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Review

# Phosphate homeostasis in the yeast *Saccharomyces cerevisiae*, the key role of the SPX domain-containing proteins

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ABSTRACT

**In the yeast *Saccharomyces cerevisiae*, a working model for nutrient homeostasis in eukaryotes, inorganic phosphate (Pi) homeostasis is regulated by the PHO pathway, a set of phosphate starvation induced genes, acting to optimize Pi uptake and utilization. Among these, a subset of proteins containing the SPX domain has been shown to be key regulators of Pi homeostasis. In this review, we summarize the recent progresses in elucidating the mechanisms controlling Pi homeostasis in yeast, focusing on the key roles of the SPX domain-containing proteins in these processes, as well as describing the future challenges and opportunities in this fast-moving field.**


Inorganic phosphate (Pi) is an essential macronutrient required in numerous biological processes such as biomolecule synthesis, energy metabolism, and protein modification. For most organisms the availability of Pi in the environment is a growth limiting factor. In the budding yeast *Saccharomyces cerevisiae*, Pi sensing, acquisition, and storage are mainly mediated by the phosphate-responsive signaling pathway, referred to as the PHO pathway [1–4]. Under Pi deficiency, the PHO operon, which controls a set of genes aimed at increasing Pi uptake and Pi use efficiency, is triggered. Under prolonged Pi deficient conditions, both growth and cell division of *S. cerevisiae* will eventually be arrested, referred as the quiescent $G_0$-state. In budding yeast, the Pi acquisition system is composed of four cell membrane localized Pi transporters, with the two low affinity Pi transporters, namely Pho87, Pho90 being ubiquitously expressed [5], while the two high affinity Pi transporters, Pho84 and Pho89 are regulated by the PHO pathway (Table 1) [1,2,6–8]. Pho91, initially considered as part of the low Pi affinity uptake system was recently shown to be localized on the tonoplast and involved in exporting Pi from the vacuole to the cytosol [9]. Interestingly, Pho87, Pho90 and Pho91 possess the SPX domain, a domain often present in proteins involved in regulating Pi homeostasis [10]. In addition to these three Pi transporters, the budding yeast genome encodes seven additional proteins harbouring the SPX domain, namely, three of the four yeast polyphosphate synthase subunits, Vacuolar Transporter Chaperone 2 (Vtc2), Vtc3 and Vtc4, the cyclin dependent kinase (CDK) inhibitor Pho81, the glycerophosphocholine phosphodiesterase 1 (Gde1), the Suppressor of Yeast *gpa1* (Syg1), and Ydr089 (Table 1). Surprisingly, all the yeast proteins harbouring the SPX domain, with the exception of Syg1 and Ydr089, have been shown to be key regulators in maintaining Pi homeostasis in yeast.

The SPX domain, initially identified in yeast, is named after the *S*uppressor of *Y*east *gpa1* (*S*yg1), where a truncated version of Syg1 has a negative effect on the mating pheromone signal in yeast, the yeast *P*hosphatase 81 (*P*ho81), a cyclin dependent kinase inhibitor, and the human *X*enotropic and *P*olytropic *R*etrovirus receptor1 (*X*pr1) and is located within the first 150–300 N-termini amino acids. Although the structure of this ~200 amino acids long hydrophilic domain is variable, it can be subdivided into three well conserved regions of 30–40 amino acids, separated with regions of low similarity [10] (Fig. 1). Surprisingly, despite the conservation of this domain among all the major eukaryotes, from *Caenorhabditis elegans* and Drosophila to plants and mammals, our knowledge on the function of the SPX domain is still scarce. The SPX domain of the yeast Syg1 was for long the only evidence of the involvement of the SPX domain in protein interaction, interacting with the β subunit of the G-protein heterotrimer, ultimately suppressing the

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**Table 1**
List of the yeast SPX domain-containing proteins and their characteristics. N, nucleus; PM, plasma membrane; V, vacuolar membrane; C, cytosol.

| Standard name | Systematic name | Alias | Description | Function | Role of the SPX domain | Main localization | References |
|---|---|---|---|---|---|---|---|
| Pho81 | YG233C | Vac6 | Cyclin-dependent kinase inhibitor | Under Pi starvation, in association with inositiol heptakisphoaphate, negatively regulates the activity of the Pho80–Pho85 complex | May be involved in protein interaction with some Pho85 cyclins. Acts as an auto-inhibitory domain | N | [2–4,8,27–36] |
| Pho87 | YCR037C | | Low affinity $Na^+$ + Pi transporter | Pi sensor & transporter | Physically interacts with Spl2, resulting in endocytosis and vacuolar targeting | PM | [1–4,8,9,38–44] |
| Pho90 | YJL198W | | Low affinity $Na^+$ + Pi transporter | Pi transporter | Physically interacts with Spl2. Required for vacuolar targeting | PM | [1–4,8,9,38,39,47] |
| Pho91 | YNR013C | | Vacuolar Pi transporter | Transport Pi from the vacuole to the cytosol | May be required for ubiquitination | V | [1–4,8,9] |
| Vtc2 | YFL004W | Phm1 | Vacuolar transporter chaperone 2 | Involved in vacuolar polyphosphate accumulation, membrane trafficking, microautophagy and non-autophagic vacuolar fusion | | V | [1,50–54] |
| Vtc3 | YPL019C | Phm2 | Vacuolar transporter chaperone 3 | Involved in vacuolar polyphosphate accumulation, membrane trafficking, microautophagy and non-autophagic vacuolar fusion | | V | [1,50–54] |
| Vtc4 | YJL012C | Phm3 | Vacuolar transporter chaperone 4 | PolyP synthesizing enzymes; regulates membrane trafficking; role in non-autophagic vacuolar fusion | Not required for catalytic activity | V | [1,50–54] |
| Gde1 | YPL110C | | Glycerophosphocholine phosphodiesterase | Hydrolyzes GroPCho to choline and glycerolphosphate, for use as a phosphate source and as a precursor for phosphocholine synthesis | | C ? | [59–61] |
| Syg1 | YIL047C | | Plasma membrane protein of unknown function | Truncation and overexpression suppresses lethality of G-alpha protein deficiency | Interacts with the β subunit of the Gprotein, suppressing the lethality of the G-protein α-subunit deficiency | PM? | [11] |
| Ydr089 | Ydr089W | | Protein of unknown function | | | | |

lethality of the G-protein α-subunit deficiency (Table 1) [11]. Since then, two additional studies, in yeast and *Arabidopsis thaliana*, have shown a role for the SPX domain in protein interaction [12,13]. In addition, a strong link between proteins harbouring the SPX domain and Pi homeostasis has been observed in yeast and plants. In plants, although none of these proteins have yet been characterized as Pi transporters, several proteins possessing the SPX domain have been shown to be major regulators of Pi homeostasis, being involved in Pi signaling, remobilization, export [10,14–20]. Moreover, recent work in both yeast and plants report that the SPX domain itself could be involved in fine-tuning of Pi transport and signaling, through mechanisms such as physical interactions with other proteins [12,13,21].

In this review, we summarize and highlight the recent advances in understanding the mechanisms regulating Pi homeostasis in yeast, focusing principally on the key roles of the proteins possessing the SPX domain in these processes, as well as addressing the future challenges that remain in this area.

## 1. Pho81, a negative regulator of the PHO pathway

Under limiting Pi conditions, the activation of the PHO pathway is mediated by the transcription factor Pho4, in cooperation with its co-activator Pho2 [22,23], resulting in increased expression of multiple genes involved in the acquisition, uptake and storage of Pi [1,3,8,24,25]. The activity of Pho4 has been shown to be regulated by the phosphorylation of several serine residues, mediated by the Pho80–Pho85 cyclin dependent kinase (CDK) complex [26].

Pho81, a cyclin dependent kinase inhibitor (CKI), has been shown to be a major regulator of the PHO pathway, negatively regulating the activity of the Pho80–Pho85 kinase complex (Fig. 2, Table 1). Pho81 is constitutively associated with the Pho85–Pho80 complex, independently of the Pi status, and mainly interacts via the Pho80 cyclin subunit [27,28]. Interestingly, recent studies demonstrated that upon Pi starvation, the levels of inositol hepta*kis*phosphate (IP7), an evolutionary conserved metabolite [29], increased and that IP7 could allosterically interact with the tertiary complex of Pho81–Pho85–Pho80, ultimately inducing a conformational change of Pho81, preventing Pho4 to access the kinase active site of Pho80–Pho85 [30,31]. As a consequence, unphosphorylated Pho4 accumulates in the nucleus and activates the phosphate starvation responsive genes, encoding proteins such as Pho84 and Pho89, the two high affinity Pi transporters, Pho5, a secreted phosphatase, and Spl2 a negative regulator of the low affinity transporters [1] (Fig. 2). Conversely, under Pi replete conditions, the lack of IP7 accumulation prevents Pho81 from inhibiting the kinase activity of Pho85–Pho80, thus enabling the phosphorylation of Pho4 [31]. Upon phosphorylation of the latter transcription factor, the Pho4–Pho2 complex is disassembled and Pho4 is exported from the nucleus to the cytoplasm, where it is unable to activate transcription of the phosphate starvation inducible genes. Furthermore, it has been shown that the expression of Pho81 is itself controlled by Pho4, thus creating a positive feedback loop where a low-Pi signal results in enhanced expression of Pho81 [32,33]. The tight regulation observed for the Pho81–Pho85–Pho80 complex in association with IP7 has been shown to be reversible and rapid, making it a perfect mechanism to face fluctuating and transient changes in Pi availability in the environment [31].

Interestingly, the SPX domain of Pho81 is not required for the inhibition of the kinase activity of the Pho85–Pho80 complex [31,34]. Indeed, only a small domain of Pho81, namely the minimum domain, constituted of 80 amino acids (amino acids

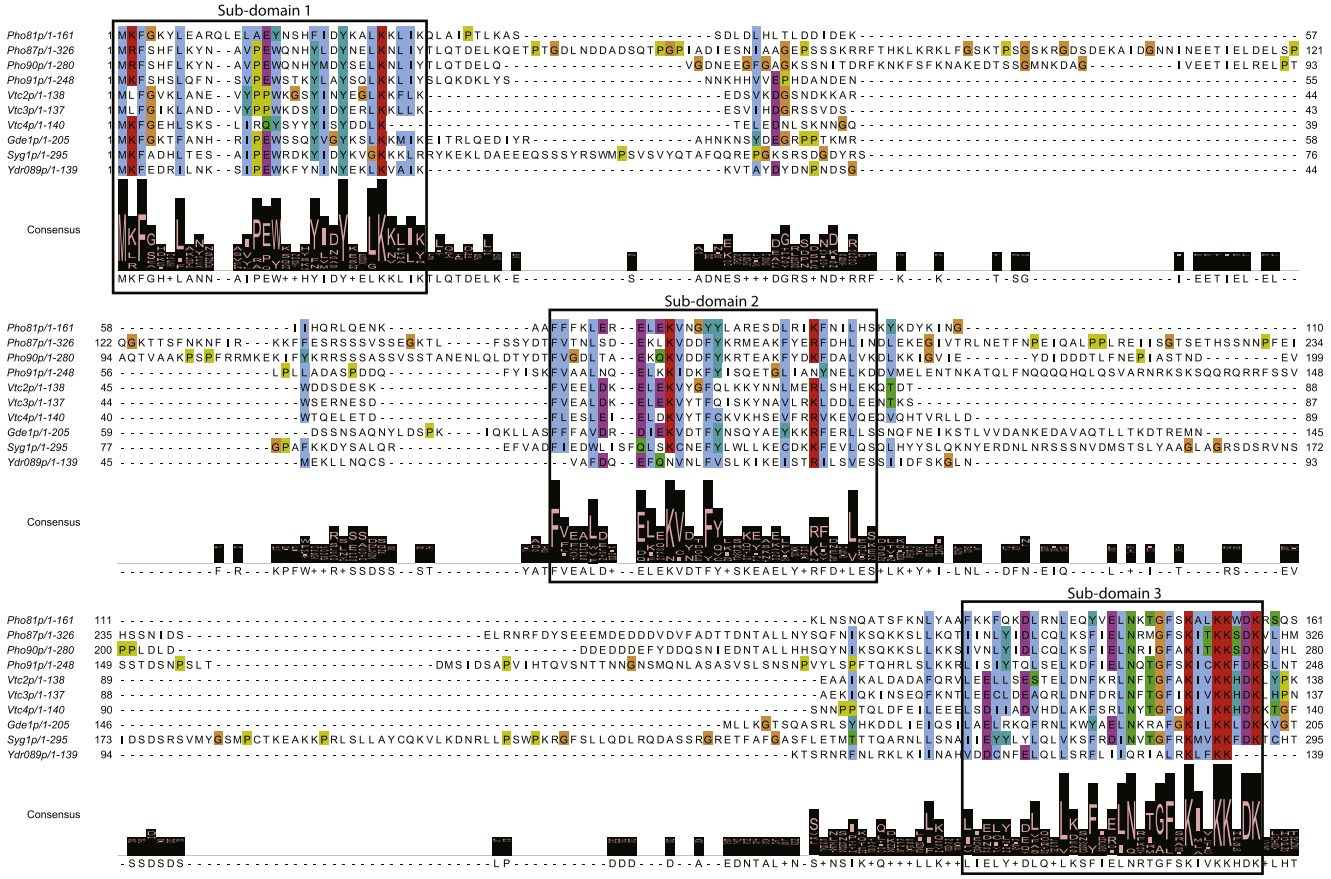


**Fig. 1.** The yeast SPX domain. The SPX domain of all the yeast SPX domain-containing proteins was identified and aligned using the Conserved Domain Database and Jalview soft-wares, respectively. The three well conserved SPX sub-domains are highlighted by a black box.

645–724) at the C-terminus is necessary and sufficient to interact with the Pho85–Pho80 complex [34]. In addition, it has been shown that this minimum domain can be subdivided into two subdomains, the first one being required for constitutive binding of Pho81 with the Pho85–Pho80 complex, while the second one is responsible for the binding and the inhibition of the activity of the Pho85–Pho80 complex, only in presence of IP7 [31].

The presence of Pho81 has also been reported in other pathways than the PHO pathway, negatively regulating the Pcl7–Pho85 complex [35], and another Pho85–cyclin complex involved in trehalose metabolism [35,36]. However, while in the PHO pathway the minimum domain is sufficient to inhibit the kinase activity of the multifunctional Pho85 cyclin dependent kinase, Swinnen et al. (2005) [36] demonstrated that the full length of Pho81 was required to inhibit a Pho85–cyclin complex(es), in order to maintain proper regulation of the trehalose metabolism under Pi limiting conditions. A similar observation was made with the post-diauxic shift controlled stress responsive genes, involved in general stress response [36]. Thus, it appears that others domains than the minimum domain are required for proper activity of Pho81. Interestingly, in addition to the SPX domain, Pho81also harbours several ankyrin repeats, also involved in mediating protein–protein interactions [37]. Therefore it would be interesting to decipher the role of these domains in the function of Pho81. It is noteworthy that the N-terminal region of Pho81, which includes the SPX domain acts as an auto-inhibitory domain, which can suppress, in trans, the activity of a truncated Pho81 [33]. Altogether, it appears that Pho81 is involved in negatively regulating various cyclin dependent kinases in response to Pi starvation, through potential protein interaction via the SPX domain.

## 2. The Pi transporters: Pho87, Pho90 and Pho91

The yeast genome encodes five Pi transporters, Pho84, Pho89, Pho87, Pho90 and Pho91, with, Pho87, Pho90 and Pho91, harbouring the SPX domain at their N-termini and belonging to the divalent anion symporter family [8]. While these three phosphate transporters were initially thought to form the low affinity Pi uptake system in yeast, it has recently been shown that, unlike Pho87 and Pho90 which are localized to the plasma membrane, Pho91 was localized to the vacuolar membrane and was involved in exporting Pi from the vacuole to the cytosol [9]. In addition, it has been demonstrated that Pho87 and Pho90 were non-redundant Pi transporters, with Pho90 being the most important Pi transporter under high Pi conditions, in cells lacking the high affinity transporters [38], while Pho87 is mainly involved in Pi sensing [38–40]. Indeed despite Pho84 being the major Pi transporter involved in Pi sensing, Pho87, in the absence of either Pho4 alone or in combination with Pho84, becomes essential for growth recovery from Pi starvation and rapid signaling through the activation of the PKA pathway, in the presence of glucose [38,40,41]. The PKA pathway is a signaling pathway that plays a major role in the nutrient dependent control of growth, metabolism and stress resistance [24,42]. However, the activation of the PKA signaling pathway by glycerol-3-phosphate only requires Pho84 [43]. Thus, Pho84 is considered as a transceptor, having both nutrient transport and signaling functions, while the transceptor capacity of Pho87 still remains to be confirmed [3,4,43].

In addition, Pinson et al. (2004) [39] showed that under high Pi conditions, mutation in PHO87, PHO90 or PHO91 altered the regulation of Pi repressed genes, such as the high Pi affinity transporter

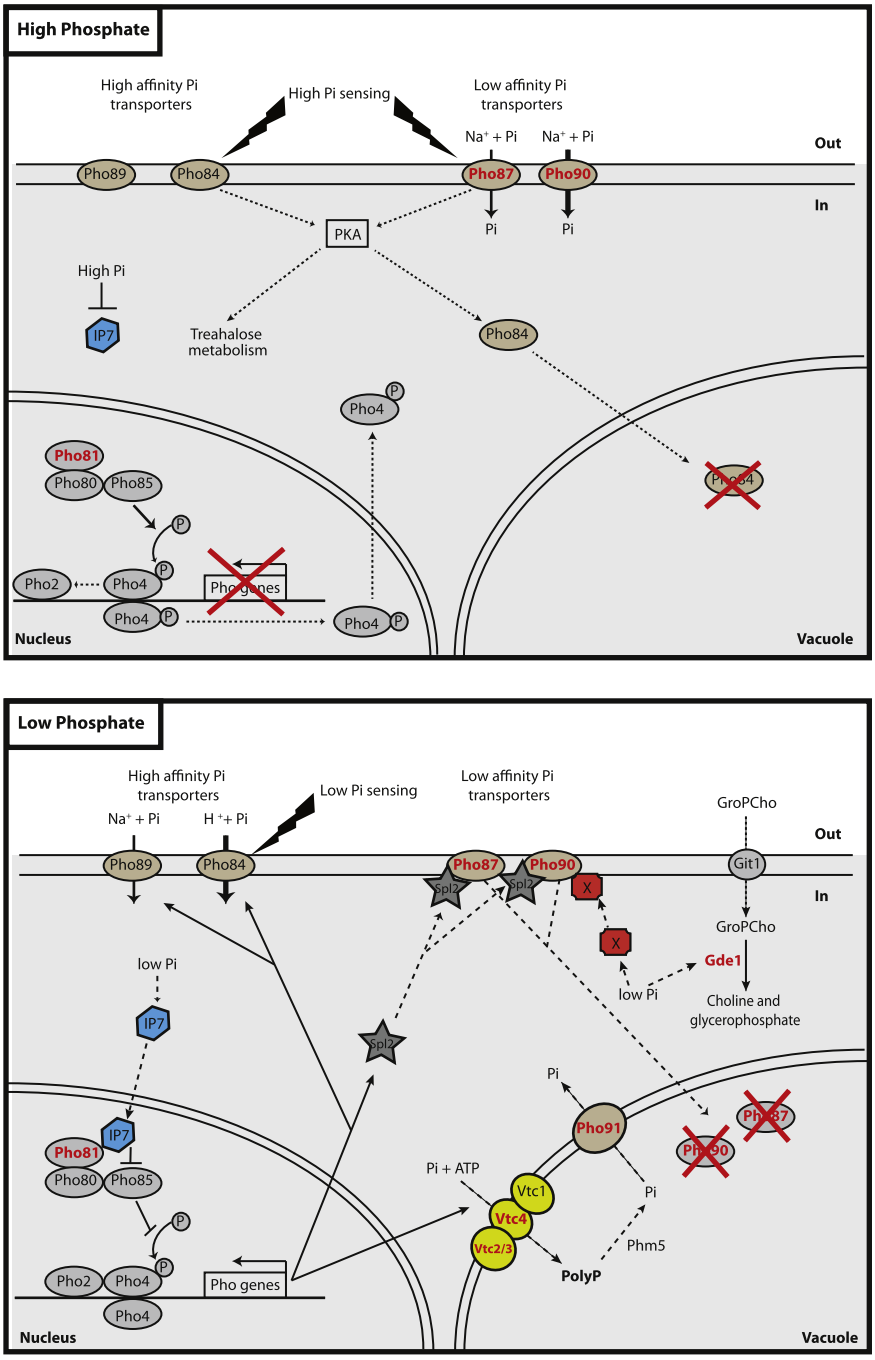


**Fig. 2.** Schematic representation of the function of the SPX domain-containing proteins in the regulation of phosphate homeostasis in yeast. Under normal/high Pi conditions, the low affinity Pi transport system is responsible for Pi uptake, while Pho84 is targeted to the vacuole, where it is degraded. The Pho81–Pho85–Pho80 complex is active, phosphorylating Pho4, thus inactivating the PHO pathway. Under Pi limiting condition, IP7, in association with Pho81, prevents the phosphorylation of Pho4, thus activating the PHO pathway. Consequently, Pho87 and Pho90 are targeted to the vacuole for degradation, in a mechanism requiring their SPX domain. Concomitantly, the Vtc complex is also induced, resulting in increased polyP synthesis in the vacuole. Gde1, is responsible for the hydrolysis of GroPCho into choline and glycerophosphate, which can be used as a source of Pi for cell growth. Negative and positive regulatory effects are indicated by flat-ended dashed lines and arrow-heads, respectively. IP7, inositol heptakisphosphate; polyP, polyphosphate; X, unknown protein; GroPCho, glycerophosphocholine.

PHO84 and the regulatory factor SPL2, without disturbing Pi uptake capacity. The existence of a complex feedback mechanism enabling the yeast cells to switch from low to high Pi affinity transporters, and reciprocally, depending on internal Pi availability has recently been demonstrated [44]. Under low internal Pi concentration the PHO pathway is activated, Pho4 is dephosphorylated and thus activating the transcription of Pho84, resulting in higher Pi uptake into yeast cells, generating a negative feedback loop. Concomitantly, low internal Pi concentration also activates Spl2, which negatively regulates the activity of Pho87 and Pho90 [44], thus creating a positive feedback mechanism. Surprisingly, since the signaling of these two feedback mechanisms is mediated by the PHO pathway, the two Pi uptake systems are mutually exclusive, therefore only one type of Pi transport system can function at a time [44]. Such a tight regulation of the low affinity system might come from its intrinsic property. Indeed, it has been shown that

Pho87 and Pho90, if not properly regulated, could mediate Pi efflux out of the cell under Pi starvation [45]. Thus under limiting external Pi conditions, it is important to turn off the low affinity Pi uptake, in order to prevent Pi leakage from the cell. Interestingly, a recent study provided evidence of the advantages of possessing both low and high affinity transporters, prolonging preparation for starvation and facilitating subsequent recovery [46].

Recently, Hurlimann et al. (2009) [13] showed that Spl2, a protein induced by Pi starvation, could negatively regulate the activity of Pho87 and Pho90, specifically by interacting with the SPX domain of Pho87 and Pho90. In the same study, using Pho87 and Pho90 proteins devoid of the SPX domain, the authors showed that removal of the SPX domain did not alter protein expression levels or localization. However, deletion of the SPX domain increased the catalytic activity of these two transporters, resulting in unrestricted phosphate accumulation, until cells were no longer able to grow [13]. Interestingly, Ghillebert et al. (2011) [38] showed that under Pi limiting conditions, Pho87 and Pho90 were endocytosed and targeted to the vacuole, dependently and independently of Spl2, respectively. Indeed, inactivation of cell surface proteins, such as transporters and signaling receptors, in response to changes in nutrient availability by ubiquitin-mediated endocytosis and vacuolar targeting is a common strategy employed by cells [38]. Furthermore, the vacuolar targeting of both Pho87 and Pho90 strictly required the presence of the SPX domain [38]. Although a previous study showed that Spl2 could interact with the SPX domain of Pho90, the vacuolar targeting of Pho90 upon Pi stress does not seem to require interaction with Spl2 [45]. These apparently contradictory results might arise from the different experimental conditions used in the two studies. It has also been shown that Pho87 and Pho90 can also be targeted to the vacuole under conditions other than Pi deficiency, such as carbon-source starvation or nitrogen deficiency, which are independent from the PHO signaling pathway suggesting that other SPX-interacting proteins, besides Spl2, are involved in this mechanism [38]. However, independently of the conditions tested, the vacuolar targeting of both Pho87 and Pho90 strictly required the presence of their N-terminal SPX domain. Moreover, another study performed by Estrella et al. (2008) [47] also showed that Pho87 and Pho91, in response to Pi deficiency, were ubiquitinated in an E3 ubiquitin ligase Rsp5-dependent manner before being targeted for endocytosis and degraded in the vacuole [47]. Consequently, in order to shed further light on the mechanisms regulating the activity of Pho87, Pho90 and Pho91, a key step would be the complete identification of the mechanisms involved in the vacuolar targeting of Pho87 and Pho90, identifying their interacting partner proteins as well as defining the key role of the SPX domain in these processes.

The high affinity Pi transport system, composed of Pho84 and Pho89, is required for Pi uptake under Pi limiting condition [6,7]. Pho84, a proton coupled Pi symporter, is the main Pi transceptor in yeast, being involved in scavenging Pi from external media, at an acidic pH optimum of 4.5, as well as sensing external Pi levels through the activation of the PKA pathway, [6,40]. Under Pi sufficient condition, Pho84 is phosphorylated and ubiquitinated, resulting in its internalization and vacuolar degradation [48]. Pho89 has recently been demonstrated to be a sodium coupled Pi transporter, being mainly active at alkaline pH [49].

## 3. The Vtc complex, a role in polyphosphate synthesis

The Vacuolar Transporter Chaperon Complex (Vtc), consisting of Vtc1, Vtc2, Vtc3 and Vtc4 (also referred as Phm4, Phm1, Phm2 and Phm3, respectively) has been implicated in several membrane-related processes such as vacuolar membrane fusion, V-ATPase stability and trafficking, micro-autophagy and vacuolar polyphosphate homeostasis [1,50–54]. Inorganic polyphosphate (polyP), a linear chain of three to thousands of Pi residues linked by high-energy phosphoanhydride bonds, occurs in all living organisms. While in prokaryotes and lower plants, polyP is usually a store of phosphate, in fungi that form mycorrhizas it is the main form of phosphate that is transported from the extraradical hyphae to the intraradical hyphae [55]. In yeast, polyP is mainly stored in the vacuoles and can be hydrolysed to Pi by polyphosphatases such as Phm5 [1,56]. Interestingly, the Vtc protein family is only found in protists, fungi and diatoms but does not seem to be present in plants or animals. The four members of the yeast Vtc family possess a Vtc domain and a domain of unknown function as well as a transmembrane domain. However, only Vtc2, Vtc3 and Vtc4 harbour the N-terminal SPX domain (Fig. 2, Table 1). In an experiment aimed at discovering new components of the PHO regulon, Oagawa et al. (2000) [1], identified the four members of the Vtc family, as being up-regulated by Pi starvation. Various phosphate analyses showed that these genes were specifically involved in polyphosphate accumulation. Indeed deletion of VTC2 and VTC3 significantly reduced total polyP accumulation after polyP surplus, a condition where yeast accumulates large quantities of polyP in vacuoles under conditions of high Pi preceded by a period of Pi starvation [1]. In addition, deletion of VTC1 and VTC4 completely abolished polyP accumulation [1]. Only recently, Hothorn and co-workers (2009), using X-ray crystallography, identified Vtc4 as a polyP synthesizing enzyme (Fig. 2). Interestingly, despite the Vtc4 fragment used for this study being devoid of the SPX domain, this protein was still active in polyP synthesis, indicating that the SPX domain is not essential for the catalytic activity [52]. PolyP is synthesized by Vtc4, using ATP as a substrate, before translocating these phosphate polymers to the vacuolar lumen [52], which is consistent with the previous reports showing that cells with altered Vtc complex had reduced polyP accumulation as well as increased ATP concentration compared to intact cells. In addition, it also appears that the Vtc complex accounts for the most of polyP synthesis in yeast [52]. In vivo, the Vtc complex has been shown to function as an heterotrimer consisting of Vtc1, Vtc4 and either Vtc2 or Vtc3, with Vtc4 being the catalytic subunit [52]. Interestingly, increasing polyP synthesis under Pi limiting conditions might appear contrasting. However this step is required to avoid the generation of a negative feedback on the activity of the high affinity Pi transporters. Indeed, under Pi deficiency, the high affinity Pi transporter system is activated resulting in increased levels of intracellular Pi, which if not stored in the vacuole as polyP, could generate a negative feedback loop. Surprisingly, while all the Vtc proteins are mainly localized to the vacuole, independently of Pi status Vtc2 is mainly expressed around the nucleus and at the cell periphery under Pi replete conditions [1,50,52]. However under Pi limiting conditions, Vtc2 is shifted back to the vacuoles [52]. Consequently, Vtc2 has been hypothesized to be involved in the synthesis/transport of both intra- and extra-cellular polyP, depending on the Pi status.

In the parasitic protozoa *Toxoplasma gondii*, TgVtc2, a Vtc2 homolog also harbouring the SPX domain, has been identified and characterized [57]. Mutation in TgVTC2 resulted in a dramatic decrease in polyP accumulation, showing the importance and the conservation of the role of the Vtc proteins in polyP synthesis between lower eukaryotes.

Despite the SPX domain not being required for the catalytic activity of Vtc4, the function of the SPX domain in these proteins still remains to be elucidated. One hypothesis would be that the SPX domain could be involved in the formation of Vtc complex, through protein–protein interaction or being involved in signal transduction. Additionally, the SPX domain could interact with other proteins, such as proteins involved in microautophagy and membrane trafficking, explaining the wide spectrum of functions associated with the Vtc complex [50,51,53].

## 4. Gde1, a role in scavenging Pi

Under Pi deficiency, several phosphatases, catalysing the hydrolysis of phosphate esters, such as phospholipids, are induced in order to scavenge Pi from these macromolecules [58]. Among these, Gde1, the only characterized yeast glycerophosphodiester phosphodiesterase so far, is responsible for the hydrolysis of glycerophosphocholine, formed via the deacylation of the phospholipid phosphatidylcholine, into glycerophosphate and choline (Fig. 2, Table 1). Consequently, deletion and overexpression of GDE1 resulted in the accumulation and reduction of the intracellular levels of glycerophosphocholine, respectively [59]. Under optimal growth conditions, phosphatidylcholine, like other phospholipids, such as glycerophosphoinositol, can be excreted into the external medium and transported back into the cell upon nutritional stresses [60]. Extracellular phosphatidylcholine and glycerophosphoinositol have been shown to be transported across the yeast plasma membrane by the permease Git1. In addition, it has been shown that wild type yeast cells could use glycerophosphocholine as sole source of P for cell growth, with Gde1 and Git1 being required for this process, strengthening the function of Gde1 in scavenging Pi [61]. Surprisingly, several studies failed to detect any in vitro phosphodiesterase activity in cell lysates or fractions derived from wild type or GDE1 overexpressing strains, raising the hypothesis that phosphodiesterase activity was physically dependent on another protein [59,62]. Indeed, in addition to the SPX domain, which has been shown to be involved in protein interaction, Gde1, possesses ankyrins repeats, also involved in protein–protein interaction [37]. Thus, in order to shed light on some of the mechanisms controlling phospholipid turnover in yeast, and more specifically the hydrolysis of phosphatidylcholine, it would be interesting to try to identify interactors of Gde1 as well as confirming the cytoplasmic localization prediction of Gde1. In addition, experiments using various truncated version of Gde1 would be useful in order to decipher the function of the various domains of Gde1.

Several glycerophosphodiester phosphodiesterase have been identified in other organisms and have been implicated in a wide set of biological processes such as osmotic pressure, the G-protein signaling pathway, osteoblast differentiation and neuronal differentiation. Of note, despite lacking the SPX domain, two glycerophosphodiester phosphodiesterases have recently been involved in maintaining cellular phosphate homeostasis in plants [63,64].

## 5. Conclusion and perspectives

Increasing the understanding of the mechanisms controlling phosphate homeostasis in yeast is a key challenge as it represents the model organism for nutrient homeostasis in eukaryotes, and thus provides new paths for research in other organisms. Among the numerous proteins involved in regulating yeast phosphate homeostasis, a subset of key proteins harbouring the SPX domain, has been identified and characterized. Although the knowledge of the biological role of the SPX domain is still relatively scarce, being only involved so far in protein interaction and signaling, it is conserved between various organisms, and is present in proteins principally involved in regulating phosphate homeostasis. Surprisingly, proteins possessing the SPX domain regulate a broad variety of processes involved in Pi homeostasis, such as Pi transport, sensing, signaling, remobilization as well as polyP synthesis, confirming the key function of this domain. Nonetheless, despite the increasing importance of the SPX domain-containing proteins in controlling Pi homeostasis, this domain still remains poorly studied. Thus, a first step to increase our knowledge on the regulatory function of the SPX domain would be to determine to which extent this domain is involved in protein interaction and signaling, and if these properties can be extended to all proteins harbouring an SPX domain. Additionally, identifying the important and conserved sub-domains as well as key amino acids of the SPX domain may also be informative for assessing the function of the SPX domain in yeast.

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