SAGE-Hindawi Access to Research
Enzyme Research
Volume 2011, Article ID 103980, 7 pages
doi:10.4061/2011/103980

*Review Article*

# Inorganic Phosphate as an Important Regulator of Phosphatases

**Claudia Fernanda Dick,[1,2,3] André Luiz Araújo Dos-Santos,[2,3] and José Roberto Meyer-Fernandes[2,3]**

[1] *Instituto de Microbiologia Professor Paulo de Góes, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brazil*
[2] *Laboratório de Bioquímica Celular, Instituto de Bioquímica Medica, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brazil*
[3] *Instituto Nacional de Ciência e Tecnologia em Biologia Estrutural e Bioimagens, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Cidade Universitária, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brazil*

Correspondence should be addressed to José Roberto Meyer-Fernandes, meyer@bioqmed.ufrj.br

Received 25 February 2011; Accepted 3 May 2011

Academic Editor: Heung Chin Cheng



Cellular metabolism depends on the appropriate concentration of intracellular inorganic phosphate (Pi). Pi starvation-responsive genes appear to be involved in multiple metabolic pathways, implying a complex Pi regulation system in microorganisms and plants. A group of enzymes is required for absorption and maintenance of adequate phosphate levels, which is released from phosphate esters and anhydrides. The phosphatase system is particularly suited for the study of regulatory mechanisms because phosphatase activity is easily measured using specific methods and the difference between the repressed and derepressed levels of phosphatase activity is easily detected. This paper analyzes the protein phosphatase system induced during phosphate starvation in different organisms.

## 1. Introduction

Regulation of cellular processes, such as cellular differentiation, proliferation, cell death, mobility, metabolism, survival, and organization of the cytoskeleton, in response to some stimuli is fundamental to all aspects of cell life [1–4]. Protein phosphorylation is one of the most common mechanisms used to regulate these processes. Processes that are reversibly controlled by protein phosphorylation require both a protein kinase and a protein phosphatase [5–7].

Traditionally, protein kinases have been studied more intensely than protein phosphatases due to the earlier view that protein kinases confer fine regulation to protein phosphorylation, whereas protein phosphatases merely act to remove phosphate groups. Only in the last decade was it realized that protein phosphatases are also regulated by a variety of mechanisms and are of no less importance to cellular physiology than protein kinases [8]. Protein phosphatases are able to hydrolyze phosphomonoester metabolites, releasing inorganic phosphate (Pi) from these substrates [5, 9].

Phosphorus, in the form of inorganic phosphate (Pi), is one of the most important macronutrients for all organisms [7, 10–13]. It is not only used in the biosynthesis of cellular components, such as ATP, nucleic acids, phospholipids, and proteins, but it is also involved in many metabolic pathways, including energy transfer, protein activation, and carbon and amino acid metabolic processes [14]. Large amounts of phosphate are required for cell survival. In plants, Pi is essential for growth and development [15]. In fungi, the Pi signal transduction pathway regulates the expression of many phosphate responsive genes that are involved in scavenging and Pi uptake from extracellular sources [16]. In trypanosomatid parasites, Pi influences their ability to correctly grow and colonize in the invertebrate host [17, 18].

In summary, protein phosphatases and kinases are necessary for Pi homeostasis during the acquisition, storage, release, and metabolic integration of Pi [19–23]. The purpose of this paper is to summarize the regulation of phosphatases by inorganic phosphate, with an emphasis on the role of these enzymes in cell biology.

## 2. Feedback Control of Phosphatases by Inorganic Phosphate: The *PHO* Pathway

*Saccharomyces cerevisiae* has several phosphatases with different specificities, cellular location, and permeases used in Pi uptake. The set of genes responsible for these activities are coordinately repressed by the Pi concentration in the growth medium [24]. The cell acquisition, storage, release, and metabolic integration of Pi requires the participation of many essential enzymes such as extracellular acid phosphatases (APases), phosphodiesterases, Pi transporters, polyphosphate kinases, alkaline phosphatases (ALPases), and endopolyphosphatases [19–21]. The activities of these enzymes are intrinsically linked to Pi homeostasis, and they are subjected to regulation via the Pi signal transduction pathway (PHO) in response to varying Pi levels [21, 24].

In one current model for *PHO* regulation, the positive regulator (or positive factor) Pho4p, encoded by the *PH04* gene, is indispensable for transcriptional activation of *PHO* genes by its activity and location. In high Pi medium, a cyclin-dependent kinase (CDK) complex consisting of Pho80p and Pho85p inhibits Pho4p function by hyperphosphorylation. Hyperphosphorylated Pho4p remains in the cytoplasm and is unable to activate the transcription of the *PHO* genes [25, 26]. When the Pi concentration in the medium is sufficiently low, Pho81p inhibits the function of the Pho80p-Pho85p complex [26, 27], allowing Pho4p to relocate to the nucleus and activate transcription of the *PHO* genes [20, 25]. These genes encode for the high-affinity transporters Pho84p and Pho89p; secreted acid phosphatases Pho5p, Pho11p, and Pho12p; other related proteins that increase Pi recovery from extracellular sources [24, 28].

This *PHO* pathway has been described in different organisms such as plants [29–34], bacteria [35–37], and fungi [16, 24, 38, 39].

## 3. The Phosphatase System in Yeast

Initially, it was observed that a several phosphatase genes are modulated by the Pi concentration in the culture medium; thus, the PHO pathway was initially characterized by differentially expressed phosphatases [24].

In *S. cerevisiae*, the transcription of genes encoding acid and alkaline phosphatases and the Pi transporter is coordinately repressed and derepressed depending on the Pi concentration in the culture medium [20]. Most of the phosphatases synthesized under Pi limiting conditions are extracellularly located or are associated with the plasma membrane or cell wall [40].

The Pi-regulated phosphatase genes include *PHO5* [41], which encodes for the major fraction of repressible acid phosphatases (rAPase; optimum pH 3-4; EC 3.1.3.2), and its isozymes *PHO10* and *PHO11* [42]. These three rAPases are glycoproteins that are found in the cell wall or the periplasmic space. They are responsible for phosphate scavenging and work in conjunction with high-affinity transporters to acquire phosphate when the Pi concentration in the environment is low [24, 43]. The rAPase encoded by the *PHO5* gene is glycosylated during secretion across the membrane and is localized in the periplasmic space [41]. Pho5p is responsible for >90% of APase activity [43].

Because the *PHO5* gene product constitutes the bulk of the acid phosphatases, *PHO5* regulation is key to cellular phosphate homeostasis. Transcriptional activators, Pho4p and Pho2p, are required to generate the active chromatin structure in the *PHO5* promoter and stimulate transcription. Pho80p-Pho85p is a cyclin/cyclin-dependent kinase complex that phosphorylates Pho4p at multiple sites to negatively regulate Pho4p function [44]. Huang and O'Shea [45] carried out a high-throughput, quantitative, enzymatic screen of a yeast deletion collection, searching for novel mutants defective in *PHO5* expression. Among the constitutive mutants, the *pho80* and *pho85* strains showed the most elevated levels of Pho5 phosphatase activity and *PHO5* mRNA under high-phosphate conditions, consistent with their central role in the *PHO* pathway. Complete loss of the high kinase activity (Pho80p-Pho85) results in full activation of the Pho4 transcription factor, leading to full *PHO5* expression.

Another important class of phosphatases in *S. cerevisiae* is the alkaline phosphatases (ALPase; optimum pH 8; EC 3.1.3.1). *PHO8* [46] encodes for a nonspecific repressible alkaline phosphatase (rALPase). It is a vacuole-localized glycoprotein that cleaves diverse substrates to retrieve phosphate from intracellular products [24]. Pho8p is an $Mg^{2+}/Zn^{2+}$-dependent dimeric protein, similar to the ALPase in *Escherichia coli* and in mammalian cells [47]. The enzyme product of *PHO13* is a monomeric protein and is specific for p-nitrophenyl phosphate (pNPP) and histidinyl phosphate. This enzyme was strongly activated by $Mg^{2+}$ ions, with a pH optimum of 8.2 and a high specific activity for pNPP, with a mean value of $3.6 \times 10^{-5}$ M [4].

## 4. Phosphorus Stress Modulates Acid Phosphatases in Plants

Acid phosphatases (APases) may be active against a wide array of organic phosphates present in soil. These enzymes are nonspecific orthophosphoric monoester phosphohydrolases (EC 3.1.3.2) that cleave Pi from ester linkage sites. Secreted plant phosphatases sustain 50% activity over a broad pH range (4.0–7.6), maintain 80% activity over a broad temperature range (22°C–48°C), and are stable at temperatures as high as 60°C, making them ideal candidates for active soil enzymes [48–50].

APases are abundant in Arabidopsis and are represented by at least four gene families. A recent survey of the annotated Arabidopsis genome identified sequences for 1 His APase, 4 phosphatidic APases, 10 vegetative storage protein APases, and 29 purple APases [50, 51].

In recent years, there has been considerable interest in purple APases (PAPs). Comparative analysis of the structure of PAPs from higher plants and mammals has allowed for the identification of conserved sequence and structural motifs in this type of enzyme from many eukaryotic species [51, 52].

Biochemically, plant PAPs function as homodimeric proteins with a molecular mass of ~55 kDa per monomer,

whereas mammalian PAPs are typically monomeric proteins with a molecular mass of ~35 kDa [51–53]. Many PAPs are glycoproteins that are targeted to the secretory pathway [52]. One PAP from *Spirodela oligorrhiza* has been found to be glycosylphosphatidylinositol anchored in the cell [54]. Structurally, plant PAPs have two domains. The NH2-domain does not have catalytic function. The COOH-domain has the metal center and is the catalytic domain of the enzyme. Another PAP from *Lupinus albus* may contain a third domain, with a structure resembling that of sterol desaturases, at its carboxyl terminus [55]. It is not known how common the latter two forms of posttranslational modification are in PAPs from other species. PAPs are metalloenzymes that have a binuclear metal ion complex in its active site. Their characteristic pink to purple color is due to a charge transfer transition by a tyrosine residue coordinating a ferric ion [52, 56]. This enzyme can hydrolyze phosphoric acid esters and anhydrides [57].

PAPs have been isolated from *Phaseolus vulgaris* (common bean) [58], *Glycine max* (soybean) [57], *Lupinus albus* (white lupin) [59], *Lycopersicon esculentum* (tomato) [60], *Triticum aestivum* (wheat) [61], *Hordeum vulgare* (barley) [61], *Zea maize* (maize) [61], and *Oryza sativa* (rice) [61].

The plant response to Pi starvation can be divided into two categories: the specific response and the general response. The specific responses promote efficient mobilization and acquisition of Pi from growth medium and intracellular stores. The general responses allow for long-term survival by coordinating cell metabolism with nutrient availability and growth potential [14, 62]. The implementation of these strategies requires changes in the expression profiles of hundreds of genes, as demonstrated by the transcriptome analyses of *Arabidopsis thaliana* (Arabidopsis) [14, 63].

During Pi starvation, plants enhance phosphatase expression as a general response [50, 63]. Phosphatase production is linked to Pi deficiency, and a positive correlation between acid phosphatase production and Pi nutrition has been proposed [64–66]. For example, plants such as lupins, which are more efficient in acquiring Pi from the soil, produce significantly more phosphatase in comparison to cereals [66].

Wu et al. [14] analyzed the regulation of protein phosphatases in Arabidopsis and found that three genes for PAP were induced by Pi starvation. In addition, the gene At1g25230 was induced more than 2-fold, showing that this gene is responsive to Pi starvation.

In the rice genome, a total of 26 putative PAP genes were identified, and Pi starvation induced the expression of 10 rice PAP genes, suggesting that these play important roles in the acclimation of rice to low Pi conditions [67].

In *Lycopersicon esculentum* (tomato), LePS2, is induced by Pi starvation [66]. It is noted that LePS2 phosphatases represent the first cytoplasmatic phosphatases that are components of the Pi starvation response [31]. Suspending tomato cells in Pi-starved medium led to a PAP-specific activity increase of approximately 4-fold, but the PAP-specific activity remained low and constant in cells maintained in high Pi medium. The increase of PAP activity in cells growth in Pi-starved medium demonstrates that PAP could have a role in enhancing the availability and utilization of Pi and may be pivotal for mobilizing intracellular Pi by sensing a lack of Pi in tomato [60, 66].

## 5. Ectophosphatases as Pi Sensors

The plasma membrane of cells may contain enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ectoenzymes, can be measured using intact cells [17, 68–73]. Ectophosphatases and ectokinases have been detected in several microorganisms, including protozoa [69, 71, 74–78], bacteria [79–82], and fungi [83–88].

Many studies have demonstrated a role of ectophosphatases in acquiring Pi for use in the growth of various cell types [73, 74, 86, 89]. In fungi cells (*Fonsecae pedrosoi*), the depletion of Pi from the culture medium apparently induces the expression of different ectophosphatase activities [86]. The cultivation of these fungi in the absence of exogenous Pi has been shown to result in the generation of fungal cells expressing an ectophosphatase activity 130-fold higher than that expressed by fungi cultivated in the presence of Pi [85]. Trypanosomatid cells have ectophosphatases that provide Pi by hydrolyzing phosphomonoester metabolites [17, 18, 51, 60, 73, 81]. For example, in *T. rangeli*, a low Pi concentration in the growth medium induces the expression of a different ectophosphatase activity [17, 73], suggesting that this enzyme leads to hydrolyzes phosphorylated compounds present in the extracellular medium. This hydrolysis could contribute to the acquisition of Pi during the development of *T. rangeli* epimastigotes [18].

Under conditions of Pi limitation, fluorescent bacteria *Pseudomona* express a set of phosphate starvation genes [90, 91]. For example, at least 56 Pi starvation proteins are induced in the *P. putida* strain KT2442 [90], and, in the *P. fluorescens* strain DF57, induction of several phosphate starvation genes has been reported [91].

In many eukaryotes, the nucleotide pyrophosphatase/phosphodiesterase (E-NPP) family of proteins is directly responsible for phosphate hydrolysis from extracellular nucleotides. NPP1 to −3 are found in nearly all human tissue types, and these enzymes contain an alkaline ectonucleotide pyrophosphatase/phosphodiesterase type 1 domain. In *S. cerevisiae*, *NPP1* and *NPP2* are upregulated via Pi-regulated transcription [43].

## 6. Concluding Remarks

Pi is a compound that is growth limiting in various organisms when its availability is low in many ecosystems [38]. The induction of phosphatase activity in response to Pi starvation is a common phenomenon among organisms acquiring Pi from the environment. These enzymes are able to hydrolyze phosphorylated substrates to supply a source of Pi during a shortage of nutrients. In *Saccharomyces cerevisiae*, the Pi starvation signal triggers increased production of at least four types of phosphatases: (1) the acid phosphatases Pho5, Pho11, and Pho12, which are localized in periplasmic space;

(2) the alkaline phosphatase Pho8, which is localized in the vacuole; (3) the glycerol phosphatase Hor2; (4) the putative polyphosphatase Phm5, which is localized in the vacuole. All of these enzymes can contribute to increased levels of free Pi [92]. However, other functions could be attributed to these enzymes.

Del Pozo et al. [93] purified an acid phosphatase, AtACP5, that is induced by Pi starvation in *Arabidopsis thaliana*. This enzyme presents two activities, hydrolysis of phosphorylated substrates and peroxide formation. The phosphatase activity probably reflects a role in Pi mobilization; the peroxidation activity suggests that AtACP5 could also play a role in the metabolism of reactive oxygen species [34].

Taken together, Pi starvation-induced phosphatases play a role in an organism's adaptation to stress, though other roles can be found.

## Acknowledgments

The authors acknowledge the financial support given by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ). C. F. Dick and A. L. A. Dos-Santos contributed equally to this work.

## References

[1] D. Barford, A. K. Das, and M. P. Egloff, "The structure and mechanism of protein phosphatases: insights into catalysis and regulation," *Annual Review of Biophysics and Biomolecular Structure*, vol. 27, pp. 133–164, 1998.

[2] P. Cohen, "The structure and regulation of protein phosphatases," *Annual Review of Biochemistry*, vol. 58, pp. 453–508, 1989.

[3] S. Shenolikar, "Protein serine/threonine phosphatases—new avenues for cell regulation," *Annual Review of Cell Biology*, vol. 10, pp. 55–86, 1994.

[4] B. Tuleva, E. Vasileva-Tonkova, and D. Galabova, "A specific alkaline phosphatase from Saccharomyces cerevisiae with protein phosphatase activity," *FEMS Microbiology Letters*, vol. 161, no. 1, pp. 139–144, 1998.

[5] T. Hunter, "Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling," *Cell*, vol. 80, no. 2, pp. 225–236, 1995.

[6] S. Luan, "Protein phosphatases in plants," *Annual Review of Plant Biology*, vol. 54, pp. 63–92, 2003.

[7] X. J. Yang and P. M. Finnegan, "Regulation of phosphate starvation responses in higher plants," *Annals of Botany*, vol. 105, no. 4, pp. 513–526, 2010.

[8] M. A. Kutuzov and A. V. Andreeva, "Protein Ser/Thr phosphatases of parasitic protozoa," *Molecular and Biochemical Parasitology*, vol. 161, no. 2, pp. 81–90, 2008.

[9] S. K. Hanks and T. Hunter, "The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification," *FASEB Journal*, vol. 9, no. 8, pp. 576–596, 1995.

[10] T. Watanabe, N. Ozaki, K. Iwashita, T. Fujii, and H. Iefuji, "Breeding of wastewater treatment yeasts that accumulate high concentrations of phosphorus," *Applied Microbiology and Biotechnology*, vol. 80, no. 2, pp. 331–338, 2008.

[11] S. Wongwisansri and P. J. Laybourn, "Disruption of histone deacetylase gene *RPD3* accelerates *PHO5* activation kinetics through inappropriate Pho84p recycling," *Eukaryotic Cell*, vol. 4, no. 8, pp. 1387–1395, 2005.

[12] A. Kornberg, N. N. Rao, and D. Ault-Riché, "Inorganic polyphosphate: a molecule of many functions," *Annual Review of Biochemistry*, vol. 68, pp. 89–125, 1999.

[13] R. Ghillebert, E. Swinnen, P. De Snijder, B. Smets, and J. Winderickx, "Differential roles for the low-affinity phosphate transporters Pho87 and Pho90 in *Saccharomyces cerevisiae*," *Biochemical Journal*, vol. 434, no. 2, pp. 243–251, 2010.

[14] P. Wu, L. Ma, X. Hou et al., "Phosphate starvation triggers distinct alterations of genome expression in arabidopsis roots and leaves," *Plant Physiology*, vol. 132, no. 3, pp. 1260–1271, 2003.

[15] M. Lei, C. Zhu, Y. Liu et al., "Ethylene signalling is involved in regulation of phosphate starvation-induced gene expression and production of acid phosphatases and anthocyanin in Arabidopsis," *New Phytologist*, vol. 189, no. 4, pp. 1084–1095, 2010.

[16] C. Auesukaree, T. Homma, H. Tochio, M. Shirakawa, Y. Kaneko, and S. Harashima, "Intracellular phosphate serves as a signal for the regulation of the PHO pathway in *Saccharomyces cerevisiae*," *Journal of Biological Chemistry*, vol. 279, no. 17, pp. 17289–17294, 2004.

[17] C. F. Dick, A. L. A. dos-Santos, A. L. Fonseca-de-Souza, J. Rocha-Ferreira, and J. R. Meyer-Fernandes, "Trypanosoma rangeli: differential expression of ecto-phosphatase activities in response to inorganic phosphate starvation," *Experimental Parasitology*, vol. 124, no. 4, pp. 386–393, 2010.

[18] A. L. Fonseca-de-Souza, C. F. Dick, A. L. A. dos Santos, F. V. Fonseca, and J. R. Meyer-Fernandes, "Trypanosoma rangeli: a possible role for ecto-phosphatase activity on cell proliferation," *Experimental Parasitology*, vol. 122, no. 3, pp. 242–246, 2009.

[19] Y. Kaneko, Y. Tamai, A. Toh-e, and Y. Oshimal, "Transcriptional and post-transcriptional control of PHO8 expression by PHO regulatory genes in *Saccharomyces cerevisiae*," *Molecular and Cellular Biology*, vol. 5, no. 1, pp. 248–252, 1985.

[20] Y. Oshima, N. Ogawa, and S. Harashima, "Regulation of phosphatase synthesis in *Saccharomyces cerevisiae*—a review," *Gene*, vol. 179, no. 1, pp. 171–177, 1996.

[21] B. L. Persson, J. O. Lagerstedt, J. R. Pratt et al., "Regulation of phosphate acquisition in *Saccharomyces cerevisiae*," *Current Genetics*, vol. 43, no. 4, pp. 225–244, 2003.

[22] D. D. Wykoff and E. K. O'Shea, "Phosphate transport and sensing in *Saccharomyces cerevisiae*," *Genetics*, vol. 159, no. 4, pp. 1491–1499, 2001.

[23] K. Huang, I. Ferrin-O'Connell, W. Zhang, G. A. Leonard, E. K. O'Shea, and F. Quiocho, "Structure of the Pho85-Pho80 CDK-cyclin complex of the phosphate-responsive signal transduction pathway," *Molecular Cell*, vol. 30, no. 4, pp. 614–623, 2007.

[24] Y. Oshima, "The phosphatase system in *Saccharomyces cerevisiae*," *Genes and Genetic Systems*, vol. 72, no. 6, pp. 323–334, 1997.

[25] A. Kaffman, I. Herskowitz, R. Tjian, and E. K. O'Shea, "Phosphorylation of the transcription factor PHO4 by a cyclin-CDK complex, PHO80-PHO85," *Science*, vol. 263, no. 5150, pp. 1153–1156, 1994.

[26] N. Ogawa, K. I. Noguchi, H. Sawai, Y. Yamashita, C. Yompakdee, and Y. Oshima, "Functional somains of Pho81p, an Inhibitor of Pho85p protein kinase, in the transduction

pathway of Pi Signals in *Saccharomyces cerevisiae*," *Molecular and Cellular Biology*, vol. 15, no. 2, pp. 997–1004, 1995.

[27] K. R. Schneider, R. L. Smith, and E. K. O'Shea, "Phosphate-regulated inactivation of the kinase PHO80-PHO85 by the CDK inhibitor PHO81," *Science*, vol. 266, no. 5182, pp. 122–126, 1994.

[28] J. M. Mouillon and B. L. Persson, "New aspects on phosphate sensing and signalling in *Saccharomyces cerevisiae*," *FEMS Yeast Research*, vol. 6, no. 2, pp. 171–176, 2006.

[29] I. Ciereszko, H. Johansson, V. Hurry, and L. A. Kleczkowski, "Phosphate status affects the gene expression, protein content and enzymatic activity of UDP-glucose pyrophosphorylase in wild-type and pho mutants of Arabidopsis," *Planta*, vol. 212, no. 4, pp. 598–605, 2001.

[30] C. A. Ticconi, C. A. Delatorre, and S. Abel, "Attenuation of phosphate starvation responses by phosphite in Arabidopsis," *Plant Physiology*, vol. 127, no. 3, pp. 963–972, 2001.

[31] I. Stenzel, K. Ziethe, J. Schurath, S. C. Hertel, D. Bosse, and M. Köck, "Differential expression of the LePS2 phosphatase gene family in response to phosphate availability, pathogen infection and during development," *Physiologia Plantarum*, vol. 118, no. 1, pp. 138–146, 2003.

[32] E. González, R. Solano, V. Rubio, A. Leyva, and J. Paz-Ares, "Phosphate transporter traffic facilitator1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in Arabidopsis," *Plant Cell*, vol. 17, no. 12, pp. 3500–3512, 2005.

[33] F. Shimano and H. Ashihara, "Effect of long-term phosphate starvation on the levels and metabolism of purine nucleotides in suspension-cultured Catharanthus roseus cells," *Phytochemistry*, vol. 67, no. 2, pp. 132–141, 2006.

[34] J. C. Baldwin, A. S. Karthikeyan, A. Cao, and K. G. Raghothama, "Biochemical and molecular analysis of LePS2;1: a phosphate starvation induced protein phosphatase gene from tomato," *Planta*, vol. 228, no. 2, pp. 273–280, 2008.

[35] A. Torriani, "From cell membrane to nucleotides: the phosphate regulon in *Escherichia coli*," *BioEssays*, vol. 12, no. 8, pp. 371–376, 1990.

[36] R. D. Monds, P. D. Newell, J. A. Schwartzman, and G. A. O'Toole, "Conservation of the Pho regulon in Pseudomonas fluorescens Pf0-1," *Applied and Environmental Microbiology*, vol. 72, no. 3, pp. 1910–1924, 2006.

[37] M. G. Lamarche, B. L. Wanner, S. Crépin, and J. Harel, "The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis," *FEMS Microbiology Reviews*, vol. 32, no. 3, pp. 1–13, 2008.

[38] Y. Tasaki, Y. Kamiya, A. Azwan, T. Hara, and T. Joh, "Gene expression during P deficiency in Pholiota nameko: accumulation of mRNAs for two transporters," *Bioscience, Biotechnology and Biochemistry*, vol. 66, no. 4, pp. 790–800, 2002.

[39] P. F. de Gouvêa, F. M. Soriani, I. Malavazi et al., "Functional characterization of the *Aspergillus fumigatus* PHO80 homologue," *Fungal Genetics and Biology*, vol. 45, no. 7, pp. 1135–1146, 2008.

[40] R. L. Metzenberg and W. Chia, "Genetic control of phosphorus assimilation in *Neurospora crassa*: dose-dependent dominance and recessiveness in constitutive mutants," *Genetics*, vol. 93, no. 3, pp. 625–643, 1979.

[41] K. Arima, T. Oshima, I. Kubota, N. Nakamura, T. Mizunaga, and A. Toh-e, "The nucleotide sequence of the yeast PHO5 gene: a putative precursor of repressible acid phosphatase contains a signal peptide," *Nucleic Acids Research*, vol. 11, no. 6, pp. 1657–1672, 1983.

[42] K. A. Bostian, J. M. Lemire, L. E. Cannon, and H. O. Halvorson, "In vitro synthesis of repressible yeast acid phosphatase: identification of multiple mRNAs and products," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 77, no. 8, pp. 4504–4508, 1980.

[43] E. J. Kennedy, L. Pillus, and G. Ghosh, "Pho5p and newly identified nucleotide pyrophosphatases/phosphodiesterases regulate extracellular nucleotide phosphate metabolism in *Saccharomyces cerevisiae*," *Eukaryotic Cell*, vol. 4, no. 11, pp. 1892–1901, 2005.

[44] S. Wongwisansri and P. J. Laybourn, "Disruption of histone deacetylase gene RPD3 accelerates PHO5 activation kinetics through inappropriate Pho84p recycling," *Eukaryotic Cell*, vol. 4, no. 8, pp. 1387–1395, 2005.

[45] S. Huang and E. O'Shea, "A systematic high-throughput screen of a yeast deletion collection for mutants defective in *PHO5* regulation," *Genetics*, vol. 169, no. 4, pp. 1859–1871, 2005.

[46] Y. Kaneko, N. Hayashi, A. Toh-e, I. Banno, and Y. Oshima, "Structural characteristics of the PHO8 gene encoding repressible alkaline phosphatase in *Saccharomyces cerevisiae*," *Gene*, vol. 58, no. 1, pp. 137–148, 1987.

[47] C. M. L. Janeway, J. E. Murphy, A. Chaidaroglou, and E. R. Kantrowitz, "Magnesium in the active site of *Escherichia coli* alkaline phosphatase is important for both structural stabilization ajid catalysis," *Biochemistry*, vol. 32, no. 6, pp. 1601–1609, 1993.

[48] B. R. LeBansky, T. D. McKnight, and L. R. Gritting, "Purification and characterization of a secreted purple phosphatase from soybean suspension cultures," *Plant Physiology*, vol. 99, no. 2, pp. 391–395, 1992.

[49] M. Li and T. Tadano, "Comparison of characteristics of acid phosphatases secreted from roots of lupin and tomato," *Soil Science and Plant Nutrition*, vol. 42, no. 4, pp. 753–763, 1996.

[50] J. L. Tomscha, M. C. Trull, J. Deikman, J. P. Lynch, and M. J. Guiltinan, "Phosphatase under-producer mutants have altered phosphorus relations," *Plant Physiology*, vol. 135, no. 1, pp. 334–345, 2004.

[51] D. Li, H. Zhu, K. Liu et al., "Purple acid phosphatases of *Arabidopsis thaliana*," *Journal of Biological Chemistry*, vol. 277, no. 31, pp. 27772–27781, 2002.

[52] G. Schenk, L. W. Guddat, Y. Ge et al., "Identification of mammalian-like purple acid phosphatases in a wide range of plants," *Gene*, vol. 250, no. 1-2, pp. 117–125, 2000.

[53] L. W. Guddat, A. S. McAlpine, D. Hume, S. Hamilton, J. de Jersey, and J. L. Martin, "Crystal structure of mammalian purple acid phosphatase," *Structure*, vol. 15, no. 7, pp. 757–767, 1999.

[54] H. Nakazato, T. Okamoto, M. Nishikoori et al., "The glycosylphosphatidylinositol-anchored phosphatase from *Spirodela oligorrhiza* is a purple acid phosphatase," *Plant Physiology*, vol. 118, no. 3, pp. 1015–1020, 1998.

[55] S. S. Miller, J. Liu, D. L. Allan, C. J. Menzhuber, M. Fedorova, and C. P. Vance, "Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus-stressed white lupin," *Plant Physiology*, vol. 127, no. 2, pp. 594–606, 2001.

[56] T. Waratrujiwong, B. Krebs, F. Spener, and P. Visoottiviseth, "Recombinant purple acid phosphatase isoform 3 from sweet potato is an enzyme with a diiron metal center," *FEBS Journal*, vol. 273, no. 8, pp. 1649–1659, 2006.

[57] G. Schenk, Y. Ge, L. E. Carrington et al., "Binuclear metal centers in plant purple acid phosphatases: Fe-Zn in sweet potato and Fe-Zn in soybean," *Archives of Biochemistry and Biophysics*, vol. 370, no. 2, pp. 183–189, 1999.

[58] C. Liang, J. Tian, H. M. Lam, B. L. Lim, X. Yan, and H. Liao, "Biochemical and molecular characterization of PvPAP3, a novel purple acid phosphatase isolated from common bean enhancing extracellular ATP utilization," *Plant Physiology*, vol. 152, no. 2, pp. 854–865, 2010.

[59] J. Wasaki, S. Kojima, H. Maruyama, S. Haase, M. Osaki, and E. Kandeler, "Localization of acid phosphatase activities in the roots of white lupin plants grown under phosphorus-deficient conditions," *Soil Science and Plant Nutrition*, vol. 54, no. 1, pp. 95–102, 2008.

[60] G. G. Bozzo, K. G. Raghothama, and W. C. Plaxton, "Structural and kinetic properties of a novel purple acid phosphatase from phosphate-starved tomato (Lycopersicon esculentum) cell cultures," *Biochemical Journal*, vol. 377, no. 2, pp. 419–428, 2004.

[61] G. Dionisio, C. K. Madsen, P. B. Holm et al., "Cloning and characterization of purple acid phosphatase phytases from wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), maize (Zea maize L.) and rice (Oryza sativa L.)," *Plant Physiology*. In press.

[62] D. D. Lefebvre, S. M. G. Duff, C. A. Fife, C. Julien-Inalsingh, and W. C. Plaxton, "Response to phosphate deprivation in *Brassica nigra* suspension cells," *Plant Physiology*, vol. 93, no. 2, pp. 504–511, 1990.

[63] H. Yuan and D. Liu, "Signaling components involved in plant responses to phosphate starvation," *Journal of Integrative Plant Biology*, vol. 50, no. 7, pp. 849–859, 2008.

[64] A. Jungk, B. Seeling, and J. Gerke, "Mobilization of different phosphate fractions in the rhizosphere," *Plant and Soil*, vol. 155-156, no. 1, pp. 91–94, 1993.

[65] J. Ascencio, "Acid phosphatase as a diagnostic tool," *Communications in Soil Science and Plant Analysis*, vol. 25, pp. 1553–1564, 1994.

[66] J. C. Baldwin, A. S. Karthikeyan, and K. G. Raghothama, "LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato," *Plant Physiology*, vol. 125, no. 2, pp. 728–737, 2001.

[67] Q. Zhang, C. Wang, J. Tian, K. Li1, and H. Shou, "Identification of rice purple acid phosphatases related to posphate starvation signaling," *Plant Biology*, vol. 13, pp. 7–15, 2011.

[68] J. R. Meyer-Fernandes, P. M. Dutra, C. O. Rodrigues, J. Saad-Nehme, and A. H. Lopes, "Mg-dependent ecto-ATPase activity in *Leishmania tropica*," *Archives of Biochemistry and Biophysics*, vol. 341, no. 1, pp. 40–46, 1997.

[69] J. B. De Jesus, A. A. de Sá Pinheiro, A. H. C. S. Lopes, and J. R. Meyer-Fernandes, "An ectonucleotide ATP-diphosphohydrolase activity in Trichomonas vaginalis stimulated by galactose and its possible role in virulence," *Zeitschrift fur Naturforschung—Section C Journal of Biosciences*, vol. 57, no. 9-10, pp. 890–896, 2002.

[70] S. A. Gomes, A. L. Fonseca De Souza, B. A. Silva et al., "*Trypanosoma rangeli*: differential expression of cell surface polypeptides and ecto-phosphatase activity in short and long epimastigote forms," *Experimental Parasitology*, vol. 112, no. 4, pp. 253–262, 2006.

[71] A. A. de Sá Pinheiro, J. N. Amazonas, F. de Souza Barros et al., "*Entamoeba histolytica*: an ecto-phosphatase activity regulated by oxidation-reduction reactions," *Experimental Parasitology*, vol. 115, no. 4, pp. 352–358, 2007.

[72] C. E. Peres-Sampaio, E. E. de Almeida-Amaral, N. L. L. Giarola, and J. R. Meyer-Fernandes, "*Leishmania amazonensis*: effects of heat shock on ecto-ATPase activity," *Experimental Parasitology*, vol. 119, no. 1, pp. 135–143, 2008.

[73] A. L. Fonseca-de-Souza, C. F. Dick, A. L. Santos, and J. R. Meyer-Fernandes, "A $Mg^{(2+)}$-dependent ecto-phosphatase activity on the external surface of *Trypanosoma rangeli* modulated by exogenous inorganic phosphate," *Acta Tropica*, vol. 107, no. 2, pp. 153–158, 2008.

[74] E. C. Fernandes, J. R. Meyer-Fernandes, M. A. C. Silva-Neto, and A. E. Vercesi, "*Trypanosoma brucei*: ecto-phosphatase activity present on the surface of intact procyclic forms," *Zeitschrift fur Naturforschung*, vol. 52, no. 5-6, pp. 351–358, 1997.

[75] N. Sacerdoti-Sierra and C. L. Jaffe, "Release of ecto-protein kinases by the protozoan parasite *Leishmania major*," *Journal of Biological Chemistry*, vol. 272, no. 49, pp. 30760–30765, 1997.

[76] J. R. Meyer-Fernandes, M. A. Da Silva-Neto, M. Dos Santos Soares, E. Fernandas, A. E. Vercesi, and M. M. De Oliveira, "Ecto-phosphatase activities on the cell surface of the amastigote forms of *Trypanosoma cruzi*," *Zeitschrift fur Naturforschung—Section C Journal of Biosciences*, vol. 54, no. 11, pp. 977–984, 1999.

[77] A. Dos Passos Lemos, A. L. Fonseca de Souza, A. A. De Sá Pinheiro, M. De Berrêdo-Pinho, and J. R. Meyer-Fernandes, "Ecto-phosphatase activity on the cell surface of *Crithidia deanei*," *Zeitschrift fur Naturforschung*, vol. 57, no. 5-6, pp. 500–505, 2002.

[78] J. N. Amazonas, D. Cosentino-Gomes, A. Werneck-Lacerda et al., "*Giardia lamblia*: characterization of ecto-phosphatase activities," *Experimental Parasitology*, vol. 121, no. 1, pp. 15–21, 2009.

[79] J. B. Bliska, J. E. Galan, and S. Falkow, "Signal transduction in the mammalian cell during bacterial attachment and entry," *Cell*, vol. 73, no. 5, pp. 903–920, 1993.

[80] J. B. Bliska, K. Guan, J. E. Dixon, and S. Falkow, "Tyrosine phosphate hydrolysis of host proteins by an essential Yersinia virulence determinant," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 15, no. 4, pp. 1187–1191, 1991.

[81] M. Braibant and J. Content, "The cell surface associated phosphatase activity of *Mycobacterium bovis* BCG is not regulated by environmental inorganic phosphate," *FEMS Microbiology Letters*, vol. 195, no. 2, pp. 121–126, 2001.

[82] E. Madec, A. Laszkiewicz, A. Iwanicki, M. Obuchowski, and S. Séror, "Characterization of a membrane-linked Ser/Thr protein kinase in *Bacillus subtilis*, implicated in developmental processes," *Molecular Microbiology*, vol. 46, no. 2, pp. 571–586, 2002.

[83] W. N. Arnold, L. C. Mann, K. H. Sakai, R. G. Garrison, and P. D. Coleman, "Acid phosphatases of *Sporothrix schenckii*," *Journal of General Microbiology*, vol. 132, no. 12, pp. 3421–3432, 1986.

[84] M. Bernard, I. Mouyna, G. Dubreucq et al., "Characterization of a cell-wall acid phosphatase (PhoAp) in *Aspergillus fumigatus*," *Microbiology*, vol. 148, no. 9, pp. 2819–2829, 2002.

[85] L. F. Kneipp, V. F. Palmeira, A. A. S. Pinheiro et al., "Phosphatase activity on the cell wall of *Fonsecaea pedrosoi*," *Medical Mycology*, vol. 41, no. 6, pp. 469–477, 2003.

[86] L. F. Kneipp, M. L. Rodrigues, C. Holandino et al., "Ecto-phosphatase activity in conidial forms of Fonsecaea pedrosoi is modulated by exogenous phosphate and influences fungal adhesion to mammalian cells," *Microbiology*, vol. 150, no. 10, pp. 3355–3362, 2004.

[87] I. Collopy-Junior, F. F. Esteves, L. Nimrichter, M. L. Rodrigues, C. S. Alviano, and J. R. Meyer-Fernandes, "An ectophosphatase

activity in *Cryptococcus neoformans*," *FEMS Yeast Research*, vol. 6, no. 7, pp. 1010–1017, 2006.

[88] T. Kiffer-Moreira, A. A. De Sá Pinheiro, W. S. Alviano et al., "An ectophosphatase activity in *Candida parapsilosis* influences the interaction of fungi with epithelial cells," *FEMS Yeast Research*, vol. 7, no. 4, pp. 621–628, 2007.

[89] M. Gottlieb and D. M. Dwyer, "Protozoan parasite of humans: surface membrane with externally disposed acid phosphatase," *Science*, vol. 212, no. 4497, pp. 939–941, 1981.

[90] M. Givskov, L. Eberl, and S. Molin, "Responses to nutrient starvation in *Pseudomonas putida* KT2442: two- dimensional electrophoretic analysis of starvation- and stress-induced proteins," *Journal of Bacteriology*, vol. 176, no. 16, pp. 4816–4824, 1994.

[91] K. Leopold, S. Jacobsen, and O. Nybroe, "A phosphate-starvation-inducible outer-membrane protein of *Pseudomonas fluorescens* Ag1 as an immunological phosphate-starvation marker," *Microbiology*, vol. 143, no. 3, pp. 1019–1027, 1997.

[92] N. Ogawa, J. DeRisi, and P. O. Brown, "New components of a system for phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae* revealed by genomic expression analysis," *Molecular Biology of the Cell*, vol. 11, no. 12, pp. 4309–4321, 2000.

[93] J. C. Del Pozo, I. Allona, V. Rubio et al., "A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilising/oxidative stress conditions," *Plant Journal*, vol. 19, no. 5, pp. 579–589, 1999.