD, Wu P, Shou H, Whelan J: **The emerging importance of the SPX domain-containing proteins in phosphate homeostasis.** *New Phytol* 2012, **193**:852-841.

17. Hamburger D, Rezzonico E, MacDonald-Comber Petétot J, Somerville C, Poirier Y: **Identification and characterization of the *Arabidopsis* PHO1 gene involved in phosphate loading to the xylem.** *Plant Cell* 2002, **14**:889-902.

18. Wege S, Khan GA, Jung J-Y, Vogiatzaki E, Pradervand S, Aller I, Meyer AJ, Poirier Y: **The EXS domain of PHO1 participates in the response of shoots to phosphate deficiency via a root-to-shoot signal.** *Plant Physiol* 2016, **170**:385-400.

19. Secco D, Baumann A, Poirier Y: **Characterization of the rice PHO1 gene family reveals a key role for *OsPHO1;2* in phosphate homeostasis and the evolution of a distinct clade in dicotyledons.** *Plant Physiol* 2010, **152**:1693-1704.

20. Stefanovic A, Ribot C, Rouached H, Wang Y, Chong J, Belbahri L, Delessert S, Poirier Y: **Members of the PHO1 gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways.** *Plant J* 2007, **50**:982-994.

21. Wang Y, Secco D, Poirier Y: **Characterization of the *PHO1* gene family and the responses to phosphate deficiency of *Physcomitrella patens***. *Plant Physiol* 2008, **146**:646-656.
22. Liu JL, Yang L, Luan MD, Wang Y, Zhang C, Zhang B, Shi JS, Zhao FG, Lan WZ, Luan S: **A vacuolar phosphate transporter essential for phosphate homeostasis in *Arabidopsis***. *Proc Natl Acad Sci USA* 2015, **112**:E6571-E6578.
- Transport of Pi in and out of the vacuole plays a major role in maintaining cellular Pi homeostasis, but the transporters involved in this process remained unknown for decades. This work at last identified a major vacuolar Pi transporter that belongs to the SPX-MFS family.
23. Liu TY, Huang TK, Yang SY, Hong YT, Huang SM, Wang FN, Chiang SF, Tsai SY, Lu WC, Chiou TJ: **Identification of plant vacuolar transporters mediating phosphate storage**. *Nature Comm* 2016, **7**:11095.
- A second independent group reports the role of SPX-MFS members as vacuolar Pi transporters.
24. Wang C, Yue WH, Ying YH, Wang SD, Secco D, Liu Y, Whelan J, Tyerman SD, Shou HX: **Rice SPX-Major Facility Superfamily3, a vacuolar phosphate efflux transporter, is involved in maintaining phosphate homeostasis in rice**. *Plant Physiol* 2015, **169**:2822-2831.
- A third independent group reports the role of SPX-MFS members as vacuolar Pi transporters.
25. Kant S, Peng M, Rothstein SJ: **Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in *Arabidopsis***. *PLoS Genet* 2011, **7**:e1002021.
26. Lin W-Y, Huang T-K, Chiou T-J: **NITROGEN LIMITATION ADAPTATION, a target of microRNA827, mediates degradation of plasma membrane-localized**

- phosphate transporters to maintain phosphate homeostasis in arabidopsis.** *Plant Cell* 2013, **25**:4061-4074.
27. Park BS, Seo JS, Chua N-H: **NITROGEN LIMITATION ADAPTATION recruits PHOSPHATE2 to target the phosphate transporter PT2 for degradation during the regulation of Arabidopsis phosphate homeostasis.** *Plant Cell* 2014, **26**:454-464.
28. Duan K, Yi KK, Dang L, Huang HJ, Wu W, Wu P: **Characterization of a sub-family of Arabidopsis genes with the SPX domain reveals their diverse functions in plant tolerance to phosphorus starvation.** *Plant J* 2008, **54**:965-975.
29. Liu F, Wang Z, Ren H, Shen C, Li Y, Q. LH, Wu C-C, Lian X, Wu P: **OsSPX1 supresses the function of osPHR2 in the regulation of expression of OsPT2 and phosphate homeostasis in shoots or rice.** *Plant J* 2010, **62**:508-517.
30. Wang C, Ying S, Huang HJ, Li K, Wu P, Shou HX: **Involvement of OsSPX1 in phosphate homeostasis in rice.** *Plant J* 2009, **57**:895-904.
31. Secco D, Wang C, Shou H, Whelan J: **Phosphate homeostasis in the yeast *Saccharomyces cerevisiae*, the key role of the SPX domain-containing proteins.** *FEBS Lett* 2012, **586**:289-295.
32. Lee RB, Ratcliffe RG, Southon TE: **P-31 NMR measurements of the cytoplasmic and vacuolar pi content of mature maize roots - relationships with phosphorus status and phosphate fluxes.** *J Exp Bot* 1990, **41**:1063-1078.
33. Pratt J, Boisson A-M, Gout E, Bligny R, Douce R, Aubert S: **Phosphate (Pi) starvation effect on the cytosolic pi concentration and pi exchanges across the tonoplast in plant cells: an in vivo P-31-nuclear magnetic resonance study using methylphosphonate as a pi analog.** *Plant Physiol* 2009, **151**:1646-1657.
34. Mukherjee P, Banerjee S, Wheeler A, Ratliff LA, Irigoyen S, Garcia LR, Lockless SW, Versaw WK: **Live imaging of inorganic phosphate in plants with cellular and subcellular resolution.** *Plant Physiol* 2015, **167**:628-638.

- Reports the use of a fluorescence-based biosensor that detect changes in the concentration of Pi in the root cytosol. First such tool developed to detect Pi availability in living cells at the sub-cellular resolution level. The system can be adapted to detect Pi also in organelles.

35. Wild R, Hothorn M: **The macro domain as fusion tag for carrier-driven crystallization.** *Protein Sci* 2017, **26**:365-374.

36. Shears SB: **Inositol pyrophosphates: why so many phosphates.** *Adv Biol Reg* 2015, **57**:203-216.

37. Azevedo C, Saiardi A: **Extraction and analysis of soluble inositol polyphosphates from yeast.** *Nat Protoc* 2006, **1**:2416-2422.

38. Gerasimaite R, Pavlovic I, Capolicchio S, Hofer A, Schmidt A, Jessen HJ, Mayer A: **Inositol pyrophosphate specificity of the SPX-dependent polyphosphate polymerase VTC.** *ACS Chem Biol* 2017, **12**:648-653.

39. Lonetti A, Szijgyarto Z, Bosch D, Loss O, Azevedo C, Saiardi A: **Identification of an evolutionarily conserved family of inorganic polyphosphate endopolyphosphatases.** *J Biol Chem* 2011, **286**:31966-31974.

40. Desai M, Rangarajan P, Donahue JL, Williams SP, Land ES, Mandal MK, Phillippy BQ, Perera IY, Raboy V, Gillaspay GE: **Two inositol hexakisphosphate kinases drive inositol pyrophosphate synthesis in plants.** *Plant J* 2014, **80**:642-653.

41. Laha D, Johnen P, Azevedo C, Dynowski M, Weiss M, Capolicchio S, Mao H, Iven T, Steenbergen M, Freyer M, et al.: **VIH2 regulates the synthesis of inositol pyrophosphate InsP(8) and jasmonate-dependent defenses in Arabidopsis.** *Plant Cell* 2015, **27**:1082-1097.

42. Williams SP, Gillaspay GE, Perera IY: **Biosynthesis and possible functions of inositol pyrophosphates in plants.** *Front Plant Sci* 2015, **6**:e67.

43. Lv Q-D, Zhong Y, Wang Y, Wang Z, Zhang L, Shi J, Wu Z, Liu Y, Mao C, Yi KK, et al.: **SPX4 negatively regulates phosphate signaling and homeostasis through its interaction with PHR2 in rice.** *Plant Cell* 2014, **26**:1586-1597.
- Demonstrate that under high Pi conditions, SPX4 binding to PHR2 prevents its localization to the nucleus and thus blocks its action as a transcription factor. Under low Pi, SPX4 is unstable and degraded via the 26S proteasome.
44. Puga MI, Mateos I, Charukesi R, Wang Z, Franco-Zorilla JM, de Lorenzo L, Irigoyen ML, Masiero S, Bustos R, Rodriguez J, et al.: **SPX1 is a phosphate-dependent inhibitor of PHOSPHATE STARVATION RESPONSE 1 in Arabidopsis.** *Proc Natl Acad Sci USA* 2014, **111**:14947-14952.
- This work in Arabidopsis shows that SPX1 regulates the activity of PHR1 as a transcription factor by binding to it in a Pi-dependent fashion. It thus links cellular phosphate content to the activity of PHR1 and the phosphate starvation response.
45. Wang Z, Ruan W, Shi J, Zhang L, Xiang D, Yang C, Li C, Wu Z, Liu Y, Yu Y, et al.: **Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner.** *Proc Natl Acad Sci USA* 2014, **111**:14953-14958.
- A second group who independently shows, in rice, the Pi-dependent binding of SPX proteins to the transcription factor PHR2.
46. Kuo H-F, Chang T-Y, Chiang S-F, Wang W-D, Charng Y-Y, Chiou T-J: **Arabidopsis inositol pentakisphosphate 2-kinase, AtIPK1, is required for growth and modulates phosphate homeostasis at the transcriptional level.** *Plant J* 2014, **80**:503-515.
47. Stevensons-Paulik J, Bastidas RJ, Chiou ST, Frye RA, York JD: **Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases.** *Proc Natl Acad Sci USA* 2005, **102**:12612–12617.



48. Couso I, Evans BS, Li JT, Liu Y, Ma F, Diamond S, Allen DK, Umen JG: **Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control, and lipid metabolism in Chlamydomonas.** *Plant Cell* 2016, **28**:2026-2042.
49. Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu F-F, Sharon M, Browse J, et al.: **Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor.** *Nature* 2010, **468**:400-405.
50. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N: **Mechanism of auxin perception by the TIR1 ubiquitin ligase.** *Nature* 2007, **446**:640-645.
51. Mosblech A, Koenig S, Stenzel I, Grzegarek P, Feussner I, Heilmann I: **Phosphoinositide and inositolpolyphosphate signalling in defense responses of Arabidopsis thaliana challenged by mechanical wounding.** *Mol Plant* 2008, **1**:249-261.
52. Mosblech A, Thurow C, Gatz C, Feussner I, Heilmann I: **Jasmonic acid perception by COI1 involves inositol polyphosphates in Arabidopsis thaliana.** *Plant J* 2011, **65**:949-957.
53. Khan GA, Vogiatzaki E, Glauser G, Poirier Y: **Phosphate Deficiency Induces the Jasmonate Pathway and Enhances Resistance to Insect Herbivory.** *Plant Physiol* 2016, **171**:632-644.
54. Perez-Torres CA, Lopez-Bucio J, Cruz-Ramirez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L: **Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor.** *Plant Cell* 2008, **20**:3258-3272.
55. Kobayashi K, Masuda T, Takamiya K-I, Ohta H: **Membrane lipid alteration during phosphate starvation is regulated by phosphate signaling and auxin/cytokinin cross-talk.** *Plant J* 2006, **47**:238-248.

56. Varadarajan DK, Karthikeyan AS, P.D. M, Raghothama KG: **Phosphite, an analogue of phosphate, suppresses the coordinated expression of genes under phosphate starvation.** *Plant Physiol* 2002, **129**:1232-1240.
57. Brown NW, Marmelstein AM, Fiedler D: **Chemical tools for interrogating inositol pyrophosphate structure and function.** *Chem Soc Rev* 2016, **45**:6311-6326.
58. Uslu VV, Grossmann G: **The biosensor toolbox for plant developmental biology.** *Curr Opin Plant Biol* 2016, **29**:138-147.
59. Rouached H, Stefanovic A, Secco D, Arpat AB, Gout E, Bligny R, Poirier Y: **Uncoupling phosphate deficiency from its major effects on growth and transcriptome via PHO1 expression in Arabidopsis.** *Plant J* 2011, **65**:557-570.
60. Khan GA, Bouraine S, Wege S, Li Y, de Carbonnel M, Berthomieu P, Poirier Y, Rouached H: **Coordination between zinc and phosphate homeostasis involves the transcription factor PHR1, the phosphate exporter PHO1, and its homologue PHO1;H3 in Arabidopsis.** *J Exp Bot* 2014, **65**:871-884.
61. Kang X, Ni M: **Arabidopsis SHORT HYPOCOTYL UNDER BLUE1 contains SPX and EXS domains and acts in cryptochrome signaling.** *Plant Cell* 2006, **18**:921-934.
62. Zhou Y, Ni M: **SHB1 plays dual roles in photoperiodic and autonomous flowering.** *Dev Biol* 2009, **331**:50-57.

## Figure legends

### **Figure 1. 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  $\alpha$ -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  $\alpha_6$  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), two proteins combine SPX and a RING domain (NLA and NLA2), three combine SPX and a MFS domain (PHT5 or VPT), and eleven proteins combine SPX and an EXS domain (the PHO1 family). The functions of these proteins are indicated.

**Figure 2. Interaction of the SPX domain with sulfate and InsP6.** (A) Structure of the SPX domain of the human XPR1, a PHO1 homologue (<http://rcsb.org>, PDB-ID 5IJH, colors as in Fig. 1) bound to a sulfate ion (in bonds representation). Note that the SPX domain cannot differentiate between sulfate and Pi and that sulfate is a good structural mimic to Pi. The sulfate ion is in hydrogen bond contact (grey dotted lines) with only few of the lysine residues conserved among SPX domains. (B) Structure of the SPX domain of *Chaetomium thermophilum* Glycerophosphodiester Phosphodiesterase 1 (<http://rcsb.org>, PDB-ID 5IJJ) in complex with InsP6. The signaling molecule establishes an extensive hydrogen bond network with a tyrosine originating from the N-terminal helical hairpin motif and with the invariant lysine residues.



