Identity and functions of inorganic and inositol polyphosphates in plants

Laura Lorenzo-Orts*1, Daniel Couto*1 and Michael Hothorn 1

Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, 30 Quai E. Ansermet, Geneva 1211, Switzerland

Summary

Inorganic polyphosphates (polyPs) and inositol pyrophosphates (PP-InsPs) form important stores of inorganic phosphate and can act as energy metabolites and signaling molecules. Here we review our current understanding of polyP and InsP metabolism and physiology in plants. We outline methods for polyP and InsP detection, discuss the known plant enzymes involved in their synthesis and breakdown, and summarize the potential physiological and signaling functions for these enigmatic molecules in plants.

I. Introduction

Phosphorus is an essential nutrient for all living organisms, representing one of the nine macronutrients present in large quantities in plant tissues. Phosphorus is taken up by plants in the form of inorganic phosphate (Pi; for a list of abbreviations used in this article, see Table 1) (Fig. 1a). Pi is an essential building block for many cellular components, such as nucleic acids and membranes, is a major component of molecules that function as the energy currency of the cell, and is an important signaling molecule. Hence, Pi deficiency can affect a wide range of biological processes, ultimately affecting plant growth and development (Rouached et al., 2010).

Plants store Pi in their vacuoles and relocate it to the cytosol when intracellular Pi concentrations are low (Liu et al., 2016). Some plant tissues, such as seeds and fruits, can store Pi in the form of phytic acid (inositol hexakisphosphate, InsP6; Fig. 1b) (Secco et al., 2017). Phytic acid is one of several inositol multiphosphorylated compounds present in plants, and these compounds share the D-myo-inositol ring-bearing ester phosphate group at one or more positions. While InsP6 may represent a storage form of Pi in plants, higher phosphorylated inositol pyrophosphates act as signaling molecules in plants (Laha et al., 2015; Wild et al., 2016; Zhu et al., 2019) and many other eukaryotes (Azevedo & Saiardi, 2017).

Inorganic polyphosphates (polyPs) exist in many prokaryotes and eukaryotes, and form a major store of Pi, for example, in yeast (Urech et al., 1978). PolyPs form linear chains that vary in length from 3 to c. 1000 Pi units and are linked by energy-rich phosphoanhydride bonds (Fig. 1c). Depleting inositol pyrophosphates (PP-InsPs) in yeast results in a massive decrease in polyP concentrations (Lonetti et al., 2011). This suggests that Pi, InsP and polyP metabolism are functionally connected, especially in sessile, soil-living organisms. However, it is presently unclear whether polyPs exist in plants and whether they contribute to Pi metabolism and storage. Here, we critically review our current knowledge concerning polyPs in plants, and the roles of PP-InsPs in Pi sensing and cell signaling.

*These authors contributed equally to this work.
II. Inorganic polyphosphates

PolyPs were first described in yeast (Liebermann, 1890) and bacteria (Babes, 1895). They also form abiotically; for instance, it has been shown that volcanic activity can generate polyPs from tetraphosphorus decoxide (P₄O₁₀) (Yamagata et al., 1991). They might therefore have played a role in evolution, when Pi was mostly found as part of prebiotic phosphorous minerals. The presence of polyPs in both prokaryotes and eukaryotes suggests an ancient origin for this polymer.

1. Localization and functions of inorganic polyphosphates

Many functions have been attributed to polyPs in various organisms (Fig. 2). In bacteria, polyPs accumulate within granules in the nucleoid region (Racki et al., 2017) and can regulate various cellular processes, including growth, sporulation, response to nutrient deprivation, virulence, cell cycle and metal toxicity (Xie & Jakob, 2019). PolyPs also act as primordial chaperones in bacteria, binding to unfolded proteins and promoting their refolding (Gray et al., 2014). Furthermore, they are able to associate with calcium ions (Ca²⁺) and polyhydroxybutyrate in the bacterial membrane, forming DNA entry channels that are involved in bacterial competence (Castuma et al., 1995).

In yeast, polyPs are located mainly in the vacuole, although a minor fraction (<10%) can be found in mitochondria (Pestov et al., 2004), where they control energy metabolism and cell death (Abramov et al., 2007). In vacuoles, they act as a major storage form of Pi, energy and divalent cations, accumulating when intracellular Pi concentrations are high. Under Pi starvation, yeast cells can remodelize Pi from polyP (Shirahama et al., 1996). The vacuolar polyP store thus allows yeast cells to maintain Pi, ion (Rosenfeld et al., 2004), where they control energy metabolism and cell death (Abramov et al., 2007). In vacuoles, they act as a major storage form of Pi, energy and divalent cations, accumulating when intracellular Pi concentrations are high. Under Pi starvation, yeast cells can remodelize Pi from polyP (Shirahama et al., 1996). The vacuolar polyP store thus allows yeast cells to maintain Pi, ion (Rosenfeld et al., 2004), where they control energy metabolism and cell death (Abramov et al., 2007). In vacuoles, they act as a major storage form of Pi, energy and divalent cations, accumulating when intracellular Pi concentrations are high. Under Pi starvation, yeast cells can remodelize Pi from polyP (Shirahama et al., 1996).

In the amoeba Dictyostelium discoideum, polyPs accumulate in acidocalcisomes (Zhang et al., 2005). Acidocalcisomes are acidic vacuoles, which contain Ca²⁺ and polyP and thus play a role in several processes, including calcium signaling and autophagy (Docampo et al., 1995). In trypanosomes, polyPs also accumulate in acidocalcisomes, although they have been detected in the nucleolus and the glycosome as well (Negreiros et al., 2018).

Algae accumulate polyPs in vacuoles with similar properties to acidocalcisomes (Ruiz et al., 2001; Aksoy et al., 2014), which maintain Pi and ion homeostasis. PolyPs also play a role in nutrient deprivation; mutants lacking a component of the polyP polymerase complex are more susceptible to sulfur, phosphorus and nitrogen starvation (Aksoy et al., 2014).

In humans, polyPs have been found in different subcellular compartments, including acidocalcisomes (Ruiz et al., 2004), lysosomes (Pisoni & Lindley, 1992) and the nucleolus (Jimenez-Nuñez et al., 2012). PolyPs play a role in diverse biological functions in humans, for instance in the regulation of rRNA transcription, ion homeostasis and metal toxicity (Xie & Jakob, 2019).
biogenesis (Jimenez-Nuñez et al., 2012; Azevedo et al., 2015), coagulation (Müller et al., 2009), and bone formation (Hacchou et al., 2007).

PolyPs have been demonstrated to be covalently linked to lysine residues in yeast and human proteins involved in ribosome biogenesis (Azevedo et al., 2015; Bentley-DeSousa et al., 2018). In yeast, protein polyphosphorylation is genetically linked to inositol pyrophosphate metabolism (Azevedo et al., 2015). Whether lysine polyphosphorylation represents an important post-translational modification in plants requires further investigation.

2. Enzymes metabolizing inorganic polyphosphates

Specific polyP-metabolizing enzymes have been identified in bacteria and lower eukaryotes, as well as in humans, yet they have not been functionally characterized in multicellular organisms. Bacterial polyP kinases 1 and 2 (PPK1/2) catalyze the synthesis of polyP from ATP and/or GTP (Ahn & Kornberg, 1990; Zhang et al., 2002). PPK1 can also regenerate ATP from polyP (Ahn & Kornberg, 1990). In yeast, polyP synthesis is carried out by the vacuolar transporter chaperone (VTC) complex, which contains several subunits (Hothorn et al., 2009). The catalytic subunit Vtc4 contains a tunnel-shaped (TRIPHOSPHATE TUNNEL METALLOENZYME, TTM) catalytic domain, which catalyzes the synthesis of polyP from ATP. The growing polymer is translocated into the vacuole by means of a trans-membrane domain, to which several subunits contribute (Hothorn et al., 2009; Gerasimaitė et al., 2014). VTC is conserved in all unicellular eukaryotes, including *Chlamydomonas* (Aksoy et al., 2014) and trypanosomes (Lander et al., 2013). *Dictyostelium discoideum* can produce polyP via a protein homolog of the bacterial PPK1 (Zhang et al., 2005), and an actin-related protein named DdPPK2, which forms filaments concurrently with the synthesis of polyP (Gómez-García & Kornberg, 2004).

Specific inorganic polyphosphatasases have been discovered in prokaryotes and eukaryotes. In bacteria, long-chain polyPs can be sequentially hydrolyzed by exopolyphosphatase 1 (PPX1; Akiyama et al., 1993). PPX1 belongs to the same protein superfamily as actin, HSP70 chaperones and sugar kinases, and hydrolyzes both polyP and the alarmone guanosine pentaphosphate (pppGpp; Kuroda et al., 1999). The short-chain inorganic polyphosphatase ygiF from *Escherichia coli* hydrolyzes tripolyphosphate into pyrophosphate and Pi (Kohn et al., 2012; Martinez et al., 2015). ygiF contains an N-terminal TTM domain with structural homology to yeast Vtc4 and a C-terminal conserved α-helical domain (CHAD). In yeast, PPX1 belongs to the DHH

| Clade  | polyP localization | polyP functions                                                                 |
|-------|-------------------|---------------------------------------------------------------------------------|
| Bacteria | polyP granules or acidocalcisomes | Cell cycle, sporulation, motility, biofilm formation, metal toxicity, stress responses |
| Fungi | Vacuoles | *P*<sub>i</sub> homeostasis, cell cycle, ion and pH homeostasis |
|        | Cell wall | *P*<sub>i</sub> source for mycorrhiza, antimicrobial compound, toxicity |
|        | Mitochondria | Energy metabolism, cell death |
| Animals | Mitochondria | Energy metabolism, cell death |
|        | Acidocalcisomes | *P*<sub>i</sub> and ion homeostasis, coagulation, bone formation |
|        | Nucleus/nucleolus | rRNA biogenesis |
|        | Lysosomes | Gliotransmitter, pH buffer and protection from hydrolyses |
| Algae | Vacuoles | *P*<sub>i</sub> and ion homeostasis, response to nutrient deprivation |
|        | Cell wall | Protective functions during cytokinesis |
| Plants | Nucleus/nucleolus? | ? |

Fig. 2 Localizations and functions of polyPs in different organisms. Subcellular localization of inorganic polyphosphates (polyPs) is shown schematically on the left. A table summarizing the functions reported in the different cellular compartments is shown on the right.
phosphatase family and hydrolyzes the terminal Pi from short-chain polyPs (Wurst & Kornberg, 1994). Human H-prune belongs to the same protein family as yeast PPX1 and can hydrolyze polyP (preferentially short-length polyP) as well as nucleoside tetraphosphates (Tammenkoski et al., 2008). Members of the nudix hydrolase family (NUDTs), which have been reported to cleave InsPs, can also cleave polyPs (Lonetti et al., 2011). In addition, the human purple acid phosphatase ACP5 is able to cleave polyP, with a substrate preference for short-chain polyPs (Harada et al., 2013).

There are also endopolyphosphatases that release Pi from an internal or terminal position in a polyP chain. PPN1 localizes to the yeast cytosol and belongs to the calcineurin-like phosphatase family (Kumble & Kornberg, 1995), while PPN2 is a metallophosphatase in the vacuolar lumen (Gerasimaité & Mayer, 2017).

3. Inorganic polyphosphate metabolizing enzymes and binding proteins in plants

No bona fide PPK1, PPK2 or Vtc4 orthologs have been identified in higher eukaryotes, including plants, and polyP synthesis is therefore poorly understood in these organisms (Kumble & Kornberg, 1995; Pavlov et al., 2010). However, several conserved proteins with significant homology to bacterial or animal inorganic polyphosphatases are present in plant genomes, including the model plant Arabidopsis thaliana (Table 2).

AtTTM3 shares structural homology to both the catalytic domain of the polyP polymerase Vtc4 (Hothorn et al., 2009) and the E. coli short-chain polyP phosphatase ygiF (Martinez et al., 2015). Enzymatic and structural characterization of AtTTM3, which is conserved in the entire plant lineage (Lorenzo-Orts et al., 2019b), revealed that it is a bona fide ortholog of bacterial ygiF, hydrolyzing short-chain polyPs using a conserved catalytic mechanism based on two metal ion centers (Moeder et al., 2013; Martinez et al., 2015). AtTTM3 is a broadly expressed, soluble protein localized in the cytosol and nucleus (Lorenzo-Orts et al., 2019b). Genetic characterization of AtTTM3 initially revealed a function in root growth (Moeder et al., 2013). Subsequent analyses, however, revealed that TTM3 is transcribed into and translated from a bicistronic mRNA, encoding both TTM3 and the cell division protein 26 (CDC26; Lorenzo-Orts et al., 2019b). While CDC26 is essential for Arabidopsis embryo development and regulates plant growth, AtTTM3 seems to be dispensable under favorable growth conditions (Lorenzo-Orts et al., 2019b). While the physiological roles of AtTTM3 remain unclear, it is of note that functional connections between polyPs and cell cycle regulation are well established in other prokaryotes and eukaryotes (Bru et al., 2016; Racki et al., 2017). There are at least two additional TTM proteins in plants (AtTTM1 and AtTTM2), where the TTM domain is located at the C-terminus of a uridine kinase domain (Ung et al., 2014, 2017). Both enzymes have been reported to have pyrophosphatase activity, but it is not known which domain harbors this activity (Ung et al., 2014, 2017).

A putative yet thus far uncharacterized ortholog of bacterial exopolyphosphatase is present in the Arabidopsis genome as a single-copy gene (At1g09195) and appears to be conserved among many plant species. PPX enzymes have been reported to cleave both polyP and pppGpp (Kuroda et al., 1999). Enzymes catalyzing the synthesis and breakdown of pppGpp in plants (van der Biezen et al., 2000) have been shown to function in the chloroplast, where pppGpp accumulates under stress conditions (Chen et al., 2014). It has been speculated that PPX could cleave pppGpp, regulating stress responses (Boniecka et al., 2017). However, the enzymatic

### Table 2 Arabidopsis thaliana orthologs of polyP polymerases and phosphatases from bacteria, yeast and Dictyostelium discoideum.

| Organism     | Enzyme | Protein domain | Arabidopsis protein homologs | Secondary structure | Example       |
|--------------|--------|----------------|------------------------------|--------------------|---------------|
| **PolyP polymerases** |        |                | Arabidopsis protein homologs |                    |               |
| Bacteria     | PPK1   | Phospholipase D | –                            | Phospholipase D domain: c. 40 | At1g55180    |
|              | PPK2   | Thymidine kinase| –                            | Thymidine kinase: 2 | At3g07800 (TKA1) |
| Yeast        | Vtc4   | VTC            | –                            | CYTH/TTM domain: 3 | At2g11890 (TTM3) |
| Dictyostelium| PPK1   | Phospholipase D | –                            | Phospholipase D domain: c. 40 | At1g55180    |
|              | DdPPK2 | Actin-related protein 1 | – | – | – |
| **Polyphosphatases** |        |                | Arabidopsis protein homologs |                    |               |
| Bacteria     | PPX    | (actin, HSP70, sugar kinases) | 1 | PPX domain: 1 | At1g09195 |
|              | ygiF   | CYTH           | –                            | CYTH/TTM domain: 3 | At2g11890 (TTM3) |
| Yeast        | PPX1   | DHH phosphoesterases | – | DHH domain: 5; DHHa1 domain: 7 | At1g53345 |
|              | PPN1   | Calcineurin-like phosphoesterases | – | Calcineurin-like phosphoesterases: 190 | At3g03305 |
|              | PPN2   | Metallophosphatase | – | – | – |
| Human        | H-prune| DHH phosphoesterases | – | DHH domain: 5; DHHa1 domain: 7 | At1g53345 |
|              | DIPP1/2/3 | Nudix hydrolases | 2 | Nudix hydrolase domain: c. 100 | At4g25434 (NUDX10) |
|              | ACP5   | Purple acid phosphatases | c. 10 | Purple acid phosphatases: 16 | At2g01880 (PAP7) |

The numbers of proteins with primary and secondary structure homology to inorganic polyphosphate (polyP)-metabolizing enzymes is listed. Putative polyP-metabolizing enzymes from Arabidopsis were identified by sequence homology using a protein–protein BLAST search (Altschul et al., 1990), or by similarities in domain architecture using InterPro (Mitchell et al., 2019). Note that the biochemical activities for many of the Arabidopsis proteins listed have not been experimentally assessed.
properties and physiological functions of AtPPX require further investigation.

In Arabidopsis, there are 28 putative NUDTs (Yoshimura & Shigeoka, 2015). Human NUDTs have been shown to hydrolyze polyP, InsPs and other nucleoside polyphosphates (Lonetti et al., 2011). Plant NUDTs act on a large variety of substrates, including ADP-ribose (Ogawa et al., 2005), nucleotides modified by reactive oxygen species (Jemth et al., 2019), and the alarmone pppGpp (Ito et al., 2012). The large number of NUDTs in Arabidopsis and the diverse substrate specificity of NUDT family members render their contribution to polyP metabolism difficult to assess. This is even more true for plant purple acid phosphatases (PAPs), metalloenzymes which hydrolyze a wide range of Pi esters and anhydrides. In Arabidopsis, there are 29 predicted PAPs, although only 28 of them have been shown to be at least transcribed (Tran et al., 2010). Among them, AtPAP15 was reported to have phytase activity (Zhang et al., 2008), but polyP was not tested as potential substrates.

Recently, we have characterized CHAD, present for example in the bacterial short-chain polyphosphatase ygiF, as a specific polyP-binding module (Lorenzo-Orts et al., 2019a). CHADs can be found in the three domains of life and co-localize with polyP granules in bacteria (Tumlirsch & Jendrossek, 2017), yet they do not exhibit enzymatic activity towards polyP (Martinez et al., 2015). Interestingly, the plant Ricinus communis has a CHAD-containing protein (Lorenzo-Orts et al., 2019a). Its high structural and sequence similarity with bacterial CHAD domains and its singularity in the plant kingdom suggests that RcCHAD might have been acquired by Ricinus via horizontal gene transfer from a soil-living bacterium. RcCHAD is expressed in Ricinus and binds polyPs with low micromolar affinity and high selectivity (Lorenzo-Orts et al., 2019a). As with other genes acquired by horizontal gene transfer, RcCHAD may have evolved a new cellular function or may bind polyP as in bacteria.

4. Inorganic polyphosphate detection in cells and tissues

Early studies report the detection of polyPs in different algae and mosses and in higher plants, such as spinach (leaves) and cotton (seeds) (Keck & Stich, 1957; Miyachi, 1961; Tewari & Singh, 1964). In line with this, electron-dense granules inside and outside plant vacuoles have been interpreted as polyP bodies in transmission electron microscopy (TEM) sections (Mamun et al., 2005). Here, we will briefly review the different methods available for the identification and quantification polyP stores in cells and tissues to put these findings in context.

The yeast polyphosphatase PXP1, which releases the terminal Pi from polyP molecules, has been used to estimate polyP concentrations in cell extracts. The product of this reaction can be estimated with malachite green, which allows Pi concentrations to be quantified via a simple colorimetric reaction (Bru et al., 2017). This assay may be sensitive to contamination by free Pi or other Pi-containing metabolites. Alternatively, the reverse reaction of PPK1 causes conversion of ADP to ATP, which can be quantified via luciferase-based assays (Ault-Riché et al., 1998).

Nuclear magnetic resonance (NMR) spectroscopy has long been used to detect the presence of polyP in cells by detecting the magnetic field of $^{31}$P atoms (Klein et al., 1988; Bental et al., 1991). This method has been used in plants, but polyPs were not detected (Pratt et al., 2009). The sensitivity and resolution of this method are limited, however, hindering the detection of metabolites present at low concentrations (Gowda & Raftery, 2015).

Using TEM, polyP bodies appear as electron-dense granules, and they have been observed in palm seeds and rice anthers (DeMason & Stillman, 1986; Mamun et al., 2005). One way to determine the composition of these granules is by combining electron microscopy with energy-dispersive X-ray microanalysis. This technique allows for the detection of atoms based on their emission spectra upon X-ray excitation, and it has been used to analyze the composition of polyP granules in bacteria (Alvarez & Jerez, 2004) and plants (DeMason & Stillman, 1986; Otegui et al., 2002).

Raman microscopy has been successfully adopted for the detection of polyP in various organisms, including bacteria (Majed et al., 2009) and microalgae (MoudrÍková et al., 2017). In plants, phytic acid (InsP$_6$) could be detected in the aleurone and endosperm tissue of wheat grains using this method, but no specific signal was reported for polyP (Kolozsvári et al., 2015). Although this technique offers a high specificity, Kolozsvári et al. suggest that the sensitivity of the method needs to be further improved.

Besides specifically staining nucleic acids, 4′,6-diamidino-2-phenylindole (DAPI) has been extensively used to detect polyP in cells. When bound to DNA, DAPI emits light at a wavelength of 461 nm. DAPI can also bind to RNA (emitting light at c. 500 nm) (Kapuscinski, 1990) and to Pi-rich compounds, including inositol polyphosphates and polyP (emitting light at c. 550 nm) (Kolozsvári et al., 2014). Thus, DAPI cannot distinguish between inorganic and inositol polyphosphate molecules (Kolozsvári et al., 2014).

Since DAPI can bind to different Pi-containing compounds, novel fluorescent dyes have been reported that bind polyPs with higher specificity. JC-D7 and JC-D8 bind polyP and heparin but do not bind to other Pi-containing molecules such as nucleic acids, nucleotides, or sodium phosphate (Angelova et al., 2014). However, both JC-D7 and JC-D8 dyes have lower affinities for polyP than for DAPI, and binding to inositol polyphosphates was not assessed (Angelova et al., 2014). We recently used the JC-D7 dye to stain polyP in plant cells (Zhu et al., 2019). While plant cells expressing the E. coli polyP kinase PPK1 revealed the presence of JC-D7-stained polyP granules in the cytosol, no polyP granules could be detected in wild-type Arabidopsis plants, in the tmm3-1 mutant (see section 2.3; Lorenzo-Orts et al., 2019b) or in other monocotyledonous and dicotyledonous plants. Specific staining could only be shown in O. sativa var. indica, where the VTC complex generates a polyP store in acidocalcisomes (Aksoy et al., 2014). These findings suggest that polyPs are either absent in higher plants, do not accumulate to high concentrations, may be present only in specific cell types or organs, or are only produced in response to certain environmental stimuli. Our observations cannot explain the fact that the inorganic polyphosphatase TTM3 is a broadly expressed enzyme in Arabidopsis (Moeder et al., 2013; Lorenzo-Orts et al., 2019b). It is worth noting that cytosolic accumulation of...
PPK1-generated polyP granules in plants is toxic (Zhu et al., 2019), as previously reported in yeast (Gerasimaitė et al., 2014).

The polyP-binding domain (PPXc) of the E. coli polyphosphatase PPIX has been used to detect polyPs in cells via immunofluorescence. Initially reported in yeast (Saito et al., 2005), PPXc has been further used to specifically detect polyP in cells from different organisms, including humans (Jimenez-Nuñez et al., 2012; Moreno-Sanchez et al., 2012) and trypanosomatids (Negréiros et al., 2018). We have generated a fluorescent protein fusion of PPXc, which when expressed in isolation localizes to the cytoplasm and nucleus in Arabidopsis and Nicotiana benthamiana (Zhu et al., 2019). The fusion protein, however, localizes to JC-D7-stained polyP granules in PPK1-expressing transient lines, suggesting that it can be used to label polyP pools in higher plants (Zhu et al., 2019).

A fluorescent protein-tagged version of the polyP-binding protein RcCHAD in Arabidopsis and N. benthamiana showed a specific localization to the nucleus and nucleolus (Lorenzo-Orts et al., 2019a). Co-expression with E. coli PPK1 relocalized RcCHAD to PPK1-generated cytoplasmic polyP granules, suggesting that CHAD domains may be suitable for polyP detection in plant cells and that there may be a nuclear/nucleolar polyP store in higher plants (Lorenzo-Orts et al., 2019a). Further experiments are required to substantiate this finding, but it is of note that nucleolar polyP stores have been reported in human cells (Jimenez-Nuñez et al., 2012) and trypanosomes (Negréiros et al., 2018). Furthermore, nucleolar proteins implicated in ribosome biogenesis in yeast and humans are known to be targets of polyphosphorylation (Azevedo et al., 2015; Bentley-DeSousa et al., 2018), and it is therefore possible that RcCHAD may bind to nucleolar proteins carrying this post-translational modification.

5. Inorganic polyphosphates in symbiotic interactions

Approx. 90% of all plant species possess roots that are associated with fungi, forming symbioses known as mycorrhizas. Plant roots provide carbohydrates to the fungus, while the fungus offers mineral nutrients (mainly P and N) to the plant (Bonfante & Genre, 2010). Interestingly, mycorrhizal fungi can accumulate c. 60% of the total Pi content in the form of polyP (Hijikata et al., 2010). Hence, most of the Pi transported to plant cells is derived from fungal polyP pools (Chiu & Paszkowski, 2019). During colonization, the fungal polyP content increases, with short-chain polyP being more abundant (Ohtomo & Saito, 2005). How Pi and/or polyPs are transported into the apoplast is currently unknown. One possibility is that the fungal VTC complex (Hothorn et al., 2009) can polymerize polyP at the plasma membrane into the apoplast, where the polymer may be hydrolyzed to Pi by polyphosphatases and taken up by the root via mycorrhiza-inducible Pi transporters (Chiu & Paszkowski, 2019).

Perspectives PolyPs have been reported in many prokaryotic and eukaryotic organisms. In the green lineage, the presence of significant polyP stores has been firmly established in algae (Werner et al., 2007a; Aksoy et al., 2014), while the microscopy and biochemical extraction methods used in the early studies on higher plants are now known to lack specificity towards polyPs (Keck & Stich, 1957; Miyachi, 1961; Tewari & Singh, 1964). The identity of the polyP bodies reported in plant TEM sections has likewise not been validated by other methods (DeMason & Stillman, 1986; Mamun et al., 2005). We have recently used a polyP-specific dye and a polyP-binding protein to detect polyPs in plants in a genetically validated manner (Zhu et al., 2019). Our experiments suggest that higher plants may not contain large polyP stores in organized vacuoles/acidocalcisomes, at least in the species, tissues and experimental conditions tested (Zhu et al., 2019). We found, however, that two polyP-binding domains (PPXc and RcCHAD) localize to the nucleus and nucleolus in plant cells (Lorenzo-Orts et al., 2019a; Zhu et al., 2019). These findings certainly require further validation, but they nevertheless indicate that polyP stores may exist in these compartments. We speculate that lower concentrations of polyPs may not be detectable by fluorescent dyes (Angelova et al., 2014; Kolozsvari et al., 2014), by 31P NMR (Pratt et al., 2009) or by Raman spectroscopy (Kolozsvari et al., 2015). However, they might be detectable using high-affinity polyP-binding domains, which have previously been used to stain polyPs in different compartments in animal cells (Jimenez-Nuñez et al., 2012). Taken together, these findings indicate that the presence of polyPs in higher plants has not been firmly established. However, a number of biochemically validated or putative polyP-binding proteins and polyP-metabolizing enzymes are present in plants, some of which are evolutionarily conserved (see section 2.3). This raises the question of what the metabolic and physiological functions of these enzymes might be, and whether or not they act on polyP substrates in vivo. Further improvement of the polyP detection methods and in-depth characterization of known and novel polyP-metabolizing enzymes may yield a final answer to the question of whether polyPs exist in plants.

III. Inositol pyrophosphates

1. Inositol pyrophosphates: metabolism

Inositol polyphosphates (InsPs) are another class of Pi-rich metabolites present in all eukaryotes; they are versatile Pi storage metabolites present in all eukaryotes; they are versatile Pi storage and signaling molecules that control a wide range of biological processes (Azevedo & Saiardi, 2017; Shears, 2018). InsPs are based on a myo-inositol ring that may be sequentially and reversibly phosphorylated at each of its six carbon positions, giving rise to six different InsP species, termed InsP1 to InsP6 according to the number of phosphate groups they possess (Fig. 3). The different InsP derivatives have distinct properties, and they can be distinguished by a prefix that indicates the position of the phosphate on the ring (e.g. 1,4,5-InsP3 represents myo-inositol 1,4,5-trisphosphate; Fig. 3).

InsPs may be further phosphorylated on existing phosphate groups to give rise to inositol pyrophosphates (PP-InsPs) containing one or more high-energy pyrophosphate groups. InsPs and PP-InsPs are present in all eukaryotes, including plants, at varying concentrations depending on the organism and cell type. Some plants accumulate high concentrations of InsP6, especially in seeds, where it is thought to act as a Pi...
The reaction product can be further phosphorylated into InsP8 by the reciprocal or inositol hexakisphosphate kinase (IP6K) enzymes, respectively. Each at positions 1 or 5 by diphosphoinositol pentakisphosphate kinase (PPIP5K) enzymes. The identity of an IP6K-like enzyme in plants remains unknown.

Fig. 3 Synthesis of inositol pyrophosphates. Inositol hexakisphosphate (InsP6) is converted to inositol pyrophosphate (InsP7) upon phosphorylation at positions 1 or 5 by diphosphoinositol pentakisphosphate kinase (PPIP5K) or inositol hexakisphosphate kinase (IP6K) enzymes, respectively. Each reaction product can be further phosphorylated into InsP8 by the reciprocal enzyme. The identity of an IP6K-like enzyme in plants remains unknown.

2. Mechanisms of inositol pyrophosphate signaling

Different mechanisms by which PP-InsPs control cell signaling have been established. The binding of PP-InsPs to target proteins may influence protein–protein interactions and/or allosterically regulate their activity, and they may compete for the same binding site as membrane-localized phosphoinositides (PIPs). In addition, PP-InsPs may directly phosphorylate proteins as a signaling-competent post-translation modification. Each of these cases is discussed in the paragraphs that follow, and they are illustrated in Fig. 4.

Protein–protein interactions Signaling proteins are typically part of dynamic complexes that determine their signaling competence, activation state and subcellular localization. Post-translational modifications or binding of small molecules such as InsPs or PP-InsPs can greatly influence the interactions between two or more proteins. InsP6,6,6 were initially shown to promote activation of mammalian casein kinase 2 (CK2; Solyakov et al., 2004), a ubiquitous kinase with multiple targets. InsP binding to CK2 interferes with the binding of its negative regulator Nopp140 (Lee et al., 2013). Importantly, the PP-InsP 5-InsP7 binds CK2 more tightly than InsP6, and is thus more potent in its activation of CK2 to promote p53-dependent cell death in human cancer cells (Rao et al., 2014). Since 5-InsP7 binds to the substrate-binding site of CK2, it is tempting to speculate as to whether PP-InsPs can modulate the substrate specificity of protein kinases. CK2 but not Nopp140 is conserved in all eukaryotes, including plants. Whether plant CK2 isoforms can bind PP-InsPs to regulate interaction with their substrates is presently unknown.

As well as blocking protein–protein interactions, InsPs and PP-InsPs may also promote them. This holds true, for example, for PP-InsP sensing SPX (SYG1/Pho81/XPR1) domains interacting with PHOSPHATE STARVATION RESPONSE (PHR) transcription factors (Wild et al., 2016). SPX domains are found in all eukaryotes, where they are located at the N-terminus of transporters or enzymes, or, in the case of plants, exist as ‘stand-alone’ modules. These stand-alone SPX proteins interact with PHR transcription factors only in the presence of InsPs/PP-InsPs (Wild et al., 2016). SPX-PPH complexes preferably bind highly phosphorylated InsPs, with the PP-InsP 5-InsP7 binding 10 times more tightly compared to InsP6 (Wild et al., 2016). When in complex with PHR, SPX proteins prevent the transcription factor from binding DNA and from activating transcription of Pi-starvation genes (Puga et al., 2014; Qi et al., 2017). How different PP-InsPs may affect SPX-PPH complexes, and which one is preferred in vivo, has not yet been determined (see the ‘PP-InsPs and nutrient sensing’ section).

Allosteric regulation SPX domains and PP-InsPs also play a regulatory role in the yeast phosphate signal transduction (PHO) pathway, specifically in the regulation of the Pho85–Pho80 CDK-cyclin complex. Here, the SPX domain-containing CDK inhibitor Pho81 constitutively associates with Pho85–Pho80, but it inhibits Pho80–Pho85 only in the presence of PP-InsPs (Lee et al., 2007). Pho81 interacts primarily with Pho80 at a site that is distant from both the substrate docking site on Pho80 and from the Pho85 kinase active site (Huang et al., 2007). PP-InsP binding to the
Pho81–Pho85–Pho80 complex is thus likely to induce conformational changes in Pho81 that allow it to block either substrate binding to Pho80 or access to the active site on Pho85. Intriguingly, PP-InsP binding and Pho85–Pho80 inhibition are independent of the Pho81 SPX domain (Lee et al., 2008), whose function in this pathway has not yet been fully investigated.

PolyP synthesis by the yeast VTC complex is activated by several PP-InsP isoforms (InsP6 has a minor effect) that bind to the SPX domains of Vtc2–5 (Wild et al., 2016; Gerasimaite et al., 2017). The exact underlying mechanism requires further investigation, but it appears that the allosteric PP-InsP–SPX interaction induces conformational changes in VTC proteins, facilitating ATP hydrolysis and polyP synthesis. Notably, alanine substitutions on key PP-InsP binding lysines from Vtc3 and Vtc4 result in auto-active variants that are capable of synthesizing polyP in the absence of PP-InsPs (Wild et al., 2016). Structural analysis of such mutants may be the key to understanding how PP-InsPs stimulate the VTC complex.

**Competition with membrane-bound phosphoinositides** Phosphoinositides (PIPs) are ubiquitous in eukaryote membranes, where they do not have a structural role, but rather regulate membrane–cytosol communication (for a review, see Schink et al., 2016). Similarly to soluble InsPs, the inositol ring on PIPs can be reversibly phosphorylated at different positions to yield distinct species with specific membrane localizations. Different PIP species can influence cell signaling by providing an anchoring point for both soluble proteins and cytoplasmic domains of membrane proteins. Pleckstrin homology (PH) domains are responsible for the translocation and membrane anchoring of several proteins by selective binding to PIPs (Lemmon, 2007). Snyder and collaborators demonstrated that InsP7 could specifically compete with 3,4,5-PIP3 for binding to the PH domain of several mammalian and Dictyostelium proteins (Luo et al., 2003). A physiological role was suggested for this mechanism, where 5-InsP7 inhibits activation of the PH domain-containing Akt kinase by blocking its translocation to the membrane during insulin signaling (Chakraborty et al., 2010). Remarkably, 5-InsP7 and InsP6 are more efficient at competing with PIP3 for the PH domain of human PBD1 than 1-InsP7 and 1,5-InsP8 in vitro (Gokhale et al., 2013), suggesting a high degree of specificity. Since PH domains are abundant and diverse and occur fused to a variety of proteins in plants, it is important to assess their affinities for PIPs, InsPs and PP-InsPs.
InsPs. No plant PH domain has, to the best of our knowledge, been tested for its ability to bind PP-InsPs.

Other protein domains can mediate binding to PIPs, and thus PIP vs PP-InsP competition might not be an exclusive feature of PH domains. For example, the synaptotagmin-1 (Syt1) C2AB domains bind to 4,5-PIP$_2$ clusters during synaptic vesicle fusion (Park et al., 2015). In turn, 5-InsP$_7$ inhibits synaptic vesicle fusion by binding to the C2AB domains of Syt1 (Lee et al., 2016), but whether this is a clear case of competition for the same binding site remains to be shown. Competition between PP-InsPs and PIPs may be a mechanism for the rapid adjustment of the localization of proteins shuttling between the cytosol and membrane. Determining how widespread this mechanism is and how it is influenced by local PP-InsP pools represents an interesting challenge for the future.

**Non-enzymatic protein pyrophosphorylation** PP-InsPs have the ability to non-enzymatically pyrophosphorylate proteins by transferring their β-phosphoryl group onto phosphoserine residues. Several yeast proteins have been shown to be pyrophosphorylated in vitro (Saiardi et al., 2004; Bhandari et al., 2007; Wu et al., 2016), and two studies have implicated this non-canonical post-translational modification during intracellular vesicle trafficking (Azevedo et al., 2009; Chanduri et al., 2016). Protein pyrophosphorylation has the potential to affect the function of target proteins and directly transduce PP-InsP metabolism into a signaling output; however, it has not yet been possible to directly identify pyrophosphorylated proteins from cell extracts. Advances in mass spectrometry methods (Penkert et al., 2017) are likely to overcome this obstacle soon. Functional studies of non-enzymatic processes are inherently problematic, but the fact that PP-InsP-mediated pyrophosphorylation requires pre-phosphorylation of the serine residue (typically by casein kinase 2 and possibly other kinases) further hinders its in vivo characterization, since mutating the substrate serine would prevent the distinction of the kinase-mediated phosphorylation and polyphosphorylation. An interesting observation in a recent study where synthetic pyrophospho-peptides were hydrolyzed after incubation with cell lysates suggests the presence of a protein pyro-phosphatase (Yates & Fiedler, 2015). Identification of such a specific phosphatase may provide a good genetic tool with which to address pyrophosphorylation in vivo.

3. Inositol pyrophosphate signaling in plants

As outlined above (section 3.2), PP-InsPs control a wide range of cellular processes, many of which could also be present in plants. However, important roles for PP-InsPs in plants are only just emerging, and the contribution of PP-InsPs for plant cell signaling and physiology requires investigation. Interestingly, the few examples of PP-InsP-mediated plant signaling events point to an involvement in plant-specific processes and/or signaling cascades (Fig. 5).

**PP-InsPs and nutrient sensing** Eukaryotic SPX domains were recently described as high-affinity PP-InsP sensors (Wild et al., 2016). SPX-containing proteins have long been associated with Pi sensing and metabolism in eukaryotes (Secco et al., 2012). SPX domains harbor a highly basic binding surface that shows great affinity to PP-InsPs (KD values are in the micromolar to millimolar range) (Wild et al., 2016), implicating PP-InsPs in the regulation of SPX-mediated Pi signaling. In fact, deletion of Kcs1 and/or Vip1 results in PP-InsP depletion in yeast, and the mutant strains are impaired in several aspects of Pi signaling, including Pi acquisition, polyP synthesis and Pi starvation responses (Saiardi et al., 2004; Lee et al., 2007; Mulugu et al., 2007; Lonetti et al., 2011; Gerasimata et al., 2017). Two Arabidopsis PPIP5K homologs, VIH1 and VIH2, are capable of suppressing yeast vip1 null-mutant phenotypes (Desai et al., 2014; Laha et al., 2015). Mutation of VIH2 in Arabidopsis, which shows a broader and stronger expression pattern compared to VIH1, eliminates production of InsP$_8$ (Laha et al., 2015). Intriguingly, vip2 mutant plants accumulate slightly more InsP$_7$, a feature that is also observed in vip1 yeast mutants (Norman et al., 2018). This most likely reflects an accumulation of the 5-InsP$_7$ isomer that is not further converted into 1,5-InsP$_8$, and implies that plants must encode for a yet unknown IP6K-like enzyme capable of synthesizing this isoform. While single vip1 and vip2 Arabidopsis mutants exhibit rather mild developmental phenotypes, the vip1 vip2 double mutant is seedling lethal (Zhu et al., 2019). By using this double mutant, it was possible to uncover an essential role for VIH1/2-generated PP-InsPs in plant phosphate starvation responses (Zhu et al., 2019). The vip1 vip2 seedlings show constitutive high expression of Pi starvation marker genes and over-accumulate Pi. The lethal phenotype is largely caused by over-activation of Pi starvation responses (PSR), as a vip1 vip2 phr1 phl1 quadruple mutant suppresses the vip1 vip2 seedling phenotypes (Zhu et al., 2019). This reveals a critical role of VIH1/2-derived PP-InsP as negative regulators of PSR, and places their kinases genetically upstream of PHR1/PHL1. Depletion of PP-InsP precursors should result in Pi-related phenotypes similar to those of vip1 vip2. This is indeed the case for mutants affecting ITPK1 (phosphorylation of InsP$_3$ and InsP$_4$ on position 1) and ITPK1 (conversion of InsP$_5$ into InsP$_6$), which also display phosphate over-accumulation and developmental phenotypes (Kuo et al., 2018).

In the current working model (Fig. 5b), the PP-InsPs produced by VIH1/2 during Pi-sufficient conditions are sensed by stand-alone SPX proteins, which can then sequester PHR1/PHL1 to prevent them from accessing their target promoters (Puga et al., 2014; Wang et al., 2014; Wild et al., 2016). Under Pi-starvation, VIH1/2-derived PP-InsP concentrations are expected to drop. Without PP-InsPs the SPX-PHR1/PHL1 complex is no longer stable, and the transcription factors are set free to activate expression of Pi starvation genes. A similar mechanism is likely to have been adopted in other Pi-dependent pathways, as OsSPX1/2 interact with the PHR1-related RLI1 transcription factor to regulate Pi-dependent leaf-inclination in rice (Ruan et al., 2018).

PP-InsPs are likely to be directly involved in the regulation of Pi transport in plants, as two Pi-transporter families possess SPX domains: the SPX-EXS and the SPX-MFS subfamily of vacuolar Pi transporters (Secco et al., 2012) (Fig. 5b). Mutation of the PP-InsP-binding lysine cluster on the SPX domain of PHO1, the prototypical Arabidopsis SPX-EXS, abrogates its ability to transport Pi from roots to shoots, causing a dramatic decrease in shoot Pi
content (Wild et al., 2016). Similarly, mutations on the SPX domain of XPR1, a PHO1 homolog and the sole human SPX-containing protein, are responsible for primary familial brain calcification disorders that are most likely caused by Pi transport deficiencies (Chande & Bergwitz, 2018). The mechanism by which PP-InsP binding to the SPX domain activates these transporters has not yet been established.

It remains unclear which PP-InsP species is the most prevalent during Pi signaling in plants. NMR experiments have confirmed that the Arabidopsis VIH2 synthesizes both 1-InsP7 (from InsP6) and 1,5-InsP8 (from 5-InsP7) (Zhu et al., 2019). In yeast, the Pho80–Pho85–Pho81 CDK complex could be inhibited by cell extracts from kcs1 but not vip1 mutants (Lee et al., 2007), suggesting that 1-InsP7 is sufficient for this action. However, kcs1 mutants still exhibit deficiencies in Pi responses (Saiardi et al., 2004), indicating that other pathways may require different PP-InsPs. This was demonstrated in the case of polyP synthesis in yeast, which is impaired in kcs1 but not in vip1 mutants (Lonetti et al., 2011; Gerasimaite et al., 2017). Interestingly, 1-InsP7 and 5-InsP7 showed similar competence in terms of activating the VTC complex in vitro, while 1,5-InsP8 was more effective (Gerasimaite et al., 2017). It is thus evident that yeast, and likely plants, specifically integrate distinct PP-InsP isoforms to control Pi signaling, increasing its plasticity and complexity.

SPX domains have equally been implicated in nitrogen signaling, thus suggesting a broader role for PP-InsPs in nutrient-acquisition pathways. The rice NLP3, a key transcription factor for nitrogen responses, is controlled by OsSPX4, which is in

![Speculative model of inositol pyrophosphate signaling in plants.](image-url)

Fig. 5 Speculative model of inositol pyrophosphate signaling in plants. Inositol pyrophosphates (PP-InsPs) may control multiple aspects of plant signaling and physiology, namely responses to nutrient availability and hormone signaling. (a) PP-InsP levels are expected to fluctuate according to nutrient availability, namely phosphate (Pi) and nitrogen (N), and to yet unidentified internal or external stimuli. Diphosphoinositols pentakisphosphate kinase (PPIP5K) enzymes (VIP1 HOMOLOG1/2 (VIH1/2) in Arabidopsis thaliana) can phosphorylate or dephosphorylate PP-InsPs in reactions respectively catalyzed by their kinase or phosphatase domains; how the activities of each domain are balanced and how these enzymes integrate upstream signaling cues remains unknown. (b) PP-InsPs are sensed by the SPX (SYG1/Pho81/XPR1) domains of multiple proteins. Stand-alone SPX proteins bind to PHR transcription factors only in the presence of PP-InsPs, preventing them from regulating the transcription of Pi-starvation response (PSR) genes. In addition, SPX proteins prevent NODULE INCEPTION PROTEIN-LIKE PROTEIN3 (NL3P3) relocalization to the nucleus, where it regulates N-starvation response (NSR) genes. The Ubiquitin (Ub) E2 conjugase and PHOSPHATE2 (PHO2) and the Ub E3 ligase NITROGEN LIMITATION ADAPTATION (NLA) ubiquitinate and regulate the accumulation of multiple target proteins, including PHOSPHATE1 (PHO1) and the PHOSPHATE TRANSPORTER (PHT) family of Pi-transporters; the nitrate (NO3) transporter NRT1.7; and the transcription factor ORESARA1 (ORE1), a key regulator of senescence-associated genes (SAGs). The mechanistic details of PP-InsP-binding to NLA to SPX-containing Pi-transporters have not yet been investigated. (c) The auxin and jasmonic-acid (JA) receptors use InsPs of PP-InsPs as cofactors that facilitate ligand recognition and complex formation with their respective co-receptors, Aux/IAA and JAZ proteins; these are then ubiquitinated by the TRANSPORT INHIBITOR RESPONSE1 (TIR1)/CORONATINE INSENSITIVE1 (COI1)-Skp/Cullin/F-box (SCF) complex and targeted for degradation, allowing auxin- and JA-signaling activation.
turn ubiquitinated and degraded in a nitrogen-dependent manner (Hu et al., 2019). Moreover, the Ub E3-ligase NLA, which contains an N-terminal SPX domain, controls nitrogen transport and nitrogen-dependent senescence by regulating the Ub-mediated degradation of nitrogen transporters and ORE1 (a key transcription factor for leaf senescence) (Peng et al., 2007; Liu et al., 2017; Park et al., 2018). Interestingly, NLA also controls the concentration of Pi transporters (Lin et al., 2013; Park et al., 2014; Yue et al., 2017), making it a hub that integrates signaling for both of these nutrients (Fig. 5b). How PP-InsP affect these pathways has not been directly tested thus far.

In addition, PP-InsPs have also been implicated in the regulation of target of rapamycin (TOR)-mediated carbon metabolism in Chlamydomonas (Couso et al., 2016), and in the control of mitochondrial function and ATP homeostasis in yeast and human cells (Gu et al., 2017a). How PP-InsPs are able to integrate such a diversity of signaling pathways is far from understood. In yeast, contradictory results account for both increases and decreases in the concentrations of PP-InsPs following Pi deprivation (Lee et al., 2007; Lonetti et al., 2011; Wild et al., 2016). Human cell lines showed a dramatic decrease in PP-InsP concentrations that were rapidly restored upon Pi replenishment (Gu et al., 2017b). Interestingly, the same study showed an oscillation of cellular ATP concentrations in response to Pi availability. The mechanisms behind PP-InsP fluctuation remain completely unknown, but it is tempting to speculate that cellular ATP concentrations may play a role, since yeast Vip1 and human PPIPK2 kinase activities depend on high ATP concentrations (Gu et al., 2017b; Zhu et al., 2019). Moreover, since these enzymes exhibit both PP-InsP kinase and phosphatase activity, it will be important to determine the contributions of each function to the maintenance of PP-InsP concentrations (Fig. 5a).

**Auxin and jasmonic acid signaling** The receptors for the phytohormones auxin and jasmonic acid (JA) – respectively TIR1 (and AFBs) and COI1 – share a common structure and mode of action. They are both leucine-rich-repeat (LRR)-containing F-box proteins that function as part of Skp1/Cullin/F-box (SCF) ubiquitin E3 ligase complexes to promote ligand-dependent degradation of their cognate co-receptors and transcription repressors: Aux/IAA members for auxin, and the JAZ protein family in the case of JA (Pérez & Goossens, 2013) (Fig. 5c). TIR1 co-crystallized with an InsP$_6$ molecule derived from the insect cell expression host (Tan et al., 2007), and that mutation of InsP$_6$-binding residues disrupts the formation of a signaling-active auxin-TIR1-AUX/IAA complex (Calderón Villalobos et al., 2012). This suggests that either InsP$_6$, or a PP-InsP acts as a co-factor for TIR1, possibly facilitating auxin binding and subsequent co-receptor recruitment. Although no auxin-related phenotype has been reported thus far for VIH1/2-depleted plants, genetic manipulation of the InsP pathway altered auxin signaling (Zhang et al., 2007, 2011; Chen & Xiong, 2010). This may, however, be caused by defects in PIP synthesis, InsP$_3$, or calcium signaling, in addition to changes in PP-InsP metabolism.

Similarly, the COI1-JAZ-JA/coronatbin receptor complex was co-crystallized in the presence of InsP$_5$ (Sheard et al., 2010) and that mutation of COI1 InsP-binding residues disrupts complex formation (Mosblech et al., 2011; Laha et al., 2016). Remarkably, InsP$_6$, and especially InsP$_5$, are more potent enablers of the assembly of the JA-receptor complex than InsP$_3$ (Laha et al., 2015, 2016). Computational modeling predicted InsP$_8$ to be a more favorable ligand for COI1, inducing conformational changes that stabilize formation of the JA-COI1-JAZ receptor complex (Cui et al., 2018). Genetic disruption of VHI2 impaired JA-dependent responses against herbivore larvae and necrotrophic pathogens (Laha et al., 2015). Since VHI2 synthesizes both 1-InsP$_7$ and 1,5-InsP$_9$, it remains unclear which PP-InsP species is the preferred co-factor for COI1. Consistently, Arabidopsis ipk1 mutants (which fail to accumulate InsP$_6$, while overaccumulating InsP$_3$) have been found to be more susceptible to necrotrophic fungi, behaving similarly to vih2 and coi1 mutants (Stevenson-Paulik et al., 2005; Murphy et al., 2008; Laha et al., 2016). However, the same mutants were also reported to be hyper-responsive to externally-applied JA and more resistant to herbivores (Mosblech et al., 2011). Additional investigation is thus required to fully elucidate the roles of PP-InsPs in JA signaling.

InsPs have been further implicated in plant immune signaling by the characterization of the Xanthomonas campestris effector protein XopH as phytase (InsP$_6$, phosphatase) (Blüher et al., 2017). Ectopic expression of XopH in N. benthamiana indeed resulted in reduced InsP$_6$, InsP$_7$ and InsP$_8$ concentrations (Blüher et al., 2017).

4. **Research tools and perspectives**

Due to their low abundance and highly-charged nature, detection and quantification of PP-InsP concentrations in vivo still poses a major challenge. Available quantitative methods can distinguish the different isomers; however, these typically involve feeding cells/organisms with radio-labeled PP-InsP precursors (for a review, see Brown et al., 2016), followed by (bio)chemical extraction and chromatographic separation of the derived InsPs/PP-InsPs. An accurate and systematic picture of the dynamics of PP-InsPs under different conditions is still a distant vision. Nevertheless, a variety of tools has emerged that provide exciting research possibilities.

Most signaling mechanisms described so far require binding of PP-InsPs to a given protein. Thus, identifying PP-InsP-interacting proteins is critical to uncover the pathways and processes governed by these molecules. The Fiedler lab at FMP Berlin devised an ingenious protocol for the synthesis of a non-hydrolyzable 5-InsP$_7$ analogue that carries a bisphosphonate group (PCP) in place of the usual pyrophosphate (Wu et al., 2016). This analogue was then linked to an affinity resin, allowing for protein pull-down, much like a co-immunoprecipitation approach, using amine-tethered or biotinylated InsPs, were employed to identify putative PP-InsP binding proteins (Jiao et al., 2015; Gregory et al., 2016). These tagged InsPs versions provide the added benefit of enabling attachment to a chip for SPR detection methods (Gregory et al., 2016), allowing for precise quantification of InsP–protein interactions that is critical to accurately distinguish PP-InsP isomer-binding preferences.
Genetic ablation of PP-InsP-producing enzymes often results in pleiotropic phenotypes and severe developmental defects that make it difficult to isolate specific processes or pathways. Delivering PP-InsPs directly into live cells may be a good alternative; however, due to their polar nature they do not easily cross membranes. A membrane-permeable photo-caged version of InsP3 was engineered to allow UV pulse-controlled InsP3 release in the cytosol, demonstrating that these molecules activate cytosolic calcium signaling within minutes (Li et al., 1998). More recently, a 5-InsP7 photo-caged analogue provided real-time evidence for the translocation of the Akt PH-domain from the plasma membrane to the cytoplasm (Pavlovic et al., 2016). These innovative approaches create exciting possibilities to study and visualize the effects of PP-InsPs in single cells in a real-time fashion.

Studying PP-InsP-mediated protein pyrophosphorylation presents perhaps the most demanding challenge. Without the possibility of genetically dissecting this process, one has to rely on in vitro biochemical methods; nevertheless, some sort of in vivo validation will be required to fully characterize pyrophosphorylation as a signaling mechanism. A promising new mass spectrometry technique was able to distinguish peptide pyrophosphorylation in vitro (Penkert et al., 2017) and may finally allow its detection from cell lysates. In addition, a fluorescent sensor designed to specifically bind diphasphate esters may provide agility in the identification and relative-quantification of protein pyrophosphorylation (Williams & Fiedler, 2015).

A combination of these emerging new tools with traditional plant genetics and biochemical approaches may uncover the cellular functions of PP-InsPs in plants and their underlying signaling mechanisms. Should the presence of polyP be substantiated in higher plants, it will also be interesting to study the potential metabolic interactions between polyPs and PP-InsPs.

Acknowledgements

We would like to apologize to our colleagues for not having been able to cite all the relevant literature in this review. We thank members of the Hothorn lab for critically reading our manuscript. This work was supported by an ERC starting grant from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement no. 310856 and the Howard Hughes Medical Institute International Research Scholar Award, both of which were awarded to MH.

ORCID

Daniel Couto https://orcid.org/0000-0003-0223-1497
Michael Hothorn https://orcid.org/0000-0002-3597-5698
Laura Lorenzo-Orts https://orcid.org/0000-0001-9532-630X

References

Abramov AY, Fraley C, Diao CT, Winkfein R, Colicos MA, Duchen MR, French RJ, Pavlov EV. 2007. Targeted polyphosphatase expression alters mitochondrial metabolism and inhibits calcium-dependent cell death. Proceedings of the National Academy of Sciences, USA 104: 18091–18096.
Ahn K, Kornberg A. 1990. Polyphosphate kinase from Escherichia coli. Purification and demonstration of a phosphoenzyme intermediate. The Journal of Biological Chemistry 265: 11734–11739.
Akiyama M, Crooke E, Kornberg A. 1993. An exopolyphosphatase of Escherichia coli. The enzyme and its ppp gene in a polyphosphate operon. The Journal of Biological Chemistry 268: 633–639.
Aksou M, Pootakham W, Grossman AR. 2014. Critical function of a Chlamydomonas reinhardtii putative polyphosphate polymerase subunit during nutrient deprivation. The Plant Cell 26: 4214–4229.
Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.
Alvarez S, Jerez CA. 2004. Copper ions stimulate polyphosphate degradation and phosphate efflux in Acidithiobacillus ferrooxidans. Applied and Environmental Microbiology 70: 5177–5182.
Angelova PR, Agrawalla BK, Elustondo PA, Gordon J, Shiba T, Abramov AY, Chang Y-T, Pavlov EV. 2014. In situ investigation of mammalian inorganic polyphosphate localization using novel selective fluorescent probes JC-D7 and JC-D8. ACS Chemical Biology 9: 2101–2110.
Aulé-Riché D, Fraley CD, Tseng C-M, Kornberg A. 1998. Novel assay reveals multiple pathways regulating stress-induced accumulations of inorganic polyphosphate in Escherichia coli. Journal of Bacteriology 180: 1841–1847.
Azevedo C, Burton A, Ruiz-Mateos E, Marsh M, Saiardi A. 2009. Photolabile InsPs directly into live cells may be a good alternative; however, due to it difficult to isolate specific processes or pathways. Delivering PP-InsPs in single cells in a real-time fashion. Studying PP-InsP-mediated protein pyrophosphorylation presents perhaps the most demanding challenge. Without the possibility of genetically dissecting this process, one has to rely on in vitro biochemical methods; nevertheless, some sort of in vivo validation will be required to fully characterize pyrophosphorylation as a signaling mechanism. A promising new mass spectrometry technique was able to distinguish peptide pyrophosphorylation in vitro (Penkert et al., 2017) and may finally allow its detection from cell lysates. In addition, a fluorescent sensor designed to specifically bind diphasphate esters may provide agility in the identification and relative-quantification of protein pyrophosphorylation (Williams & Fiedler, 2015).

A combination of these emerging new tools with traditional plant genetics and biochemical approaches may uncover the cellular functions of PP-InsPs in plants and their underlying signaling mechanisms. Should the presence of polyP be substantiated in higher plants, it will also be interesting to study the potential metabolic interactions between polyPs and PP-InsPs.

Acknowledgements

We would like to apologize to our colleagues for not having been able to cite all the relevant literature in this review. We thank members of the Hothorn lab for critically reading our manuscript. This work was supported by an ERC starting grant from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement no. 310856 and the Howard Hughes Medical Institute International Research Scholar Award, both of which were awarded to MH.

ORCID

Daniel Couto https://orcid.org/0000-0003-0223-1497
Michael Hothorn https://orcid.org/0000-0002-3597-5698
Laura Lorenzo-Orts https://orcid.org/0000-0001-9532-630X

References

Abramov AY, Fraley C, Diao CT, Winkfein R, Colicos MA, Duchen MR, French RJ, Pavlov E. 2007. Targeted polyphosphatase expression alters mitochondrial metabolism and inhibits calcium-dependent cell death. Proceedings of the National Academy of Sciences, USA 104: 18091–18096.
Ahn K, Kornberg A. 1990. Polyphosphate kinase from Escherichia coli. Purification and demonstration of a phosphoenzyme intermediate. The Journal of Biological Chemistry 265: 11734–11739.
Akiyama M, Crooke E, Kornberg A. 1993. An exopolyphosphatase of Escherichia coli. The enzyme and its ppp gene in a polyphosphate operon. The Journal of Biological Chemistry 268: 633–639.
Aksou M, Pootakham W, Grossman AR. 2014. Critical function of a Chlamydomonas reinhardtii putative polyphosphate polymerase subunit during nutrient deprivation. The Plant Cell 26: 4214–4229.
Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.
Alvarez S, Jerez CA. 2004. Copper ions stimulate polyphosphate degradation and phosphate efflux in Acidithiobacillus ferrooxidans. Applied and Environmental Microbiology 70: 5177–5182.
Angelova PR, Agrawalla BK, Elustondo PA, Gordon J, Shiba T, Abramov AY, Chang Y-T, Pavlov EV. 2014. In situ investigation of mammalian inorganic polyphosphate localization using novel selective fluorescent probes JC-D7 and JC-D8. ACS Chemical Biology 9: 2101–2110.
Aulé-Riché D, Fraley CD, Tseng C-M, Kornberg A. 1998. Novel assay reveals multiple pathways regulating stress-induced accumulations of inorganic polyphosphate in Escherichia coli. Journal of Bacteriology 180: 1841–1847.
Azevedo C, Burton A, Ruiz-Mateos E, Marsh M, Saiardi A. 2009. Photolabile InsPs directly into live cells may be a good alternative; however, due to it difficult to isolate specific processes or pathways. Delivering PP-InsPs in single cells in a real-time fashion. Studying PP-InsP-mediated protein pyrophosphorylation presents perhaps the most demanding challenge. Without the possibility of genetically dissecting this process, one has to rely on in vitro biochemical methods; nevertheless, some sort of in vivo validation will be required to fully characterize pyrophosphorylation as a signaling mechanism. A promising new mass spectrometry technique was able to distinguish peptide pyrophosphorylation in vitro (Penkert et al., 2017) and may finally allow its detection from cell lysates. In addition, a fluorescent sensor designed to specifically bind diphasphate esters may provide agility in the identification and relative-quantification of protein pyrophosphorylation (Williams & Fiedler, 2015).

A combination of these emerging new tools with traditional plant genetics and biochemical approaches may uncover the cellular functions of PP-InsPs in plants and their underlying signaling mechanisms. Should the presence of polyP be substantiated in higher plants, it will also be interesting to study the potential metabolic interactions between polyPs and PP-InsPs.
Castuma CE, Huang R, Kornberg A, Reutsch RN. 1995. Inorganic polyphosphates in the acquisition of competence in Escherichia coli. The Journal of Biological Chemistry 270: 12980–12983.

Chakraborty A. 2018. The inositol pyrophosphate pathway in health and disease. Biological Review 93: 1203–1227.

Chakraborty A, Koldobskiy MA, Bello NT, Maxwell M, Potter JJ, Juluri KR, Maag D, Kim S, Huang AS, Dailey MJ et al. 2010. Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain. Cell 143: 897–910.

Chande S, Bergwitz C. 2018. Role of phosphate sensing in bone and mineral metabolism. Nature Review Endocrinology 14: 637.

Chanduri M, Rai A, Malla AB, Wu M, Fiedler D, Malik R, Bhandari R. 2016. Inositol hexakisphosphate kinase 1 (IP6K1) activity is required for cytoplasmic dynein-driven transport. Biochemical Journal 473: 3031–3047.

Chen H, Xiong L. 2010. myo-Inositol-1-phosphate synthase is required for polar auxin transport and organ development. The Journal of Biological Chemistry 285: 24238–24247.

Chen J, Bang WY, Lee Y, Kim S, Lee KW, Son YS, Kim DW, Ahktar S, Bahk JD. 2014. AtObgC-AtRSH1 interaction may play a vital role in stress response signal transduction in Arabidopsis. Plant Physiology and Biochemistry 74: 176–184.

Chiu CH, Paszkowski U. 2019. Arabidopsis inositol phosphate kinases IPK1 and ITPK1 constitute a responsive signal transduction pathway. The Journal of Biological Chemistry 294: 2401–2405.

Chakraborty A, Uematsu T, Ueda O, Usui Y, Uematsu S, Takahashi M, Uchihata H, Kazawo Y, Shiba T, Kurihara S et al. 2007. Inorganic polyphosphate: a possible stimulant of bone formation. Journal of Dental Research 86: 893–897.

Harada K, Isho H, Kazawo Y, Miyazaki S, Doki K, Kubo T, Akagawa Y, Shiba T. 2013. Polyphosphate-mediated inhibition of tartrate-resistant acid phosphatase and suppression of bone resorption of osteoclasts. PLoS ONE 8: e78612.

Hijikata N, Murase M, Tani C, Ohtomo R, Osaki M, Ezawa T. 2010. Polyphosphate has a central role in the rapid and massive accumulation of phosphorus in extraradical mycelium of an arbuscular mycorrhizal fungus. New Phytologist 186: 285–289.

Hothorn M, Neumann H, Lenherz ED, Wehner M, Rybin V, Hassa PO, Uittenweiler A, Reinhardt M, Schmidt A, Seiler J et al. 2009. Catalytic core of a membrane-associated eucaytolic polyphosphate polymerase. Science 324: 513–516.

Hu B, Jiang Z, Wang W, Qiu Y, Zhang Z, Liu Y, Li A, Gao X, Liu L, Qian Y et al. 2019. Nitrartrate-NRT1.1a/SPX4 cascade integrates nitrogen and phosphorus signaling networks in plants. Nature Plants 5: 401.

Huang K, Ferrin-O’Connell J, Zhang W, Leonard GA, O’Shea EK, Quicho FA. 2007. Structure of the Pho85–Pho80 C20D-cyclin complex of the phosphate-responsive signal transduction pathway. Molecular Cell 28: 614.

Itô D, Kato T, Maruta T, Tamoji M, Yoshimura K, Shigeoka S. 2012. Enzymatic and molecular characterization of Arabidopsis ppGpp pyrophosphohydrolase, AtNUDX26. Biosience, Biotechnology, and Biochemistry 76: 2236–2241.

Jemth A-S, Scalleti E, Carter M, Helledge T, Stenmark P. 2019. Crystal structure and substrate specificity of the 8-oxo-dGTPase hydroxylase NUDT1 from Arabidopsis thaliana. Biochemistry 58: 887–899.

Jiao C, Summerlin M, Bruzik KS, Hanakahi L. 2015. Synthesis of biotinylated inositol hexakisphosphate to study DNA double-strand break repair and affinity capture of IP6-binding proteins. Biochemistry 54: 6312–6322.

Jimenez-Nunez MD, Moreno-Sanchez D, Hernandez-Ruiz L, Benitez-Rondán A, Ramos-Amaya A, Rodriguez-Bayona B, Medina F, Brieva JA, Ruiz FA. 2012. Myeloma cells contain high levels of inorganic polyphosphate which is associated with nuclear transcription. Haematologica 97: 1264–1271.

Kapustinski J. 1990. Interactions of nucleic acids with fluorescent dyes: spectral properties of condensed complexes. Journal of Histochemistry and Cytochemistry 38: 1323–1339.

Keck K, Stich H. 1957. The widespread occurrence of polyphosphate in lower plants. Annals of Botany 21: 611–619.

Klein G, Cotter DA, Martin JB, Bof M, Satre M. 1988. Germination of Dicotyledon dicotyledon spores. A phosphorus-31 NMR analysis. Biochemistry 27: 8193–8203.

Kohn G, Delvaux D, Lakaye B, Servais A-C, Scholer G, Fillet M, Elias B, Kohn G, Derochette J-M, Crommen J, Wins P. 2012. The AtObgC-AtRSH1 interaction may play a vital role in stress response signal transduction in Arabidopsis. The Plant Cell 24: 1329–1334.

Kolozvari B, Firth S, Saiardi A. 2015. Raman spectroscopy detection of phyric acid in plant seeds reveals the absence of inorganic polyphosphate. Molecular Plant 8: 826–828.

Kolozvari B, Parisi F, Saiardi A. 2014. Inositol phosphates induce DAPI fluorescence shift. Biochemical Journal 460: 377–385.

Kumble KD, Kornberg A. 1995. Inorganic polyphosphate in mammalian cells and tissues. The Journal of Biological Chemistry 270: 5818–5822.

Kuo H-F, Hsu Y-Y, Lin W-C, Chen K-Y, Munnik T, Breazeal CA, Chiong T-J. 2018. Arabidopsis inositol phosphate kinases IPK1 and ITPK1 constitute a metabolic pathway in maintaining phosphate homeostasis. The Plant Journal95: 613–630.

Kuroda A, Tanaka S, Ikeda T, Kato J, Takiguchi N, Ohtake H. 1999. Inorganic polyphosphate kinase is required to stimulate protein degradation and for adaptation to amino acid starvation in Escherichia coli. Proceedings of the National Academy of Sciences, USA 96: 14264–14269.
Miyachi S. 1961. Inorganic polyphosphate in spinach leaves. The Journal of Biochemistry 50: 367–371.

Moeder W, García-Peit/ C, Ung H, Fucile G, Samuel MA, Christendat D, Yoshioka K. 2013. Crystal structure and biochemical analyses reveal that the Arabidopsis triphosphate tunnel metalloenzyme AtTTM3 is a triphosphatase involved in root development. The Plant Journal 76: 615–626.

Moreno-Sánchez D, Hernandez-Ruiz L, Ruiz FA, Docampo R. 2012. Polyphosphate is a novel pro-inflammatory regulator of mast cells and is located in acidocalcisomes. The Journal of Biological Chemistry 287: 28435–28444.

Mosblech A, Thurow C, Gatz C, Feusner I, Heilmann I. 2011. Jasmonic acid perception by COI1 involves inositol polyphosphates in Arabidopsis thaliana. The Plant Journal 65: 949–957.

Moudrıkova S, Sadovsky A, Metzger S, Nedbal L, Mettler-Altmann T, Mojzes P. 2017. Quantification of polyphosphate in microalgae by Raman microscopy and by sequence analysis of an enzymatic assay. Analytical Chemistry 89: 12060–12013.

Müller F, Mutch NJ, Schenk WA, Smith SA, Esterl E, Sprock HM, Schmidbauer S, Gahl WA, Morrissey JH, Renne T. 2009. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. Cell 139: 1143–1156.

Mulugo S, Bai W, Fridy PC, Bastidas RJ, Otto JC, Dollins DE, Haystead TA, Ribeiro AA, York JD. 2007. A conserved family of enzymes that phosphorylate inositol hexakisphosphate. Science 316: 106–109.

Murphy AM, Otto B, Brealey CA, Carr JP, Hanke DE. 2008. A role for inositol hexakisphosphate in the maintenance of basal resistance to plant pathogens. The Plant Journal 56: 638–652.

Nagadoussi RS, Landor N, Huang G, Cordeiro CD, Smith SA, Morrissey JH, Docampo R. 2018. Inorganic polyphosphate interacts with nucleolar and glycosylated proteins in transcripanosomes. Molecular Microbiology 110: 973–994.

Norman KL, Shively CA, Rocha AJDL, Mutlu N, Basu S, Cullen PJ, Kumar A. 2018. Inositol polyphosphates regulate and predict yeast pseudohyphal growth phenotypes. PLoS Genetics 14: e1007493.

Ogawa T, Ueda Y, Yoshimura K, Shigeoka S. 2005. Comprehensive analysis of cytosolic Nudix hydrolases in Arabidopsis thaliana. The Journal of Biological Chemistry 280: 25277–25283.

Ohtomo R, Saito M. 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. New Phytologist 167: 571–578.

Otegui MS, Capp R, Staehelin LA. 2002. Developing seeds of Arabidopsis store different minerals in two types of vacuoles and in the endoplasmic reticulum. The Plant Cell 14: 1311–1327.

Park BS, Seo JS, Chuha N-H. 2014. NITROGEN LIMITATION ADAPTATION recruits PHOSPHATE2 to target the phosphate transporter PT2 for degradation during the regulation of Arabidopsis phosphate homeostasis. The Plant Cell 26: 454–464.

Park BS, Yao T, Seo JS, Wong ECC, Mitsuda N, Huang C-H, Chuha N-H. 2018. Arabidopsis NITROGEN LIMITATION ADAPTATION regulates ORE1 homeostasis during senescence induced by nitrogen deficiency. Nature Plants 4: 898.

Park Y, Seo JB, Fraind A, Pérez-Lara A, Yavuz H, Han K, Jung S-R, Kattan I, Walla PJ, Choi M et al. 2015. Synaptotagmin-1 binds to PiPP-containing membrane but not to SNAREs at physiological ionic strength. Nature Structural & Molecular Biology 22: 815–823.

Pavlov E, Aschar-Sobbi R, Campanella M, Turner RJ, Gómez-García MR, Abramov AY. 2010. Inorganic polyphosphate and energy metabolism in mammalian cells. The Journal of Biological Chemistry 285: 9420–9428.

Pavlovic I, Thakor DT, Vargas JR, McKinlay CJ, Hauke S, Anstaett P, Camuña RC, Bigler L, Gasser G, Schulz C et al. 2016. Cellular delivery and photochemical release of a caged inositol-polyphosphate induces PH-domain translocation in cells. Nature Communication 7: 10622.

Peng M, Hannam C, Gu H, Bi Y-M, Rothstein SJ. 2007. A mutation in NLA, which encodes a RING-type ubiquitin ligase, disrupts the adaptability of Arabidopsis to nitrogen limitation. The Plant Journal 50: 320–337.

Penkert M, Yates LM, Schimmann M, Perlman D, Fiedler D, Krause E. 2017. Unambiguous identification of serine and threonine polyphosphorylation using neutral-loss-triggered electron-transfer/higher-energy collision dissociation. Analytical Chemistry 89: 3672–3680.

Perez AC, Goossens A. 2013. Jasmonate signalling: a corypt of auxin signalling? Plant, Cell & Environment 36: 2071–2084.
Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle knowledge gained in Arabidopsis to potential biotechnological applications in H. 2017.

Rearrangement of the phosphate homeostasis of the yeast Saccharomyces cerevisiae. Plant and Cell Physiology 2003; 44:1090–1093.

Solyakov L, Cain K, Tracey BM, Jukes R, Riley AM, Potter BVL, Tobin AB. 2004. Regulation of Casein Kinase-2 (CK2) activity by inositol phosphates. Journal of Biological Chemistry 279: 43403–43410.

Stevenson-Paulik J, Bastidas RJ, Chiou S-T, Frye RA, York JD. 2005. Generation of phytate-free seeds in Arabidopsis through disruption of inositol phosphate kinases. Proceedings of the National Academy of Sciences, USA 102: 12612–12617.

Tammenkoski M, Koivula K, Cusanielli E, Zollo M, Steegborn C, Baykov AA, Lahti R. 2008. Human metastasis regulator protein H-prune is a short-chain exopolyphosphatase. Biochemistry 47: 9707–9713.

Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N. 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446: 640–645.

Tewari KK, Singh M. 1964. Acid soluble and acid insoluble inorganic polyphosphates in Coccus reflexus. Phytochemistry 3: 341–347.

Tran HT, Hurley BA, Plaxton WC. 2010. Feeding hungry plants: the role of purple acid phosphatases in phosphate nutrition. Plant Science 179: 14–27.

Tumurlish T, Jendrossek D. 2017. Proteins with CHADs (conserved histidine alpha-betical domains) are attached to polyphosphate granules in vivo and constitute a novel family of polyphosphate-associated proteins (phosins). Applied and Environmental Microbiology 83: e03399-16.

Ung H, Karia P, Ebine K, Ueda T, Yoshioka K, Moeder W. 2017. Triphosphate tunnel metalloenzyme function in senescence highlights a biological diversification of this protein superfamily. Plant Physiology 175: 473–485.

Ung H, Moeder W, Yoshioka K. 2014. Arabidopsis Triphosphate Tunnel Metalloenzyme 2 is a negative regulator of the salicylic acid-mediated feedback amplification loop for defense responses. Plant Physiology 166: 1009–1021.

Urech K, Dürr M, Boller Th, Wiemenk A, Schwencke J. 1978. Localization of polyphosphate in vacuoles of Saccharomyces cerevisiae. Archives of Microbiology 116: 275–278.

Wang H, Falck JR, Hall TMT, Shears SB. 2011. Structural basis for an inositol pyrophosphate kinase surmounting phosphate crowding. Nature Chemical Biology 8: 111–116.

Wang Z, Ruazi W, Shi J, Zhang L, Xiang D, Yang C, Li C, Wu Z, Liu Y, Yu Y et al. 2014. Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. Proceedings of the National Academy of Sciences, USA 111: 14953–14958.

Werner TP, Amrhein N, Freimoser FM. 2007a. Inorganic polyphosphate occurs in the cell wall of Chlamydomonas reinhardtii and accumulates during cytokinesis. BMC Plant Biology 7: 51.

Werner TP, Amrhein N, Freimoser FM. 2007b. Specific localization of inorganic pyrophosphate (poly P) in fungal cell walls by selective extraction and immunohistochemistry. Fungal Genetics and Biology 44: 845–852.

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

Williams FJ, Fiedler D. 2015. A fluorescent sensor and gel stain for detection of pyrophorylated proteins. ACS Chemical Biology 10: 1958–1963.

Wilson MSC, Livermore TM, Saïardi A. 2013. Inositol pyrophosphates: between signalling and metabolism. Biochemical Journal 452: 369–379.

Wu M, Chong LS, Perlman DH, Resnick AC, Fiedler D. 2016. Inositol polyphosphates interact with signaling and metabolic networks via two distinct mechanisms. Proceedings of the National Academy of Sciences, USA 113: e6757–e6765.

Wurst H, Kornberg A. 1994. A soluble exopolyphosphatase of Saccharomyces cerevisiae. Purification and characterization. The Journal of Biological Chemistry 269: 10996–11001.

Xie I, Jakob U. 2019. Inorganic polyphosphate, a multifunctional polyanionic protein scaffold. The Journal of Biological Chemistry 294: 2180–2190.

Yamagata Y, Watanabe H, Saïth M, Namba T. 1991. Volcanic production of polyphosphates and its relevance to prebiotic evolution. Nature 352: 516.
Yates LM, Fiedler D. 2015. Establishing the stability and reversibility of protein pyrophosphorylation with synthetic peptides. ChemBioChem 16: 415–423.

Yoshimura K, Shigeoka S. 2015. Versatile physiological functions of the Nudix hydrolase family in Arabidopsis. Bioscience, Biotechnology, and Biochemistry 79: 354–366.

Yue W, Ying Y, Wang C, Zhao Y, Dong C, Whelan J, Shou H. 2017. OsNLA1, a RING-type ubiquitin ligase, maintains phosphate homeostasis in Oryza sativa via degradation of phosphate transporters. The Plant Journal 90: 1040–1051.

Zhang H, Gómez-García MR, Brown MRW, Kornberg A. 2005. Inorganic polyphosphate in Dictyostelium discoideum: influence on development, sporulation, and predation. Proceedings of the National Academy of Sciences, USA 102: 2731–2735.

Zhang H, Ishige K, Kornberg A. 2002. A polyphosphate kinase (PPK2) widely conserved in bacteria. Proceedings of the National Academy of Sciences, USA 99: 16678–16683.

Zhang J, Vanneste S, Brewer PB, Michniewicz M, Grones P, Kleine-Vehn J, Løfke C, Teichmann T, Bielach A, Cannoot B et al. 2011. Inositol trisphosphate-induced Ca2+ signaling modulates auxin transport and PIN polarity. Developmental Cell 20: 855–866.

Zhang W, Gruszewski HA, Chevone BI, Nessler CL. 2008. An Arabidopsis purple acid phosphatase with phytase activity increases foliar ascorbate. Plant Physiology 146: 431–440.

Zhang Z-B, Yang G, Arana F, Chen Z, Li Y, Xia H-J. 2007. Arabidopsis inositol polyphosphate 6-/3-Kinase (AtIpk2[b]) is involved in axillary shoot branching via auxin signaling. Plant Physiology 144: 942–951.

Zhu J, Lau K, Puschmann R, Harmel RK, Zhang Y, Verena P, Gaugler P, Broger L, Dutta AK, Jessen HJ et al. 2019. Two bifunctional inositol pyrophosphate kinases/phosphatases control plant phosphate homeostasis. eLife 8: e43582.

Zhu J, Loubery S, Broger L, Lorenzo-Orts I, Utz-Pugin A, Chang Y-T, Hothorn M. 2019. A genetically validated approach to detect inorganic polyphosphates in plants. bioRxiv: 630129.