Full Paper

PHO8 gene coding alkaline phosphatase of Saccharomyces cerevisiae is involved in polyphosphate metabolism

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Introduction

Inorganic polyphosphates (polyP) are linear chains of 10–1,000 phosphate (Pi) residues linked by high-energy phosphoanhydride bonds. In eukaryotic organisms, polyP has been shown to be localized in various organelles, such as vacuoles (Allan and Miller, 1980), on the cell surfaces of yeasts (Tijssen et al., 1982), and in acidocalcisomes (Marchesini et al., 2002; Rodrigues et al., 2002; Ruiz et al., 2001a, b). It was reported that vacuolar polyphosphates are probably the natural substrate of alkaline phosphatase in Candida utilis (Fernandez et al., 1981). The purpose of this study was to confirm the relationship between alkaline phosphatase (ALP) and polyP metabolism in arbuscular mycorrhizal fungi. Our previous study suggests that a correlation exists between the amount of polyP and detectable ALP activity in arbuscular mycorrhizal (AM) fungi (Funamoto et al., 2007), which are obligate symbiotic microorganisms belonging to Glomeromycota (Schüßler et al., 2001) and which form associations with plant roots in a host nonspecific manner.

AM fungi absorb Pi from the soil via extraradical hyphae. Absorbed Pi is then converted to polyP and translocates to arbuscules formed in the cortical cells of the plant root (Smith and Read, 1996). Enzymatic-histochemical experiments showed that ALP activity were detected in arbuscules (Ezawa et al., 1995; Gianinazzi et al., 1979; Tisserant et al., 1993). Additionally, it has been speculated that hydrolyzation of polyP occurs in arbuscules. ALP has occasionally been considered to be involved in polyP metabolism. However, analysis using the ALP specific inhibitor, Be2+, suggests that ALP of AM fungi has a high substrate specificity for sugar-phosphates, such as glucose-6-Pi and trehalose-6-Pi (Ezawa et al., 1999). Previously, our studies have demonstrated that ALP activity was high in arbuscules and polyP accumulation was low (Funamoto et al., 2007). Furthermore, when the expression of the AM-inducible Pi transporter gene of host plants was suppressed, the expression of AM ALP gene (GiALP) (Aono et al., 2004), which bears a high similarity to the Pi-deficient-induced type ALP gene (PHO8) of S. cerevisiae, was suppressed, and the ALP activity decreased and polyP accumulated in mature arbuscules (Funamoto et al., 2007). These results point to the hypothesis that ALP may play some role in polyP metabolism in arbuscules. However, it is difficult to obtain direct evidence to verify this hypothesis because conventional molecular biology methods, such as transformation and gene disruption, cannot be used in the case of AM fungi.
**S. cerevisiae** has two types of ALPs. One is a non-specific ALP enzyme encoded by the *PHO8* gene and is a membrane-bound vacuolar protein (Clark et al., 1982; Kaneko et al., 1987; Klionsky and Emr, 1989). The other is a specific ALP encoded by the *PHO13* gene (Kaneko et al., 1989). *PHO8* transcription increases as a consequence of Pi starvation (Kaneko et al., 1985; Oshima, 1997), whereas *PHO13* is constitutively expressed, independent of the Pi concentration (Kaneko et al., 1989). Pho8p cleaves diverse substrates in order to liberate Pi from intracellular products. It has been suggested that the most important substrate for Pho8p is fructose-2,6-bisphosphate (Plankert et al., 1991). Some phosphopeptides are also known to be substrates for Pho8p (Donella-Deana et al., 1993). Pho13p demonstrated significant hydrolytic activity only against *p*-nitrophenylphosphate, phosphorylated histone II-A, and casein. It has been speculated that the physiological role of *PHO13* might involve reversible protein phosphorylation (Tuleva et al., 1998).

Two genes, *PPX1* and *PPN1*, encoding polyP-metabolizing enzymes, have been identified in yeast. *PPX1* encodes exopolyphosphatase (PPX) that can hydrolyze the end of the polyP chain (Wurst et al., 1995). PPX, encoded by the *PPX1* gene, is localized in the cytosol and soluble mitochondrial fraction, but PPX found in the nuclei, vacuoles, and mitochondrial membranes are not encoded by this gene (Lichko et al., 2003). *PPN1* (*PHM5*) encodes a bi-functional enzyme acting as an endopolyphosphatase (PPN) that can catalyze the internal cleavage of polyP to progressively shorter lengths (Kumble and Kornberg, 1996; Ogawa et al., 2000; Sethuraman et al., 2001) and as a PPX (Shi and Kornberg, 2005).

In this study, we have conducted comparisons of the polyP content and chain length between the *pho8* deletion (Δpho8) mutant and the wild type (WT) in order to confirm that ALP, encoded by the *PHO8* gene, is involved in polyP metabolism in *S. cerevisiae*.

### Materials and Methods

**Strains and culture conditions.** The yeast strains we used were the wild-type strain, BY4741 (MATa his3 leu2 met15 ura3) (Brachmann et al., 1998), and the pho8 deletion mutant, BY4741Δpho8, which were both purchased from ATCC (Manassas, VA, USA). These strains were grown at 30°C under aerobic conditions in a YPD medium (1% yeast extract, 2% peptone and 2% glucose) or a low Pi YPD medium (YPD (-Pi)). The YPD (-Pi) medium was made according to a previously established method (Werner et al., 2005), which was based on the method of Kaneko et al. (1982). Growth was measured by determining the optical density at 600 nm (OD<sub>600</sub>). When sampling cells, 1.0 OD<sub>600</sub> unit cells (1 OD<sub>600</sub> unit is defined as the number of cells that give an OD<sub>600</sub> of 1 in 1 ml) were collected by centrifugation at 4°C and washed twice with ice-cold distilled water for polyP analysis, or with ice-cold 25 mM Tris-HCl (pH 8.5) containing 10 mM MgSO<sub>4</sub>, for ALP activity analysis. The cells were collected by centrifugation again and frozen in liquid nitrogen and stored at −80°C until further use.

**Extraction of polyP.** A variety of methods to extract polyP from *S. cerevisiae* have been proposed, as reviewed by Werner et al. (2005). One of the popular methods is cell disruption with glass beads in a slightly alkaline buffer followed by phenol/chloroform, chloroform, and ether extraction (Sethuraman et al., 2001). To simplify this method, we omitted the glass bead disruption, and directly treated cells with chloroform followed by slightly alkaline buffer extraction. The extraction efficiency of this method is not significantly different from, and possibly even higher than, that of the glass bead method (data not shown). The details of this method are as follows. Two hundred μL of chloroform was added to the frozen cells and mixed vigorously by vortexing. Chloroform was removed by decomposition with centrifugation, the cells were then suspended in 1 mL of 50 mM Tris-HCl (pH 8.0), and centrifuged at 10,000 g for 5 min at 4°C. A portion of the supernatant was subsequently used for polyP purification and the other portion was used to quantify proteins.

**Purification of polyP.** Extracted polyP was purified with an ion-change column (Vivapure Mini D&Q spin columns, VIVASCIENCE, USA) and equalized with 50 mM Tris-HCl (pH 8.0). Four hundred μL of polyP extracted solution was loaded onto the column and centrifuged at 2,000 g for 1 min. The column was washed twice with 50 mM Tris-HCl (pH 8.0) and extracted with 400 μL of 50 mM Tris-HCl (pH 8.0) containing 0.5 M NaCl.

**Quantification of polyP.** PolyP was quantified by a PPX assay (Wurst et al., 1995) with the following modifications using recombinant *S. cerevisiae* PPX prepared by Ohtomo et al. (2008). Sixty μL of purified polyP solution was added to 120 μL of reaction solution containing 20 mM Tris-HCl (pH 7.5), 5 mM magnesium acetate, 50 mM ammonium acetate, 0.2% BSA, and 5000 units PPX, and incubated for 60 min at 37°C. The reaction was stopped by incubation for 5 min at 95°C. One hundred μL of PPX-treated solution was added to the mixture of 57 μL of ammonium molybdate in 2 M H<sub>2</sub>SO<sub>4</sub> and 43 μL of 0.76 mM malachite green in 0.35% of polyvinyl alcohol, and incubated for 20 min at 25°C. The released Pi from polyP was measured at 595 nm.

**Measurement of ALP activity.** The frozen cells were resuspended in 100 μL of 25 mM Tris-HCl (pH 8.5) containing 10 mM MgSO<sub>4</sub>, and added to 100 μL of glass beads (diameter 0.5 mm). The cells were disrupted by vortexing (30 s × 6 times) on ice and centrifuged. The supernatant was used to measure ALP activity and quantify the protein. ALP activity was measured spectrophotometrically using *p*-nitrophenyl phosphate (pNPP) as the substrate. Twenty μL aliquots were added to 100 μL of the reaction solution containing 5 mM pNPP and 100 mM Tris-HCl (pH 9.0) and incubated for 30 min at 37°C. The released *p*-nitrophenol from pNPP was measured at 415 nm.

**Quantification of protein.** Proteins were quantified by the Bradford method using a Protein assay kit (Bio-Rad, USA).

**Electrophoresis of polyP.** PolyP were extracted from cells using chloroform. The extracted polyP were precipitated
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by ethanol precipitation, and resuspended with 50 mM Tris-HCl (pH 8.0) to obtain 20 mM (as Pi) polyP solution. Five μL of the concentrated polyP was mixed with loading dye solution (40% sucrose, 0.25% bromo phenol blue, and 0.25% of xylen cyanol) and loaded onto a 15% polyacrylamide gel with 1 × TBE buffer. After electrophoresis, the gel was soaked in 10% acetate-10% methanol for 10 min, stained with 0.5% toluidine blue O-25% methanol-5% glycerol-5% acetate for 15 min, and de-stained with 25% methanol-5% glycerol-5% acetate. The polyP markers used in the electrophoresis were the polyP standards prepared by Ohtomo et al. (2008).

Results

Growth, ALP activity, and polyP content of Δpho8 mutant and WT strains

Growth, ALP activity, and polyP content of Δpho8 mutant strains.

We first investigated the effect of disrupting the pho8 gene with regard to the polyP content of cells grown in Pi-sufficient conditions. Cultures of the Δpho8 mutant and WT strains were grown for 24 h in a YPD medium, then subcultured in a fresh YPD medium. After starting the subculture, the growth and ALP activity of the Δpho8 mutant and WT were observed over time. There were no obvious differences in growth between the WT and the Δpho8 mutant during the logarithmic phase (until 8 h after starting the subculture), although the OD 600 of Δpho8 was higher than that of WT in the stationary phase (Fig. 1a). The ALP activity of the WT increased for 2–6 h after subculture. The ALP activity of the Δpho8 mutant also temporarily increased, but was lower than that of WT (Fig. 1b). The difference in ALP activity between the WT and the Δpho8 mutant were constantly maintained regardless of the growth stage. Under these experimental conditions, the polyP content of the Δpho8 mutant was significantly higher than the WT at 4 h and 6 h after the first subculture of full-grown cells (Fig. 1c).

Next, we analyzed the change in the polyP contents of cells in the logarithmic phase and subcultured in a fresh medium. Δpho8 mutants and WT cells in the stationary

Fig. 1. Growth, ALP activity, and polyP content of WT and Δpho8 mutant strains.

(a)–(c) Cells grown in YPD for 24 h were subcultured in fresh YPD, and growth (a), ALP activity (b), and polyP content (c) of the cells were measured during growth after starting subculture. (d) Cells in a stationary phase were first subcultured in YPD and then subcultured again in YPD at 4 h after the first subculture. The polyP contents of the cells were measured during growth. Open circles denote WT and closed circles denote Δpho8 mutant. The results were normalized with the total protein for ALP activity and polyP content. The values are means ± standard deviations of three replicates.
phase in a YPD medium were first subcultured in a fresh YPD medium. At 4 h after the first subculture, cells were in the logarithmic phase and subcultured again in a fresh YPD medium. As a result, the polyP contents of the Δpho8 mutant were significantly higher than the WT for 4–6 h after the second subculture (Fig. 1d).

Distribution of the polyP chain length of the Δpho8 mutant and WT strains in the logarithmic phase
Cultures of the Δpho8 mutant and WT strains were grown for 24 h in a YPD medium, then subcultured in a fresh YPD medium. At 6 h after starting the subculture, cells were harvested and polyP were extracted from these cells. The extracted polyP was concentrated and 100 nmol (as Pi) of polyP were electrophoresed in each lane on a polyacrylamide gel. This result showed no difference in the distribution of the polyP chain length between the Δpho8 mutant and WT, and the accumulated polyP of both strains were less than 100 Pi residue (Fig. 2). RNAs were also detected in the electrophoresis image, and the signals of RNAs extracted from Δpho8 mutant cells were weaker than those from WT cells (Fig. 2).

The effects of the polyP overplus condition on the polyP content and ALP activity
It has been established that polyP is rapidly accumulated in yeast cells after transfer from a Pi-deficient medium to a Pi-sufficient medium, and this phenomenon has been termed polyP “overplus” (Harold, 1966). To investigate the effect of PHO8 gene disruption on polyP “overplus”, WT and Δpho8 mutants grown overnight in a YPD (-Pi) medium were subcultured in fresh YPD (-Pi) medium and grown for 2 h. Thereafter, cells were supplemented with 10 mM (as Pi) potassium phosphate buffer (pH 5.8). ALP activity and the polyP content of cells were measured immediately, or 2 h after, the addition of Pi. Under overplus growth conditions, the ALP activity of the WT strain decreased, while the ALP activity of the Δpho8 mutant increased (Table 1), suggesting that the ALP activity of Pho8p was repressed, and that of the enzyme other than Pho8p was induced by Pi addition. On the other hand, the amount of the polyP increase 2 h after Pi addition, was not significantly different between the Δpho8 mutant (15 μmol/mg protein) and the WT strain (15 μmol/mg protein) (Table 1).

Discussion
One of the most important characteristics of the PHO8 gene is that their expression is induced by Pi deficiency. The present study focused on the relationship between ALP coded by the PHO8 gene and polyP metabolism in yeast grown under Pi-sufficient conditions. Under the Pi-sufficient conditions, ALP activity by Pho8p was low, but detectable (Fig. 1b), as reported by Kaneko et al. (1982). It has been reported that WT cells in the stationary phase subcultured in a fresh YPD medium increase their polyP content during the logarithmic and early stationary phases, but that the polyP content decreases soon afterwards (Werner et al., 2005). We also found that the polyP contents of WT and Δpho8 mutant strains increased for 2–10 h after subculture of full-grown cells in a fresh YPD medium (Fig. 1c). Under this experimental condition, the polyP content of the Δpho8 mutant was higher than that of WT. Furthermore, when cells in a logarithmic phase were subcultured in a fresh YPD medium, the polyP con-
tent of the Δpho8 mutant was also higher than that of WT (Fig. 1d). Thus, these results suggest that the Δpho8 mutant accumulated much higher polyP levels than WT, regardless of the cell growth conditions for subculture. On the contrary, under overplus conditions, the Δpho8 mutant and WT showed an equivalent increase in polyP content (Table 1). This suggests that ALP has no effect on polyP synthesis under over plus conditions.

Electrophoresis of polyP extracted from Δpho8 mutants and WT in a logarithmic phase demonstrated that there were no differences between the Δpho8 mutant and WT on the distribution of polyP chain length and that the accumulated polyP length was less than 100 (Fig. 2). It should be noted that a method to extract longer chain polyP from yeast has been developed (Vagabov et al., 2008) and such longer polyP might not be extracted by the method used in this study. The extracted polyP from Δpho8 mutants and WT were precipitated by ethanol and the same amounts of polyP from both strains were loaded. Consequently, equivalent signals of polyP from both strains were detected. On the contrary, the RNA signal from the Δpho8 mutant was weaker than that of WT. This result suggests that the Δpho8 mutant had much more polyP per RNA than wild type, which supports the quantitative test data for polyP using the enzymatic method, as shown in Fig. 1. The results suggest that ALP encoded by PHO8 gene affects the polyP content, but not the chain length, although there remains the possibility that longer chain polyP, which could not be extracted by the present method, might be affected by ALP. Additionally, ALP encoded by PHO8 gene participates in polyP homeostasis under normal culture conditions in a YPD medium.

Ppx1p and Ppn1p are the most well-known enzymes involved in polyP metabolism in yeast. The activity of Ppx1p under Pi-sufficient conditions are lower than those under Pi deficient conditions (Kulakovskaya et al., 2004). Even under Pi-sufficient conditions, the Δppx1 mutant in the early logarithmical phase accumulated six times the amount of polyP compared to WT, although polyP lengths were not affected (Sethuraman et al., 2001). The activity of PPN1 under Pi-sufficient conditions was also low during a logarithmical phase (Sethuraman et al., 2001; Shi and Kornberg, 2005), but the Δppn1 mutant accumulated long-chained-polyP in vacuoles 2.5 times higher than WT (Lichko et al., 2006). Our present study shows that ALP, encoded by PHO8, affects polyP accumulation in the logarithmical phase under Pi-sufficient conditions, albeit ALP activity is low. These data suggest that polyP-related enzymes could have important roles in the homeostasis of polyP metabolism under Pi-sufficient conditions.

It was reported that ALP, encoded by a PHO8 gene, had substrate specificity for sugar phosphates, such as glucose-6-phosphate, fructose-2,6-bisphosphate, fructose 1,6-diphosphate (Plankert et al., 1991; Toh-e et al., 1976), phosphoprotein, and phosphopeptide (Donella-Deana et al., 1993). In S. cerevisiae, it is thought that polyP synthesis requires energy generated from glycolysis because polyP accumulation was affected by sugar concentration in the medium (Werner et al., 2005). It was also reported that fructose-2,6-bisphosphate 6-phosphatase, which controls glycolysis, was encoded by the PHO8 gene (Plankert et al., 1991). Thus, the possibility remains that Pho8p links polyP metabolism with glycolysis.

Our previous and present studies suggest that ALP is involved in polyP metabolism in lower eukaryotes, including AM fungi and yeast. However, AM fungi and yeast are phylogenetically distant, and the experiments on the relationship between ALP and polyP have not been carried out using organisms other than AM fungi and yeast. In the future, it will be necessary to investigate how ALP is involved in polyP metabolism in a variety of lower eukaryotes.

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References

Allan, R. A. and Miller, J. J. (1980) Influence of S-adenosylmethionine on DAPI-induced fluorescence of polyphosphate in the yeast vacuole. Can. J. Microbiol., 26, 912–920.
Aono, T., Maldonado-Mendoza, I., Dewbre, G., Harrison, M., and Saito, M. (2004) Expression of alkaline phosphatase genes in arbuscular mycorrhizas. New Phytol., 162, 525–534.
Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J. et al. (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast, 14, 115–132.
Clark, D. W., Tkacz, J. S., and Lampen, J. O. (1982) Asparagine-linked carbohydrate does not determine the cellular location of yeast vacuolar nonspecific alkaline phosphatase. J. Bacteriol., 152, 865–873.
Donella-Deana, A., Ostojic, S., Pinna, L. A., and Barbaric, S. (1993) Specific dephosphorylation of phosphopeptides by the yeast alkaline phosphatase encoded by PHO8 gene. Biochim. Biophys. Acta, 1177, 221–228.
Ezawa, T., Saito, M., and Yoshida, T. (1995) Comparison of phosphatase localization in the intraradical hyphae of arbuscular mycorrhizal fungi, Glomus spp. and Gigaspora spp. Plant and Soil, 176, 57–63.
Ezawa, T., Kuwahara, S. Y., Sakamoto, K., Yoshida, T., and Saito, M. (1999) Specific inhibitor and substrate specificity of alkaline phosphatase expressed in the symbiotic phase of the arbuscular mycorrhizal fungus, Glomus etunicatum. Mycologia, 91, 636.
Fernandez, M. P., Gascon, S., and Schwencke, J. (1981) Some enzymatic properties of vacuolar alkaline phosphatase from yeast. Curr. Microbiol., 6, 121–126.
Funamoto, R., Saito, H., Oyaizu, H., Saito, M., and Aono, T. (2007) Simultaneous in situ detection of alkaline phosphatase activity and polyphosphate in arbuscules within arbuscular mycorrhizal roots. Func. Plant Biol., 34, 803–810.
Gianinazzi, S., Gianinazzi-Pearson, V., and Dexeheimer, J. (1979) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by Glomus mosseae (Nicol. & Gerdz.). New Phytol., 82, 127–132.
Harold, F. M. (1966) Inorganic polyphosphates in biology: structure, metabolism, and function. Bacteriol. Rev., 30, 772–794.
Kaneko, Y., Toh-e, A., and Oshima, Y. (1982) Identification of the genetic locus for the structural gene and a new regulatory gene for the synthesis of repressible alkaline phosphatase in Saccharomyces cerevisiae. Mol. Cell Biol., 2, 127–137.
Kaneko, Y., Tamai, Y., Toh-e, A., and Oshima, Y. (1985) Transcriptional and post-transcriptional control of PHO8 expression by PHO regulatory genes in Saccharomyces cerevisiae. Mol. Cell Biol., 5, 248–252.
Kaneko, Y., Hayashi, N., Toh-e, A., Banno, I., and Oshima, Y. (1987) Structural characteristics of the PHO8 gene encoding repressible alkaline phosphatase in Saccharomyces cerevisiae. Gene, 58, 137–148.
Kaneko, Y., Toh-e, A., Banno, I., and Oshima, Y. (1989) Molecular char-
acetylation of a specific p-nitrophenylphosphatase gene, PHO13, and its mapping by chromosome fragmentation in Saccharomyces cerevisiae. Mol. Gen. Genet., 220, 133–139.

Klionsky, D. J. and Emr, S. D. (1989) Membrane protein sorting: biosynthesis, transport and processing of yeast vacuolar alkaline phosphatase. EMBO J., 8, 2241–2250.

Kulakovskaya, T. V., Andreeva, N. A., Trilisenko, L. V., Vagabov, V. M., and Kulaev, I. S. (2004) Two exopolyphosphatases in Saccharomyces cerevisiae cytosol at different culture conditions. Process Biochem., 39, 1625–1630.

Kumble, K. D. and Kornberg, A. (1996) Endopolyphosphatases for long chain inorganic polyphosphate in yeast and mammals. J. Biol. Chem., 271, 27146–27151.

Lichko, L. P., Andreeva, N. A., Kulakovskaya, T. V., and Kulaev, I. S. (2003) Exopolyphosphatases of the yeast Saccharomyces cerevisiae. FEMS Yeast Res., 3, 233–238.

Lichko, L. P., Kulakovskaya, T. V., Pestov, N. A., and Kulaev, I. S. (2006) Inactivation of the PPN1 gene exerts different effects on the metabolism of inorganic polyphosphates in the cytosol and the vacuoles of the yeast Saccharomyces cerevisiae. Microbiology, 75, 253–258.

Marchesini, N., Ruiz, F. A., Vieira, M., and Docampo, R. (2002) Acidocalcisomes are functionally linked to the contractile vacuole of Dictyostelium discoideum. J. Biol. Chem., 277, 8146–8153.

Ogawa, N., DeRisi, J., and Brown, P. O. (2000) New components of a system for phosphate accumulation and polyphosphate metabolism in Saccharomyces cerevisiae revealed by genomic expression analysis. Mol. Biol. Cell, 11, 4309–4321.

Ohtomo, R., Sekiguchi, Y., Kojima, T., and Saito, M. (2008) Different chain length specificity among three polyphosphate quantification methods. Anal. Biochem., 383, 210–216.

Oshima, Y. (1997) The phosphatase system in Saccharomyces cerevisiae. Genes Genet. Syst., 72, 323–334.

Plankert, U., Purwin, C., and Holzer, H. (1991) Yeast fructose-2,6-bisphosphate 6-phosphatase is encoded by PHO8, the gene for non-specific repressible alkaline phosphatase. Eur. J. Biochem., 196, 191–196.

Rodrigues, C. O., Ruiz, F. A., Rohloff, P., Scott, D. A., and Moreno, S. N. I. (2002) Characterization of isolated acidocalcisomes from Toxoplasma gondii tachyzoites reveals a novel pool of hydrolyzable polyphosphate. J. Biol. Chem., 277, 48650–48656.

Ruiz, F. A., Marchesini, N., Seufferheld, M., Govindjee, and Docampo, R. (2001a) The polyphosphate bodies of Chlamydomonas reinhardtii possess a proton-pumping pyrophosphatase and are similar to acidocalcisomes. J. Biol. Chem., 276, 46196–46203.

Ruiz, F. A., Rodrigues, C. O., and Docampo, R. (2001b) Rapid changes in polyphosphate content within acidocalcisomes in response to cell growth, differentiation, and environmental stress in Trypanosoma cruzi. J. Biol. Chem., 276, 26114–26121.

Schüßler, A., Schwarztott, D., and Walker, C. (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res., 105, 1413–1421.

Sethuraman, A., Rao, N. N., and Kornberg, A. (2001) The endopolyphosphatase gene: essential in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA, 98, 8542–8547.

Shi, X. and Kornberg, A. (2005) Endopolyphosphatase in Saccharomyces cerevisiae undergoes post-translational activations to produce short-chain polyphosphates. FEBS Lett., 579, 2014–2018.

Smith, S. E. and Read, D. J. (1996) Mycorrhizal Symbiosis, 2nd ed., Academic Press.

Tijssen, J. P., Beekes, H. W., and Van Steveninck, J. (1982) Localization of polyphosphates in Saccharomyces fragilis, as revealed by 4′,6-diamidino-2-phenylindole fluorescence. Biochim. Biophys. Acta, 721, 394–398.

Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S., and Gollotte, A. (1993) In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycol. Res., 97, 245–250.

Toh-e, A., Nakamura, H., and Oshima, Y. (1976) A gene controlling the synthesis of non specific alkaline phosphatase in Saccharomyces cerevisiae. Biochim. Biophys. Acta, 428, 182–192.

Tuleva, B., Vasileva-Tonkova, E., and Galabova, D. (1998) A specific alkaline phosphatase from Saccharomyces cerevisiae with protein phosphatase activity. FEMS Microbiol. Lett., 161, 139–144.

Vagabov, V. M., Trilisenko, L. V., Kulakovskaya, T. V., and Kulaev, I. S. (2008) Effect of a carbon source on polyphosphate accumulation in Saccharomyces cerevisiae. FEMS Yeast Res., 8, 877–882.

Werner, T. P., Amrhein, N., and Freimoser, M. F. (2005) Novel method for the quantification of inorganic polyphosphate (iPoP) in Saccharomyces cerevisiae. Arch. Microbiol., 184, 129–136.

Wurst, H., Shiba, T., and Kornberg, A. (1995) The gene for a major exopolyphosphatase of Saccharomyces cerevisiae. J. Bacteriol., 177, 898–906.