PORTLAND PRESS

## Review Article

# Inorganic polyphosphate in mammals: where's Wally?

**Yann Desfougères,** 🔾 **Adolfo Saiardi and** 🔾 **Cristina Azevedo**

Medical Research Council Laboratory for Molecular Cell Biology, University College London, London, U.K.

**Correspondence:** Adolfo Saiardi (dmcbado@ucl.ac.uk)

OPEN ACCESS

Inorganic polyphosphate (polyP) is a ubiquitous polymer of tens to hundreds of ortho-phosphate residues linked by high-energy phosphoanhydride bonds. In prokaryotes and lower eukaryotes, both the presence of polyP and of the biosynthetic pathway that leads to its synthesis are well-documented. However, in mammals, polyP is more elusive. Firstly, the mammalian enzyme responsible for the synthesis of this linear biopolymer is unknown. Secondly, the low sensitivity and specificity of available polyP detection methods make it difficult to confidently ascertain polyP presence in mammalian cells, since in higher eukaryotes, polyP exists in lower amounts than in yeast or bacteria. Despite this, polyP has been given a remarkably large number of functions in mammals. In this review, we discuss some of the proposed functions of polyP in mammals, the limitations of the current detection methods and the urgent need to understand how this polymer is synthesized.

## Introduction

Arthur Kornberg and Igor Kulaev spent a large part of their careers working on inorganic polyphosphate (polyP). Convinced of the fundamental importance of this 'forgotten polymer', they and their teams developed tools to study polyP in a wide range of organisms from bacteria to mammals [1,2]. Today, polyP is recognized as a molecule of many and important functions. The large spectrum of activities in medically relevant contexts have made polyP a highly promising target for therapeutic applications. In recent years, a large number of publications have reported on the diverse effects of exogenously added synthetic polyP to cultured cells. Unfortunately, the complete lack of information on the biosynthetic machinery of mammalian polyP has hampered our understanding of the physiological relevance of this polymer in mammals. The discovery of the human polyP synthase would validate these previous findings and dramatically accelerate polyP research. However, this may be a herculean task considering that to date, more than 70 years after Arthur Kornberg and Igor Kulaev, started working on polyP, no enzyme carrying the ability to synthesize polyP in mammals has been identified.

## The enzymes that metabolize polyP

Despite the presence of polyP in all tested organisms from bacteria to mammals, the enzymology responsible for its synthesis is not conserved. Bacteria have two highly conserved enzymes capable of synthesising polyP, PPK1 and PPK2. PPK1 forms a tetramer, of 80 kDa units with structural similarities to Phospholipase D [3] but without defined domains, that catalyses the reversible transfer of ATP's gamma-phosphate to polyP [4]. PPK2 is a dual activity 40.8 kDa protein that synthesizes polyP from GTP or ATP [5] and can use polyP as a donor to convert GDP to GTP [6]. In *Dictyostelium discoideum*, two PPK activities have been originally reported, one with sequence similarity to the bacterial PPK1 that was acquired through horizontal gene transfer [7], and a novel DdPPK2 activity, that seems to be a complex of three actin-related proteins: Arp1, Arp2, and an unreported Arpx [8]. No genetic data is available confirming DdPPK2 polyP kinase activity. However, in contrast with what

was previously demonstrated [9], we recently reported that *D. discoideum* cells lacking DdPpk1 have no detectable polyP levels demonstrating that this is the sole enzyme responsible for polyP synthesis in social amoeba [10]. In fungi and several other protists, including *Trypanosoma* and *Leishmania*, polyP synthesis is performed by a different enzymatic complex associated with the membrane-integral vacuolar transporter chaperone (VTC) complex [11,12]. Here polyP is synthesized from ATP on the cytosolic face of acidocalcisome-like organelles and translocated into the lumen, a process requiring the proton gradient generated by the V-ATPase [13].

Another set of enzymes is responsible for the hydrolysis of polyP. These proteins can be classified as either endopolyphosphatases, if they degrade polyP by attacking internal phosphoanhydride bonds, or exopolyphosphatases, if they hydrolyse the terminal phosphate residue of the polymer. In bacteria, the exopolyphosphatase Ppx1 is the only characterized polyP hydrolase. This enzyme is conserved in fungi where it prevents the accumulation of cytosolic polyP [13] which, in excess is toxic. Ppx1 is a member of a broader family of phosphoesterases called DHH [14], which includes the mammalian homologue H-Prune [15]. This enzyme shares many similarities with bacterial Ppx1 and *in vitro* assays confirmed that it has strictly exopolyphosphatase activity [16]. Another potent exopolyphosphatase is alkaline phosphatase from calf intestine [17]. Yeast and mammals also possess endopolyphosphatases [18–20]. In *S. cerevisiae*, this arsenal is composed of two vacuolar-localized proteins named Ppn1 and Ppn2, and an additional enzyme localized in the cytosol and the nucleus called Ddp1. Ddp1 is a homologue of human DIPP proteins, members of the Nudix family, that also exhibit endopolyphosphatase activity [18]. Therefore, although no polyP synthase has been identified in mammals, two enzymes able to degrade polyP have been characterized, an exo- and an endopolyphosphatase. Interestingly, there are also proteins, like the NAD kinase, able to use polyP instead of ATP as phosphate donor [21].

## Functions of polyP in mammals

Despite the lack of knowledge of the biosynthetic pathway for polyP synthesis and hence of a direct way to genetically manipulate its levels in mammalian cells, it is remarkable how much is known about its functions, and how diverse these are (Figure 1). Initially detected in platelets [22] its role in blood coagulation and in the inflammatory response was soon established [23]. PolyP regulates several points in the blood-clotting cascade [24,25]. The different actions of polyP in this context seem to depend on the length of the polymer [25], adding unexpected complexity beyond the simplicity of its molecular structure. Therefore, polyP is a strong candidate to develop new therapeutic approaches to modulate blood clotting. Another area of intense research focuses on the role of polyP in bone and cartilage formation. Here again, polyP has been shown to act at different places to strategically promote the regeneration of these tissues [26,27].

Several studies have directly or indirectly demonstrated that polyP is somehow involved in cancer progression. Human MCF-7 breast cancer cells overexpressing the yeast exopolyphosphatase Ppx1 have reduced growth in serum-free media and are deficient in their response to mitogens, as they fail to activate mTOR [28]. The human exopolyphosphatase H-Prune, is often up-regulated in metastatic cancers, and is associated with cancer progression, tumour aggressiveness and advanced disease status in breast and gastric cancer [16]. PolyP has been found in substantially higher levels in myeloma cells compared with normal primary plasma cells, B cells and non-myeloma bone marrow mononuclear cells [29]. Lastly, exogenously-added polyP induces apoptosis specifically in myeloma cells and plasma cells [30].

PolyP has also been identified as a component or a regulator of membrane channels. It associates with the non-selective mitochondria permeability transition pore (mPTP) complex in the inner mitochondrial membrane [31], and with the transient receptor potential channels melastatin (transient receptor potential channel subfamily M, TRPM8) [32] or with TRPA1 (transient receptor potential channel, subfamily A, member 1) [33]. Its association with calcium ($Ca^{2+}$) and the polymer polyhydroxybutyrate (PHB), seems to be sufficient to produce a protein-free channel after insertion into lipid bilayers. This structure would be able to selectively transport calcium. PolyP and PHB have both been found to interact with TRPM8, modulating the activity of this cation channel [32]. The exact role of these associations is not entirely understood: polyP might contribute to ion-conduction through an electrostatic interaction with these proteins, helping to maintain an active conformation for channel gating, or through covalent modification of the protein by polyP (see below). Due to its affinity for calcium, polyP is also believed to buffer calcium availability within mitochondria. Free phosphate is known to play an essential role in binding calcium in the mitochondrial matrix to reduce the free calcium concentration. By artificially tagging the expression of Ppx1 to the mitochondrial matrix, the laboratory of
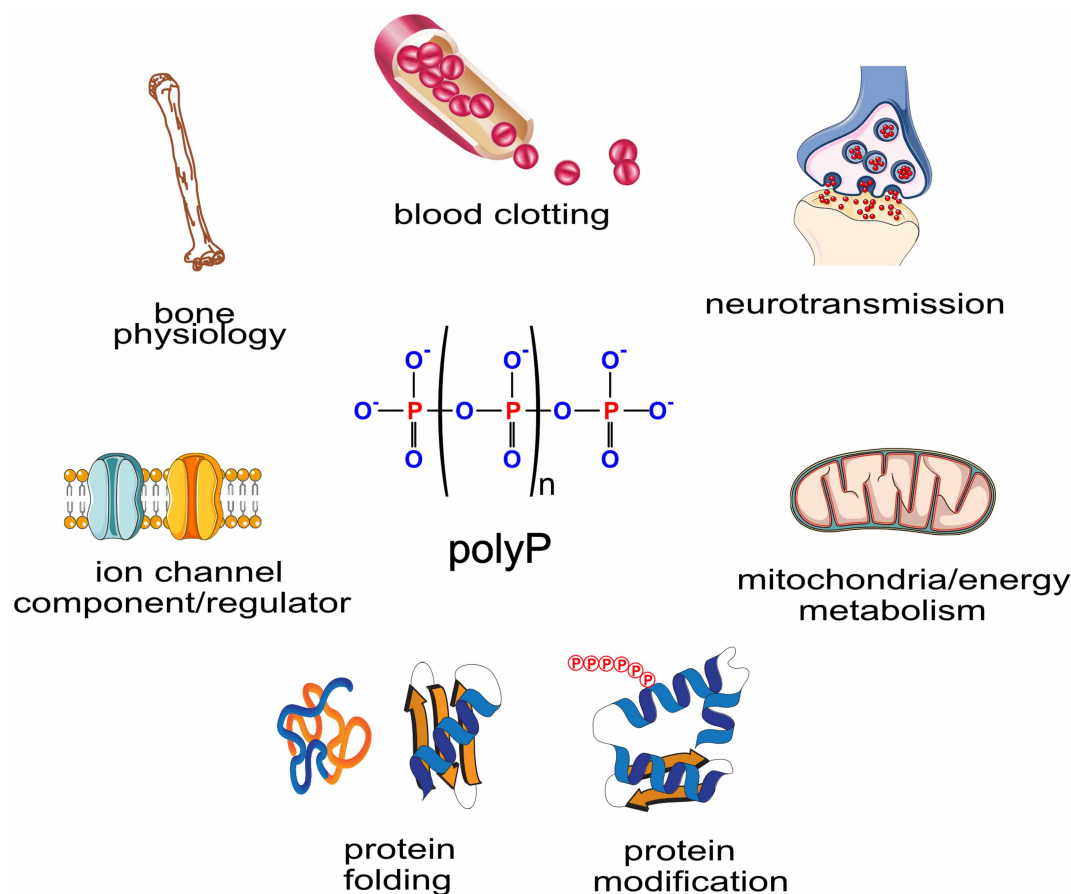
**Figure 1. polyP structure and its attributed functions in mammals.**
The polyP chemical formula is represented centrally, where 'n' can range from 1 to several hundreds. Thus a phosphate chain with at least three phosphate groups can be considered polyP. Surrounding the chemical formula are graphical representations of the different functions attributed to polyP in mammals.

E. Pavlov showed that polyP increases free calcium concentration, supposedly by decreasing the amount of calcium-phosphate precipitates [15]. A further link to mitochondrial function was recently demonstrated showing that polyP synthesis was dependent on the metabolic status of the cell, and that polyP levels correlate with cellular ATP levels [34]. Altogether, these data attribute a central role for polyP in regulating energy metabolism.

PolyP has also been described as a gliotransmitter. Upon calcium stimulation polyP is released from astrocytes (and a small proportion of neurons) and can subsequently be taken up by neurons [35]. While in the intercellular medium, polyP activates P2Y receptors, which stimulate PLC-dependent synthesis of $IP_3$. This ultimately results in the release of calcium from the endoplasmic reticulum. However, the exact role of polyP in neuronal signalling remains unknown.

Recently, further functions have been attributed to polyP. First, the discovery that polyP is a molecular chaperone in bacteria, stimulated the search for a function in pathological protein aggregation [36]. This work, undertaken by Ursula Jakob's laboratory, demonstrated that polyP nucleates fibril formation in Abeta peptide, Tau, alpha-synuclein, and huntingtin proteins involved in protein-folding diseases such as Alzheimer's, Parkinson's and Huntington's [37,38]. This nucleation is thought to decrease the abundance of the soluble toxic intermediate species. Second, our laboratory determined the ability of polyP to covalently modify proteins in a process called polyphosphorylation [39]. This new post-translational modification occurs by attachment of the polymer to a lysine residue, conferring to polyP a novel role in signal transduction. Two genome-wide screens last year identified a number of human proteins susceptible to be polyphosphorylated [40,41].

# Detection and quantification of polyP in mammalian cells and tissues

The high abundance of polyP in micro-organisms facilitates its detection and quantification. Mammalian cells possess much lower amounts of the polymer and more sensitive methods, such as [32P] radiolabelling, are therefore required. In mammals, polyP was first reported in 1963 and its characterization was performed using a set of biochemical assays. Nowadays, besides these classical assays, the semi-quantitative detection of polyP is achieved using microscopy after staining with fluorescently tagged proteins or fluorescent dyes. These approaches are now reviewed and discussed.

## Early biochemical analysis

Early studies on polyP used subcellular fractionation and biochemical assays for precise quantification. After isolation of the organ/organelle, the polyP extraction was usually performed by precipitating the proteins and releasing polyP through boiling on concentrated bases. PolyP would then be further purified by precipitation or separation on affinity columns. The resulting material would be analysed by thin-layer chromatography for its metachromatic potential, or by nuclear magnetic resonance. Quantification was often performed by orthophosphate measurement after hydrolysis in strong acid. Using these methodologies, the presence of polyP in mammalian cells was first reported in 1963 by Lynn and Brown who demonstrated that its synthesis was stimulated in rat liver mitochondria by succinate, a mitochondria swelling agent [42]. In the same year, polyP, of an estimated chain length of 2 to 500 units, was also shown to be present in rat liver nuclei [43]. In rat, the liver was estimated to have 1–2 μg of polyP per gram of tissue [44] whereas an adult brain contains a much higher amount, 15 μg [43]. In bovine, polyP was present in all tissues tested (brain, liver, pancreas, kidney cortex and spleen) with an estimated chain length between 10 to 5000 units [43]. The same study also reported the presence of polyP in rabbit erythrocytes, with the major proportion co-fractionating with membranes, suggesting that the presence of a cell nucleus is not an absolute requirement for the maintenance of a high polyP content. Although the procedures used in these studies paved the way for analysing polyP in mammalian cells, they rely on harsh extraction conditions and a non-specific quantitative assay based on the lability of the phosphoanhydride bond. These crude methods have likely led to a misattribution of the final readout, in the form of free phosphate amount, exclusively to polyP. Although the use of [32P] allows detection of lower polyP amounts, it also requires the use of defined media with low phosphate, a condition that, as demonstrated in micro-organisms, favours degradation of polyP rather than its synthesis. To overcome these drawbacks, other approaches were later developed and optimized: extraction procedures involving acidic phenol or guanidine isothiocyanate, and specific enzymatic assays reducing the need for the polyP extract to be of the highest purity. The bacterial exopolyphosphatase Ppx1 is now routinely used to degrade polyP *in vitro*. The orthophosphate residues released are then quantified by standard assays such as the malachite green. Alternatively, the reverse reaction of the polyphosphate kinase Ppk1, consisting of the transfer of phosphate from polyP to ADP, generates ATP that can be quantified with the sensitive luciferin/luciferase assay. Finally, the use of polyacrylamide gel electrophoresis (PAGE) is critical in the definitive identification of polyP, as it allows the detecting of a ladder like appearance, consequent of the stepwise increases of polyP's polymeric nature. PAGE is also essential for the estimation of the chain length of the polymer. The omission of urea from the PAGE recipe [45] considerably increases the resolution by allowing the use of gels with higher polyacrylamide concentration. PolyP detection within the gel is achieved by staining with the high molecular mass dye Toluidine Blue or the fluorescent dye DAPI. In the past two decades, with these new polyP purification methods and detection technologies, several labs have investigated the subcellular presence of polyP, and its tissue and cell line distribution. PolyP has been detected from mouse fibroblast (NIH 2T3), monkey kidney (Vero), human lymphoma (Jurkat), human embryonal kidney (193/E1A), mouse mammary gland (Mouse L) and rat adrenal phechromocytoma (PC12) cells, rat liver nucleus, plasma membrane, cytosol, mitochondria, microsomes [46], lysosomes of human fibroblasts [47], nucleolus of human myeloma cells [29], and human platelets [22]. For unknown reasons, it seems that the neural polyP is extremely labile to catabolic degradation after death whereas the polyP found in the liver is very stable [43].

## The rise of microscopy

Using microscopy to visualize polyP has several advantages. For one, it does not require the convoluted extraction procedures explained above. It also allows determination of the subcellular localization of the polymer, a difficult task when using classical sub-cellular fractionation approaches and [32P] labelling. The most

widespread dye used to visualize polyP is DAPI, which is better known for its nucleic acid binding properties. However, the ability of DAPI to bind to a large variety of molecules may hamper the detection of low amounts of polyP as in mammalian cells. Recently, more specific fluorescent dyes found to bind polyP (JC-D7 and JC-D8) allowed its visualization in living mammalian cells [48]. However, these compounds also bind to heparin, another negatively charged polymer, with almost equal affinity as polyP. Alternatively, the use of the polyP binding domain of Ppx1 (PPBD) coupled to the Green Fluorescent Protein (GFP) has also been reported [49,50]. Although this procedure requires fixing of the cells prior to incubation with the reporter, it is believed to be more specific towards polyP. Microscopy does not allow for an absolute quantification of the polyP due to heterogeneous nature of this polymer and all the dyes have different affinities for polyP according to the chain length. For most of these procedures using microscopy there is the need to fix the cells which requires extensive washing steps that may remove a substantial amount of polyP from the cells. Although microscopy has become the most popular, and unfortunately sometimes the only, technique used to detect polyP in mammalian cells, the genetic proof to ascertain polyP presence is lacking.

## Beyond observation: the search for a polyP synthase activity

Although initial studies aimed at detecting an enzymatic activity able to synthesize polyP, researchers are now focused on describing the phenotypes induced by simply adding synthetic polyP to the culture medium. The only hint reported of a possible polyP synthase in mammalian cells came from Reusch et al. twenty years ago [51]. The authors suggested that the plasma membrane calcium pump ATP2B1, purified from human erythrocytes, may function as polyP kinase by exhibiting ATP-polyphosphate transferase and polyphosphate-ADP transferase activities. Unfortunately, this old observation lacks further confirmation.

## Perspectives

- PolyP is a ubiquitous molecule with very important and diverse roles in biology, regulating a remarkable breadth of physiological functions that encompass research from haematology to neuroscience.

- The current scientific enterprise is focussed on fast and flashy publications that somehow seems to have forgotten that the mechanism of polyP synthesis in mammals is yet to be identified and characterized. While it is easy to observe the effect of adding polyP to *in vitro* assays or to culture media, these are mere phenomenological observations.

- The discovery of the polyP synthase in mammalian cells will be of instrumental importance to validate all these observations. This will require classical biochemical fractionations and assays likely requiring the use of radiolabelled [$^{32}$P]-ATP to improve sensitivity. Therefore, the quest to find the mammalian polyP synthase is still open and for the health of the research field must be accomplished soon. Only then might we be able to confidently say not only where Wally is, but also what are its actual physiological functions.

## Abbreviations

ATP, adenosine triphosphate; GTP, guanosine triphosphate; polyP, inorganic polyphosphate; PPBD, polyphosphate binding domain; Ppk, polyphosphate kinase; Ppn, endopolyphosphatase; Ppx, exopolyphosphatase; VTC, vacuolar transporter chaperone complex.

## Author Contribution

All authors contributed equally to the organization, writing and editing of the paper.

## Open Access
Open access for this article was enabled by the participation of University College London in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with JISC.

## Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

# References
1 Kulakovskaya, T. and Kulaev, I. (2013) Enzymes of inorganic polyphosphate metabolism. *Prog. Mol. Subcell. Biol.* **54**, 39–63 https://doi.org/10.1007/978-3-642-41004-8_3

2 Kornberg, A. (1995) Inorganic polyphosphate: toward making a forgotten polymer unforgettable. *J. Bacteriol.* **177**, 491–496 https://doi.org/10.1128/JB.177.3.491-496.1995

3 Zhu, Y., Huang, W., Lee, S.S.K. and Xu, W. (2005) Crystal structure of a polyphosphate kinase and its implications for polyphosphate synthesis. *EMBO Rep.* **6**, 681–687 https://doi.org/10.1038/sj.embor.7400448

4 Ahn, K. and Kornberg, A. (1990) Polyphosphate kinase from *escherichia coli*. purification and demonstration of a phosphoenzyme intermediate. *J. Biol. Chem.* **265**, 11734–9 PMID:2164013

5 Zhang, H., Ishige, K. and Kornberg, A. (2002) A polyphosphate kinase (PPK2) widely conserved in bacteria. *Proc. Natl Acad. Sci. U.S.A.* **99**, 16678–16683 https://doi.org/10.1073/pnas.262655199

6 Ishige, K., Zhang, H. and Kornberg, A. (2002) Polyphosphate kinase (PPK2), a potent, polyphosphate-driven generator of GTP. *Proc. Natl Acad. Sci. U.S.A.* **99**, 16684–8 https://doi.org/10.1073/pnas.262655299

7 Tan, S., Fraley, C.D., Zhang, M., Dailidiene, D., Kornberg, A. and Berg, D.E. (2005) Diverse phenotypes resulting from polyphosphate kinase gene (ppk1) inactivation in different strains of *Helicobacter pylori*. *J. Bacteriol.* **187**, 7687–7695 https://doi.org/10.1128/JB.187.22.7687-7695.2005

8 Gomez-Garcia, M.R. and Kornberg, A. (2004) Formation of an actin-like filament concurrent with the enzymatic synthesis of inorganic polyphosphate. *Proc. Natl Acad. Sci. U.S.A.* **101**, 15876–15880 https://doi.org/10.1073/pnas.0406923101

9 Zhang, H., Gómez-García, M.R., Brown, M.R.W. and Kornberg, A. (2005) Inorganic polyphosphate in *dictyostelium discoideum*: influence on development, sporulation, and predation. *Proc Natl Acad Sci USA*. **102**, 2731–2735 https://doi.org/10.1073/pnas.0500023102

10 Livermore, T.M., Chubb, J.R. and Saiardi, A. (2016) Developmental accumulation of inorganic polyphosphate affects germination and energetic metabolism in *dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 996–1001 https://doi.org/10.1073/pnas.1519440113

11 Hothorn, M., Neumann, H., Lenherr, E.D., Wehner, M., Rybin, V., Hassa, P.O. et al. (2009) Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. *Science* **324**, 513–516 https://doi.org/10.1126/science.1168120

12 Gomes-Vieira, A.L., Wideman, J.G., Paes-Vieira, L., Gomes, S.L., Richards, T.A. and Meyer-Fernandes, J.R. (2018) Evolutionary conservation of a core fungal phosphate homeostasis pathway coupled to development in *Blastocladiella emersonii*. *Fungal Genet. Biol.* **115**, 20–32 https://doi.org/10.1016/j.fgb.2018.04.004

13 Gerasimaitė, R., Sharma, S., Desfougères, 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 https://doi.org/10.1242/jcs.159772

14 Aravind, L. and Koonin, E.V. (1998) A novel family of predicted phosphoesterases includes *drosophila* prune protein and bacterial recJ exonuclease. *Trends Biochem. Sci.* **23**, 17–19 https://doi.org/10.1016/S0968-0004(97)01162-6

15 Solesio, M.E., Elustondo, P.A., Zakharian, E. and Pavlov, E.V. (2016) Inorganic polyphosphate (polyP) as an activator and structural component of the mitochondrial permeability transition pore. *Biochem. Soc. Trans.* **44**, 7–12 https://doi.org/10.1042/BST20150206

16 Tammenkoski, M., Koivula, K., Cusanelli, E., Zollo, M., Steegborn, C., Baykov, A.A. et al. (2008) Human metastasis regulator protein H-prune is a short-chain exopolyphosphatase. *Biochemistry* **47**, 9707–9713 https://doi.org/10.1021/bi8010847

17 Lorenz, B. and Schröder, H.C. (2001) Mammalian intestinal alkaline phosphatase acts as highly active exopolyphosphatase. *Biochim. Biophys. Acta* **1547**, 254–261 https://doi.org/10.1016/S0167-4838(01)00193-5

18 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 https://doi.org/10.1074/jbc.M111.266320

19 Kumble, K.D. and Kornberg, A. (1996) Endopolyphosphatases for long chain inorganic polyphosphate in yeast and mammals. *J. Biol. Chem.* **271**, 27146–27151 https://doi.org/10.1074/jbc.271.43.27146

20 Gerasimaitė, R. and Mayer, A. (2017) Ppn2, a novel $Zn^{2+}$-dependent polyphosphatase in the acidocalcisome-like yeast vacuole. *J. Cell Sci.* **130**, 1625–1636 https://doi.org/10.1242/jcs.201061

21 Ohashi, K., Kawai, S. and Murata, K. (2012) Identification and characterization of a human mitochondrial NAD kinase. *Nat. Commun.* **3**, 1248 https://doi.org/10.1038/ncomms2262

PORTLAND
PRESS

22  Ruiz, F.A., Lea, C.R., Oldfield, E. and Docampo, R. (2004) Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. *J. Biol. Chem.* **279**, 44250–7 https://doi.org/10.1074/jbc.M406261200

23  Smith, S.A., Mutch, N.J., Baskar, D., Rohloff, P., Docampo, R. and Morrissey, J.H. (2006) Polyphosphate modulates blood coagulation and fibrinolysis. *Proc. Natl Acad. Sci. U.S.A.* **103**, 903–908 https://doi.org/10.1073/pnas.0507195103

24  Baker, C.J., Smith, S.A. and Morrissey, J.H. (2019) Polyphosphate in thrombosis, hemostasis, and inflammation. *Res. Pract. Thromb. Haemost.* **3**, 18–25 https://doi.org/10.1002/rth2.12162

25  Morrissey, J.H. and Smith, S.A. (2015) Polyphosphate as modulator of hemostasis, thrombosis, and inflammation. *J. Thromb. Haemost.* **13**, S92–S97 https://doi.org/10.1111/jth.12896

26  Wang, Y., Li, M., Li, P., Teng, H., Fan, D., Du, W. et al. (2019) Progress and applications of polyphosphate in bone and cartilage regeneration. *Biomed. Res. Int.* **2019**, 5141204 https://doi.org/10.1155/2019/5141204

27  Omelon, S., Georgiou, J., Henneman, Z.J., Wise, L.M., Sukhu, B., Hunt, T. et al. (2009) Control of vertebrate skeletal mineralization by polyphosphates. *PLoS ONE* **4**, e5634 https://doi.org/10.1371/journal.pone.0005634

28  Wang, L., Fraley, C.D., Faridi, J., Kornberg, A. and Roth, R.A. (2003) Inorganic polyphosphate stimulates mammalian TOR, a kinase involved in the proliferation of mammary cancer cells. *Proc. Natl Acad. Sci. U.S.A.* **100**, 11249–11254 https://doi.org/10.1073/pnas.1534805100

29  Jimenez-Nunez, M.D., Moreno-Sanchez, D., Hernandez-Ruiz, L., Benitez-Rondan, A., Ramos-Amaya, A., Rodriguez-Bayona, B. et al. (2012) Myeloma cells contain high levels of inorganic polyphosphate which is associated with nucleolar transcription. *Haematologica* **97**, 1264–1271 https://doi.org/10.3324/haematol.2011.051409

30  Hernandez-Ruiz, L., González-García, I., Castro, C., Brieva, J.A. and Ruiz, F.A. (2006) Inorganic polyphosphate and specific induction of apoptosis in human plasma cells. *Haematologica* **91**, 1180–1186 PMID:16956816

31  Abramov, A.Y., Fraley, C., Diao, C.T., Winkfein, R., Colicos, M.A., Duchen, M.R. et al. (2007) Targeted polyphosphatase expression alters mitochondrial metabolism and inhibits calcium-dependent cell death. *Proc. Natl Acad. Sci. U.S.A.* **104**, 18091–6 https://doi.org/10.1073/pnas.0708959104

32  Zakharian, E., Thyagarajan, B., French, R.J., Pavlov, E. and Rohacs, T. (2009) Inorganic polyphosphate modulates TRPM8 channels. *PLoS ONE* **4**, e5404 https://doi.org/10.1371/journal.pone.0005404

33  Kim, D. and Cavanaugh, E.J. (2007) Requirement of a soluble intracellular factor for activation of transient receptor potential A1 by pungent chemicals: role of inorganic polyphosphates. *J. Neurosci.* **27**, 6500–6509 https://doi.org/10.1523/JNEUROSCI.0623-07.2007

34  Pavlov, E., Zakharian, E., Bladen, C., Diao, C.T., Grimbly, C., Reusch, R.N. et al. (2005) A large, voltage-dependent channel, isolated from mitochondria by water-free chloroform extraction. *Biophys. J.* **88**, 2614–2625 https://doi.org/10.1529/biophysj.104.057281

35  Holmström, 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 https://doi.org/10.1038/ncomms2364

36  Gray, M.J., Wholey, W.-Y., Wagner, N.O., Cremers, C.M., Mueller-Schickert, A., Hock, N.T. et al. (2014) Polyphosphate is a primordial chaperone. *Mol. Cell* **53**, 689–699 https://doi.org/10.1016/j.molcel.2014.01.012

37  Cremers, C.M., Knoefler, D., Gates, S., Martin, N., Dahl, J.-U., Lempart, J. et al. (2016) Polyphosphate: a conserved modifier of amyloidogenic processes. *Mol. Cell* **63**, 768–780 https://doi.org/10.1016/j.molcel.2016.07.016

38  Lempart, J. and Jakob, U. (2019) Role of polyphosphate in amyloidogenic processes. *Cold Spring Harb. Perspect. Biol.* **11**, a034041 https://doi.org/10.1101/cshperspect.a034041

39  Azevedo, C., Livermore, T. and Saiardi, A. (2015) Protein polyphosphorylation of lysine residues by inorganic polyphosphate. *Mol. Cell* **58**, 71–82 https://doi.org/10.1016/j.molcel.2015.02.010

40  Azevedo, C., Singh, J., Steck, N., Hofer, A., Ruiz, F.A., Singh, T. et al. (2018) Screening a protein array with synthetic biotinylated inorganic polyphosphate to define the human polyP-ome. *ACS Chem. Biol.* **13**, 1958–1963 https://doi.org/10.1021/acschembio.8b00357

41  Bentley-DeSousa, A., Holinier, C., Moteshareie, H., Tseng, Y.-C., Kajjo, S., Nwosu, C. et al. (2018) A screen for candidate targets of lysine polyphosphorylation uncovers a conserved network implicated in ribosome biogenesis. *Cell Rep.* **22**, 3427–3439 https://doi.org/10.1016/j.celrep.2018.02.104

42  Lynn, W.S. and Brown, R.H. (1963) Synthesis of polyphosphate by rat liver mitochondria. *Biochem. Biophys. Res. Commun.* **11**, 367–371 https://doi.org/10.1016/0006-291X(63)90124-4

43  Gabel, N.W. and Thomas, V. (1971) Evidence for the occurrence and distribution of inorganic polyphosphates in vertebrate tissues. *J. Neurochem.* **18**, 1229–1242 https://doi.org/10.1111/j.1471-4159.1971.tb00222.x

44  Griffin, J.B., Davidian, N.M. and Penniall, R. (1965) Studies of phosphorus metabolism by isolated nuclei. VII. identification of polyphosphate as a product. *J. Biol. Chem.* **240**, 4427–4434 PMID:5845843

45  Losito, O., Szijgyarto, Z., Resnick, A.C. and Saiardi, A. (2009) Inositol pyrophosphates and their unique metabolic complexity: analysis by gel electrophoresis. *PLoS ONE* **4**, e5580 https://doi.org/10.1371/journal.pone.0005580

46  Kumble, K.D. and Kornberg, A. (1995) Inorganic polyphosphate in mammalian cells and tissues. *J. Biol. Chem.* **270**, 5818–5822 https://doi.org/10.1074/jbc.270.11.5818

47  Pisoni, R.L. and Lindley, E.R. (1992) Incorporation of [$^{32}$P]orthophosphate into long chains of inorganic polyphosphate within lysosomes of human fibroblasts. *J. Biol. Chem.* **267**, 3626–3631 PMID:1740414

48  Angelova, P.R., Agrawalla, B.K., Elustondo, P.A., Gordon, J., Shiba, T., Abramov, A.Y. et al. (2014) In situ investigation of mammalian inorganic polyphosphate localization using novel selective fluorescent probes JC-D7 and JC-D8. *ACS Chem. Biol.* **9**, 2101–2110 https://doi.org/10.1021/cb5000696

49  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 https://doi.org/10.1128/AEM.71.10.5692-5701.2005

50  Ezawa, T., Hayatsu, M. and Saito, M. (2005) A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. *Mol. Plant Microbe Interact.* **18**, 1046–1053 https://doi.org/10.1094/MPMI-18-1046

51  Reusch, R.N., Huang, R. and Kosk-Kosicka, D. (1997) Novel components and enzymatic activities of the human erythrocyte plasma membrane calcium pump. *FEBS Lett.* **412**, 592–596 https://doi.org/10.1016/S0014-5793(97)00863-6