Enzymes of yeast polyphosphate metabolism: structure, enzymology and biological roles

Rūta Gerasimaitė and Andreas Mayer

Department of Biochemistry, University of Lausanne, Ch. des Boveresses 155, 1066 Epalinges, Switzerland

Corresponding author: Andreas Mayer

Keywords: inorganic polyphosphate, exopolyphosphatase, endopolyphosphatase, polyP polymerase, VTC complex, yeast vacuole

Abstract
Inorganic polyphosphate (polyP) is found in all living organisms. The known polyP functions in eukaryotes range from osmoregulation and virulence in parasitic protozoa to modulating blood coagulation, inflammation, bone mineralization and cellular signaling in mammals. However mechanisms of regulation and even the identity of involved proteins in many cases remain obscure. Most of the insights obtained so far stem from studies in the yeast *Saccharomyces cerevisiae.* Here, we provide a short overview of the properties and functions of known yeast polyP metabolism enzymes and discuss future directions for polyP research.

Introduction

Inorganic polyphosphate (polyP) is found in all kingdoms of life. Due to its simple chemical structure, the possibility to be synthesized abiotically, and its ubiquitous presence, polyP has been declared a "molecular" fossil, a relic of the ancient prebiotic world, which is mostly exploited by freely-living microorganisms for phosphate storage and metal chelation [1]. However, during the past several years, many exciting functions of cellular polyP in the mammals have been described. PolyP regulates bone calcification and is involved in activating the blood clotting cascade, inflammatory responses and various events of cellular signalling [2-5]. Understanding the molecular mechanisms of eukaryotic polyP synthesis and degradation appears crucial for further characterization of these intriguing activities of polyP. Although *Dictyostelium discoideum* has adopted bacterial polyphosphate kinases, probably by horizontal gene transfer, the enzymes of polyP metabolism seem not to be generally conserved between prokaryotes and eukaryotes [6]. Whereas much has been learned about bacterial polyP metabolism, our understanding of eukaryotic polyP metabolism is lagging far behind. This is best illustrated by the facts that only one type of a potential specialized polyphosphatase has been identified in mammals [7], and the role of unspecific mammalian alkaline phosphatase in polyP metabolism is still not clear [8, 9]. Most of the insights obtained so far stem from the studies in the yeast *Saccharomyces cerevisiae*, but also here the nature of some polyP-related enzyme activities has remained enigmatic. This mini-review presents a short summary of the known yeast enzymes, providing a glimpse on the high complexity of eukaryotic polyP metabolism (Figure 1, Table 1).

The VTC complex – a eukaryotic polyP polymerase

A hallmark of polyP metabolism in all eukaryotes is the sequestration of polyP in specialized membrane-enclosed compartments – acidocalcisomes or acidocalcisome-like organelles [10]. The discovery and characterization of the yeast polyP polymerase has provided insights into the mechanisms underlying this process.

The yeast polyP polymerase is a large membrane complex that has originally been designated as VTC (vacuolar transporter chaperone) [11]. Before its catalytic activity as a polyP polymerase had been established, the VTC complex was found to be required for V-ATPase stability and trafficking, yeast vacuole membrane fusion and microautophagy [11-14]. It remains to be determined whether these observations reflect the involvement of polyP in these processes, or whether the VTC complex itself performs mechanistically different tasks, as it has been demonstrated for V-ATPase [15, 16].

The VTC complex consists of four subunits: Vtc4 (catalytic) and Vtc1, Vtc2 and Vtc3 (accessory). Each subunit contains three transmembrane helixes of related sequence. Vtc1 is a small membrane protein without hydrophilic domains. Vtc2, Vtc3 and Vtc4 have two hydrophilic N-terminal domains that face the cytosol [13]: N-terminal SPX-domain and the central domain. Most proteins containing SPX domains are implicated in phosphate metabolism, which led to the proposal that SPX domains might regulate phosphate homeostasis (reviewed in [17]). In several examples, SPX domains mediate protein-protein interactions [18-21]. The central domain of Vtc2 has been crystalized and structures of the Vtc4 catalytic domain have been resolved in complex with the reaction product polyP; with phosphate; with pyrophosphate; and with the substrate analogue adenosine-5'-[(β,γ)-imido] triphosphate and $MnCl_2$ [22]. This allowed the identification of residues important for substrate binding and catalysis. The polyP is bound inside the tunnel of Vtc4, which is lined by conserved basic residues and contains the active site. The central domains of VTC proteins are structurally-related to the RNA triphosphatase Cet1.

The recombinant central (tunnel) domain of Vtc4 is catalytically active and synthesizes polyP from ATP. This catalytic activity requires metal ions, with Mn^{2+} being the most effective. It is highly stimulated by pyrophosphate (PP_i) and moderately stimulated by inorganic phosphate (P_i) and triphosphate (P_3). It has been hypothesized that PP_i might provide the primer to start the new polyP chain. The catalytic domain has very similar affinities to ATP and dATP, and the affinities to CTP and GTP are only 5-fold lower. In the crystal structure, the nucleoside part is not resolved and the

protein contacts only the triphosphate part of the substrate trinucleotide [22]. It would be interesting to know whether the intact VTC complex has a higher selectivity for ATP.
Recently, we have developed a method to monitor polyP synthesis activity by intact VTC complex using isolated vacuoles as a model system [23]. The VTC complex displays significantly higher activity when present in an intact vacuolar membrane than in the detergent-solubilized state, even though all the subunits can be co-immunoprecipitated, indicating that the complex survives the treatment. PolyP synthesis is tightly coupled to its transport into the vacuole lumen and it depends on the proton gradient across the membrane, formed by V-ATPase. As the sequestration of polyP in acidocalcisome-like organelles is a common feature of all eukaryotes, these basic properties of polyP synthesis and translocation might be similar in higher eukaryotes, even if primary sequence comparisons could not identify direct VTC homologs in plants or animals.
VTC exists as two different sub-complexes: Vtc4/Vtc3/Vtc1 and Vtc4/Vtc2/Vtc1 (Figure 1). The first sub-complex is mostly found on the vacuole membrane; the second one can also be observed in the cell periphery (likely, ER and nuclear envelope), but localizes to the vacuole under phosphate starvation. Existence of two differently regulated sub-complexes may be necessary to create differently regulated polyP pools with presumably different functions. In line with this, polyP may not only be concentrated in the acidocalcisome-like vacuole. A distinct polyP pool in the nucleus has recently been discovered that controls the localization and activity of topoisomerase 1 via polyphosphorylation [24]. The existence of polyP in mitochondria has also been described [25].
Lys458, which is essential for catalysis in Vtc4, is substituted by isoleucine in Vtc2 and by leucine in Vtc3 [22]. Consistently, no catalytic activity has been detected for Vtc2 and Vtc3 in vitro, suggesting a regulatory role for these two subunits. A potential regulatory role of Vtc2 and Vtc3 is also suggested by the fact that only few phosphorylation sites were found in Vtc4, whereas Vtc2 and Vtc3 contain numerous phosphorylated serines and threonines (Phosphogrid). All VTC subunits contain ubiquitylation sites. Substitution of the conserved arginines in Vtc1 drastically reduces polyP synthesis activity of the VTC complex. It has been proposed that the small, membrane-integral Vtc1, together with the transmembrane domains of other VTC proteins, forms a channel that transfers polyP into the organelle lumen [22, 23]. Interestingly, Vtc1 was also found in a screen for RNA binders [26] but it remains to be determined whether this is related to any physiological function.
While the sequences of the transmembrane and central domains of VTC proteins are not conserved in the multicellular organisms, SPX domains are found also in plants, flies and mammals. Vtc1 and Vtc4 proteins are also detected in trypanosomatids. The central domains of Vtc4 homologs from *Trypanosoma brucei* and *Trypanosoma cruzi* were purified and their catalytic activities were confirmed [27, 28]. Vtc4 is essential for the infectivity of *Trypanosoma brucei* and was proposed as a potential drug target. Two Homologs of VTC proteins have been identified in the phytoplankton diatom *Thalassiosira pseudonana*, where these genes were up-regulated under phosphate limitation, leading to an increase in polyP synthesis. This finding opens the way to better understand the phosphate cycle in marine ecosystems [29]. A Vtc1 homolog in the green algae *Chlamydomonas reindhardii* is important for acidocalcisome formation and for trafficking of S- and N-responsive periplasmic proteins under sulphur-deprivation [30]. The homolog of the catalytic subunit Vtc4 has also been identified, but its functions were not characterized.

The exopolyphosphatase Ppx1

The major polyphosphatase activity detectable in yeast is provided by Ppx1 [31]. Ppx1 is a soluble monomeric 40 kDa enzyme which processively hydrolyses polyP to release P_i and PP_i [31]. For optimal substrates of an average chain length of 250 P_i residues the k_{cat}/K_M approaches a diffusion-limited value. Ppx1 can hydrolyse PolyP chains as short as 3 P_i residues and does not act on PP_i and ATP. Its activity requires metal ions and prefers Mg^{2+}. The enzyme is active over a wide range of pH from 5.5 to 9. [32].
Ppx1 belongs to the DHH phosphoesterase superfamily, which includes also h-prune, the only mammalian exopolyphosphatase known to date, and the family 2 inorganic pyrophosphatases [7]. The active site of Ppx1 is structurally very similar to the active site of pyrophosphatases and is

located in a channel at the interface between two domains. Several positively charged residues, which are conserved between Ppx1 enzymes from different organisms, line the channel walls and are proposed to drive the polyP chain towards the active site [33].

Unlike most of the genes involved in yeast polyP metabolism, Ppx1 is expressed constitutively, independently from phosphate availability [34]. Deletion of Ppx1 shows little phenotypes and affects neither polyP chain length nor total polyP amount in the cell. Ppx1 was originally described as a cytosolic enzyme. Later, the exopolyphosphatase activities of the plasma membrane and mitochondria matrix were also attributed to Ppx1 [35, 36]. High throughput localisation studies found Ppx1 in cytosol, but also enriched in the nucleus [37]. This localization of the most active yeast polyphosphatase is somewhat unexpected as most of the polyP is stored inside the vacuole [38]. However, a small polyP pool might exist in the cytosol. The alternative function for Ppx1 could be to avoid extensive accumulation of polyP in the cytosol, which is harmful to the cells [23]. A further attractive possibility is that Ppx1 might act on the recently described polyphosphorylated proteins [24].

The endopolyphosphatase Ppn1

Ppn1 is expressed as a precursor peptide of 78 kDa, containing one N-terminal transmembrane helix. It localizes inside the vacuole lumen and depends on vacuolar proteases for activation [39, 40]. Ppn1 reaches the vacuole via the multi-vesicular body pathway, i.e. by ubiquitylation-dependent sorting into the lumenal vesicles of late endosomes [41]. Subsequent fusion of these endosomes with the vacuole transfers these vesicles into the vacuolar lumen where they are degraded and Ppn1 is liberated as a soluble enzyme. Expression of the truncated versions failed to complement *ppn1* deletion, indicating that the N- and C-terminal extensions are required for the activation or correct sorting of the enzyme. Four glycosylation sites are predicted and simultaneous substitution of three of them (Asn 11, 505 and 511) also inactivates the protein [40].

For biochemical characterization Ppn1 was purified from yeast [40, 42]. Its active form is a homo-tetramer, consisting of proteolytically processed monomers of 35 kDa. Ppn1 acts non-processively, preferentially hydrolyses long polyP chains in the middle to release the shorter chains and requires metal (Mn^{2+} or Mg^{2+}) for activity. Reaction end products are P_i and P_3, whereas PP_i is a potent inhibitor [42]. A recent study has revealed that, depending on the reaction conditions, Ppn1 acts preferentially as endo- or exopolyphosphatase and its endopolyphosphatase activity is inhibited by ATP, but activated by ADP [43].

PPN1 expression is induced by phosphate starvation [34]. Deletion of *PPN1* does not strongly influence the levels of polyP, but results in accumulation of longer polyP chains, lower cytosolic P_i and subtle defects of regulation of phosphate-responsive signalling (PHO) pathway [39, 44, 45].

Ddp1

Ddp1 is a 20 kDa enzyme that belongs to the Nudix hydrolase family. Ddp1 can be overexpressed and purified from *E. coli.* It has been characterized as di-adenosine and di-phosphoinositol polyphosphate hydrolase and it is an essential enzyme for the metabolism of inositol pyrophosphates [46, 47]. Unexpectedly, Ddp1 was found to preferentially hydrolyse polyphosphates in vitro, cleaving in the middle of the polyP chain to produce shorter polyP chains [48]. This property of Ddp1 is also shared by its mammalian homologs, DIPP1-3. It is possible that they play a role in the polyP metabolism of mammalian cells. Cells lacking Ddp1 show a 20% reduction of polyP levels. Like Ppx1, Ddp1 is localized in the cytosol and nucleus, where only minor quantities of polyP are found. Whether polyP is a substrate for Ddp1 in vivo it is not clear. However, it is conceivable that it might act on polyphosphorylated proteins [24].

Other yeast polyphosphatases

Polyphosphatase activity has still been detected in the cell extracts of *ppx1*Δ *ppn1*Δ strains [49]. One of the earlier studies detected two vacuolar polyphosphatases [50]. Whereas one of them was likely Ppn1, another one, activated by arginine and independent of vacuolar proteases, has remained unidentified. In a series of subsequent studies, a range of polyphosphatase activities were found in the cytosol, vacuoles, nucleus, mitochondria (membrane and lumen) and the cell envelope [35, 36,

51]. The partially purified enzymes differed by molecular weight and by their sensitivities to inhibitors. Unfortunately, the genes coding for the described activities have not yet been identified, precluding studies of the role of these enzymes. These results suggest that even in yeast not all enzymes of polyP metabolism are known. The advancing bioinformatics methods might facilitate identification of the remaining polyP enzymes in yeast and suggest candidates for the polyP metabolism machinery in higher organisms. A recent study proposed that a positively-charged semi-tunnel might be a common feature of polyP-binding proteins and offered a way to search for enzymes interacting with polyP in the absence of clear sequence homologies [52].

Conclusions and outlook

The number of proteins directly implicated in the synthesis and degradation of polyP in yeast keeps increasing. Vacuolar polyP constitutes the major pool and may be regarded as a simple phosphate storage tool, but we have virtually no information as to how its synthesis and degradation are regulated. This is particularly intriguing because polyphosphatases such as Ppn1 reside in the same compartment as the polyP that they degrade. Since the synthesis and degradation of polyP depend on phosphate availability, we should expect that cellular phosphate concentration is communicated to enzymes metabolizing polyP. Elucidating how this regulation is implemented is a major challenge for the future. A further field holding promises for interesting discoveries concerns the roles of polyP outside its acidocalcisome-like storage organelles, for example in the nucleus, the cytosol or in mitochondria. Recent results have provided fascinating new insights into the role of polyphosphorylation as a post-translational protein modification that can regulate protein-protein interactions in the nucleus. Exploring how these modifications are synthesized and removed may open a completely novel perspective on cellular regulation and signal transduction. Finally, novel roles for secreted polyP are emerging and begin to add interesting twists to the regulation of blood clotting, bone formation and inflammation in mammals, where the systems for polyP synthesis and degradation have remained essentially unexplored. The most exciting period of polyP research lies clearly ahead of us.

References

1. Kornberg, A. (1999) Inorganic polyphosphate: a molecule of many functions. Prog Mol Subcell Biol. **23**, 1-18
2. Hoac, B., Kiffer-Moreira, T., Millan, J. L. and McKee, M. D. (2013) Polyphosphates inhibit extracellular matrix mineralization in MC3T3-E1 osteoblast cultures. Bone. **53**, 478-486
3. Smith, S. A. and Morrissey, J. H. (2014) Polyphosphate: a new player in the field of hemostasis. Curr Opin Hematol. **21**, 388-394
4. Hassanian, S. M., Dinarvand, P., Smith, S. A. and Rezaie, A. R. (2015) Inorganic polyphosphate elicits pro-inflammatory responses through activation of the mammalian target of rapamycin complexes 1 and 2 in vascular endothelial cells. J Thromb Haemost. **13**, 860-871
5. Holmstrom, K. M., Marina, N., Baev, A. Y., Wood, N. W., Gourine, A. V. and Abramov, A. Y. (2013) Signalling properties of inorganic polyphosphate in the mammalian brain. Nat Commun. **4**, 1362
6. Whitehead, M. P., Hooley, P. and MR, W. B. (2013) Horizontal transfer of bacterial polyphosphate kinases to eukaryotes: implications for the ice age and land colonisation. BMC Res Notes. **6**, 221
7. Tammenkoski, M., Koivula, K., Cusanelli, E., Zollo, M., Steegborn, C., Baykov, A. A. and Lahti, R. (2008) Human metastasis regulator protein H-prune is a short-chain exopolyphosphatase. Biochemistry. **47**, 9707-9713
8. Lorenz, B. and Schroder, H. C. (2001) Mammalian intestinal alkaline phosphatase acts as highly active exopolyphosphatase. Biochim Biophys Acta. **1547**, 254-261
9. Omelon, S., Georgiou, J., Variola, F. and Dean, M. N. (2014) Colocation and role of polyphosphates and alkaline phosphatase in apatite biomineralization of elasmobranch tesserae. Acta Biomater. **10**, 3899-3910

10. Docampo, R. and Moreno, S. N. (2011) Acidocalcisomes. Cell Calcium. **50**, 113-119
11. Cohen, A., Perzov, N., Nelson, H. and Nelson, N. (1999) A novel family of yeast chaperons involved in the distribution of V-ATPase and other membrane proteins. J Biol Chem. **274**, 26885-26893
12. Muller, O., Bayer, M. J., Peters, C., Andersen, J. S., Mann, M. and Mayer, A. (2002) The Vtc proteins in vacuole fusion: coupling NSF activity to V(0) trans-complex formation. Embo J. **21**, 259-269
13. Muller, O., Neumann, H., Bayer, M. J. and Mayer, A. (2003) Role of the Vtc proteins in V-ATPase stability and membrane trafficking. J Cell Sci. **116**, 1107-1115
14. Uttenweiler, A., Schwarz, H., Neumann, H. and Mayer, A. (2007) The vacuolar transporter chaperone (VTC) complex is required for microautophagy. Mol Biol Cell. **18**, 166-175
15. Peters, C., Bayer, M. J., Buhler, S., Andersen, J. S., Mann, M. and Mayer, A. (2001) Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. Nature. **409**, 581-588
16. Strasser, B., Iwaszkiewicz, J., Michielin, O. and Mayer, A. (2011) The V-ATPase proteolipid cylinder promotes the lipid-mixing stage of SNARE-dependent fusion of yeast vacuoles. Embo J. **30**, 4126-4141
17. Secco, D., Wang, C., Arpat, B. A., Wang, Z., Poirier, Y., Tyerman, S. D., Wu, P., Shou, H. and Whelan, J. (2012) The emerging importance of the SPX domain-containing proteins in phosphate homeostasis. New Phytol. **193**, 842-851
18. Wang, Z., Ruan, W., Shi, J., Zhang, L., Xiang, D., Yang, C., Li, C., Wu, Z., Liu, Y., Yu, Y., Shou, H., Mo, X., Mao, C. and Wu, P. (2014) Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. Proc Natl Acad Sci U S A. **111**, 14953-14958
19. Puga, M. I., Mateos, I., Charukesi, R., Wang, Z., Franco-Zorrilla, J. M., de Lorenzo, L., Irigoyen, M. L., Masiero, S., Bustos, R., Rodriguez, J., Leyva, A., Rubio, V., Sommer, H. and Paz-Ares, J. (2014) SPX1 is a phosphate-dependent inhibitor of Phosphate Starvation Response 1 in Arabidopsis. Proc Natl Acad Sci U S A. **111**, 14947-14952
20. Hurlimann, H. C., Pinson, B., Stadler-Waibel, M., Zeeman, S. C. and Freimoser, F. M. (2009) The SPX domain of the yeast low-affinity phosphate transporter Pho90 regulates transport activity. EMBO Rep. **10**, 1003-1008
21. Spain, B. H., Koo, D., Ramakrishnan, M., Dzudzor, B. and Colicelli, J. (1995) Truncated forms of a novel yeast protein suppress the lethality of a G protein alpha subunit deficiency by interacting with the beta subunit. J Biol Chem. **270**, 25435-25444
22. Hothorn, M., Neumann, H., Lenherr, E. D., Wehner, M., Rybin, V., Hassa, P. O., Uttenweiler, A., Reinhardt, M., Schmidt, A., Seiler, J., Ladurner, A. G., Herrmann, C., Scheffzek, K. and Mayer, A. (2009) Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. Science. **324**, 513-516
23. Gerasimaite, R., Sharma, S., Desfougeres, Y., Schmidt, A. and Mayer, A. (2014) Coupled synthesis and translocation restrains polyphosphate to acidocalcisome-like vacuoles and prevents its toxicity. J Cell Sci. **127**, 5093-5104
24. Azevedo, C., Livermore, T. and Saiardi, A. (2015) Protein polyphosphorylation of lysine residues by inorganic polyphosphate. Mol Cell. **58**, 71-82
25. Beauvoit, B., Rigoulet, M., Guerin, B. and Canioni, P. (1989) Polyphosphates as a source of high energy phosphates in yeast mitochondria: A 31P NMR study. FEBS Lett. **252**, 17-21
26. Tsvetanova, N. G., Klass, D. M., Salzman, J. and Brown, P. O. (2010) Proteome-wide search reveals unexpected RNA-binding proteins in Saccharomyces cerevisiae. PLoS One. **5**
27. Lander, N., Ulrich, P. N. and Docampo, R. (2013) Trypanosoma brucei vacuolar transporter chaperone 4 (TbVtc4) is an acidocalcisome polyphosphate kinase required for in vivo infection. J Biol Chem. **288**, 34205-34216

28. Ulrich, P. N., Lander, N., Kurup, S. P., Reiss, L., Brewer, J., Soares Medeiros, L. C., Miranda, K. and Docampo, R. (2014) The acidocalcisome vacuolar transporter chaperone 4 catalyzes the synthesis of polyphosphate in insect-stages of Trypanosoma brucei and T. cruzi. J Eukaryot Microbiol. **61**, 155-165
29. Dyhrman, S. T., Jenkins, B. D., Rynearson, T. A., Saito, M. A., Mercier, M. L., Alexander, H., Whitney, L. P., Drzewianowski, A., Bulygin, V. V., Bertrand, E. M., Wu, Z., Benitez-Nelson, C. and Heithoff, A. (2012) The transcriptome and proteome of the diatom Thalassiosira pseudonana reveal a diverse phosphorus stress response. PLoS One. **7**, e33768
30. Aksoy, M., Pootakham, W. and Grossman, A. R. (2014) Critical function of a Chlamydomonas reinhardtii putative polyphosphate polymerase subunit during nutrient deprivation. Plant Cell. **26**, 4214-4229
31. Wurst, H. and Kornberg, A. (1994) A soluble exopolyphosphatase of Saccharomyces cerevisiae. Purification and characterization. J Biol Chem. **269**, 10996-11001
32. Tammenkoski, M., Moiseev, V. M., Lahti, M., Ugochukwu, E., Brondijk, T. H., White, S. A., Lahti, R. and Baykov, A. A. (2007) Kinetic and mutational analyses of the major cytosolic exopolyphosphatase from Saccharomyces cerevisiae. J Biol Chem. **282**, 9302-9311
33. Ugochukwu, E., Lovering, A. L., Mather, O. C., Young, T. W. and White, S. A. (2007) The crystal structure of the cytosolic exopolyphosphatase from Saccharomyces cerevisiae reveals the basis for substrate specificity. J Mol Biol. **371**, 1007-1021
34. 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
35. 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
36. Lichko, L., Kulakovskaya, T., Pestov, N. and Kulaev, I. (2006) Inorganic polyphosphates and exopolyphosphatases in cell compartments of the yeast Saccharomyces cerevisiae under inactivation of PPX1 and PPN1 genes. Biosci Rep. **26**, 45-54
37. Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S. and O'Shea, E. K. (2003) Global analysis of protein localization in budding yeast. Nature. **425**, 686-691
38. Saito, K., Ohtomo, R., Kuga-Uetake, Y., Aono, T. and Saito, M. (2005) Direct labeling of polyphosphate at the ultrastructural level in Saccharomyces cerevisiae by using the affinity of the polyphosphate binding domain of Escherichia coli exopolyphosphatase. Appl Environ Microbiol. **71**, 5692-5701
39. Sethuraman, A., Rao, N. N. and Kornberg, A. (2001) The endopolyphosphatase gene: essential in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. **98**, 8542-8547
40. Shi, X. and Kornberg, A. (2005) Endopolyphosphatase in Saccharomyces cerevisiae undergoes post-translational activations to produce short-chain polyphosphates. FEBS Lett. **579**, 2014-2018
41. Reggiori, F. and Pelham, H. R. (2001) Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. Embo J. **20**, 5176-5186
42. Kumble, K. D. and Kornberg, A. (1996) Endopolyphosphatases for long chain inorganic polyphosphate in yeast and mammals. J Biol Chem. **271**, 27146-27151
43. Andreeva, N., Trilisenko, L., Eldarov, M. and Kulakovskaya, T. (2015) Polyphosphatase PPN1 of Saccharomyces cerevisiae: switching of exopolyphosphatase and endopolyphosphatase activities. PLoS One. **10**, e0119594
44. Auesukaree, C., Homma, T., Tochio, H., Shirakawa, M., Kaneko, Y. and Harashima, S. (2004) Intracellular phosphate serves as a signal for the regulation of the PHO pathway in Saccharomyces cerevisiae. J Biol Chem. **279**, 17289-17294
45. Thomas, M. R. and O'Shea, E. K. (2005) An intracellular phosphate buffer filters transient fluctuations in extracellular phosphate levels. Proc Natl Acad Sci U S A. **102**, 9565-9570

46. Cartwright, J. L. and McLennan, A. G. (1999) The Saccharomyces cerevisiae YOR163w gene encodes a diadenosine 5', 5"'-P1,P6-hexaphosphate (Ap6A) hydrolase member of the MutT motif (Nudix hydrolase) family. J Biol Chem. **274**, 8604-8610
47. Safrany, S. T., Ingram, S. W., Cartwright, J. L., Falck, J. R., McLennan, A. G., Barnes, L. D. and Shears, S. B. (1999) The diadenosine hexaphosphate hydrolases from Schizosaccharomyces pombe and Saccharomyces cerevisiae are homologues of the human diphosphoinositol polyphosphate phosphohydrolase. Overlapping substrate specificities in a MutT-type protein. J Biol Chem. **274**, 21735-21740
48. Lonetti, A., Szijgyarto, Z., Bosch, D., Loss, O., Azevedo, C. and Saiardi, A. (2011) Identification of an evolutionarily conserved family of inorganic polyphosphate endopolyphosphatases. J Biol Chem. **286**, 31966-31974
49. Lichko, L. P., Kulakovskaya, T. V., Kulakovskaya, E. V. and Kulaev, I. S. (2008) Inactivation of PPX1 and PPN1 genes encoding exopolyphosphatases of Saccharomyces cerevisiae does not prevent utilization of polyphosphates as phosphate reserve. Biochemistry (Mosc). **73**, 985-989
50. Wurst, H., Shiba, T. and Kornberg, A. (1995) The gene for a major exopolyphosphatase of Saccharomyces cerevisiae. J Bacteriol. **177**, 898-906
51. Andreeva, N. A. and Okorokov, L. A. (1993) Purification and characterization of highly active and stable polyphosphatase from Saccharomyces cerevisiae cell envelope. Yeast. **9**, 127-139
52. Wei, Z. Z., Vatcher, G., Tin, A. H., Teng, J. L., Wang, J., Cui, Q. H., Chen, J. G. and Yu, A. C. (2015) Positively-charged semi-tunnel is a structural and surface characteristic of polyphosphate-binding proteins: an in-silico study. PLoS One. **10**, e0123713

Figure legends

Figure 1. Schematic representation of yeast enzymes involved in polyP metabolism.
In the yeast cell, polyP is mainly accumulated inside the vacuole, but small pools are found also in the cytosol, nucleus and mitochondria. The VTC complex (yeast polyP polymerase) exists as two different sub-complexes that are localised on the vacuole membrane and in the cell periphery – most likely on the membranes of endoplasmic reticulum (ER) and nuclear envelope. The known enzymes of polyP degradation are indicated: the exopolyphosphatase Ppx1 processively degrades polyP chains by cleaving phosphate residues one-by-one from the end of the chain; the endopolyphosphatases Ppn1 and Ddp1 cleave polyP chains in the middle to produce the shorter chains. Unidentified enzymes for which an activity has been found are indicated by question marks. PP-InsPP – inositol pyrophosphate, the dashed lines indicate the regulation of polyP synthesis by PP-InsP by yet unidentified mechanism.

Tables

Table 1. Yeast enzymes of polyP metabolism and their homologs described in the present review.

Protein	Organism	Function	NCBI gene ID	References
*Sc*Vtc1	*Saccharomyces cerevisiae*	accessory subunit of polyP polymerase	856803	[11, 22]
*Sc*Vtc2	*Saccharomyces cerevisiae*	accessory subunit of polyP polymerase	850544	[11, 22]
*Sc*Vtc3	*Saccharomyces cerevisiae*	accessory subunit of polyP polymerase	856088	[11, 22]
*Sc*Vtc4	*Saccharomyces cerevisiae*	catalytic subunit of polyP polymerase	853441	[11, 22]
*Tb*Vtc4	*Trypanosoma brucei*	catalytic subunit of polyP polymerase	3665506	[27]
*Tc*Vtc4	*Trypanosoma cruzi*	catalytic subunit of polyP polymerase	XM_824191 (Genebank)	[28]
*Cr*Vtc1	*Chlamydomonas reinhardtii*	vacuolar transport chaperone-like protein	Cre12.g510250	[30]
*Tp*Vtc4	*Thalassiosira pseudonana*	vacuolar transport chaperone-like protein	estExt_gwp_gw1.C_chr_240033	[29]
*Sc*Ppx1	*Saccharomyces cerevisiae*	exopolyphosphatase	856608	[31, 50]
h-prune	*Homo sapiens*	exopolyphosphatase	58497	[7]
*Sc*Ppn1	*Saccharomyces cerevisiae*	endopolyphosphatase	852063	[39, 40]
*Sc*Ddp1	*Saccharomyces cerevisiae*	endopolyphosphatase/ diadenosine hexaphosphate hydrolase/ diphosphoinositol polyphosphate phosphohydrolase	854334	[46-48]