Inorganic Polyphosphate in the Microbial World. Emerging Roles for a Multifaceted Biopolymer

Tomás Albi and Aurelio Serrano[*]

Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla Cartuja, CSIC y Universidad de Sevilla, Av. Américo Vespucio 49, 41092 Sevilla, SPAIN

*To whom correspondence should be addressed: Dr. Aurelio Serrano, Institute for Plant Biochemistry and Photosynthesis, CSIC and University of Seville, Av. Américo Vespucio 49, 41092 Seville, SPAIN. Phone: ++ 34 95 4489524. Fax: ++ 34 95 4460165. E-mail: aurelio@ibvf.csic.es. Web: http://www.ibvf.csic.es/en/bioenergetics-phosphate

ABSTRACT

Inorganic polyphosphates (polyP) are linear polymers of tens to hundreds orthophosphate residues linked by phosphoanhydride bonds. These fairly abundant biopolymers occur in all extant forms of life, from prokaryotes to mammals, and could have played a relevant role in prebiotic evolution. Since the first identification of polyP deposits as metachromatic or volutin granules in yeasts in the 19th century, an increasing number of varied physiological functions have been reported. Due to their "high energy" bonds analogous to those in ATP and their properties as polyanions, polyP serve as microbial phosphagens for a variety of biochemical reactions, as a buffer against alkalis, as a storage of Ca2+ and as a metal-chelating agent. In addition, recent studies have revealed polyP importance in signaling and regulatory processes, cell viability and proliferation, pathogen virulence, as a structural component and chemical chaperone, and as modulator of microbial stress response. This review summarizes the current status of knowledge and future perspectives of polyP functions and their related enzymes in the microbial world.

KEYWORDS

Inorganic polyphosphate; Cation chelator; Nutrient deficiency; Stress Protection; Cell signaling; Chemical chaperone.

PolyP Chemistry and Biochemistry. Enzymes Involved in PolyP Synthesis and Degradation.

1. Structure and Chemical Composition

Inorganic polyphosphates, polyP, are polymers of orthophosphate (Pi) residues linked by phosphoanhydride P-O-P bonds . They are often termed as "condensed phosphates" since they are composed by several Pi units (from three up to thousands) connected by oxygen bridges. Contrary to long-chained polyP, which are poorly soluble in water, the majority of polyP are stable in neutral aqueous solutions even at hight temperatures. Considering their chemical structure (Kulaev et al. 2005) polyP are divided into three classes: cyclic condensed phosphates (also referred as metaphosphates, $P_nO_{3n}^{n-}$, whose simplest member is cyclic-triphosphate), linear polyphosphates (or linear metaphosphates, $P_nO_{3n+1}^{(n+2)-}$, whose shortest component is tripolyphosphate), and lastly, the "ultraphosphates" or branched polyphosphates (Fig.1).

PolyP is perhaps one of the biopolymers with the highest density of negative charge. Its analogous structure to the RNA and other polyanions leads to comparable reactivity. For instance, both polymers increase fluorescence of DAPI, which can potentially provoke misinterpretations. As a result, the development of new polyP specific sensitive and selective detection techniques, and its application was critical for further progress on polyP research (Angelova et al. 2014). In fact, in spite of their discovery in the end of the 19th century (Babes 1895) and their wide occurrence, polyP was largely dismissed as a "molecular fossil" (Kornberg 1999). Fortunately, these recent studies have revealed the real physiological importance of polyP, starting an emerging interest in polyP research.

2. Natural Occurrence of PolyP

Polyphosphate is ubiquitous in living beings having being found in archaea, bacteria, algae, fungi, protists, plants, insects and mammals (Brown and Kornberg 2004; Docampo et al. 2005a; Rao et al. 2009). PolyP reserves were formely discovered in bacteria and unicellylar eukaryotes being dennoted as metachromatic or volutin granules due to their metachromatic effect - they appear red when stained with methylene blue. Later, once polyP was proven as one of its main components, they were also referred as polyphosphate granules o acidocalcisomes (Docampo et al. 2005a). Besides this widely distribution among living organisms, the amount and chemical structure of polyP reserves may vary depending the species and the particular growth

conditions. In general, prokaryotes and protists are able to accumulate polyP at higher rates than multicellular eukaryotes.

3. Enzymes Involved in PolyP Synthesis

### Synthesis of PolyP in Prokaryotic Microorganisms: Polyphosphate Kinase

Most studies concerning proteins involved in polyP biosynthesis have been focused on microorganisms, namely bacteria, including pathogenic and phosphate-accumulating strains, yeasts and parasitic protists. Based on these findings, some orthologs have been identified in microorganisms of other taxonomic groups. Nevertheless, to date there are still numerous organisms with no archetypical orthologs identifed so far, in spite of being able to accumulate high polyP levels. Consequently, it is deduced that they should have alternative pathways for polyP synthesis.

In prokaryotes -and in some microbial eukaryotes as well (Zhang et al. 2007)- polyP is mainly sinthesized by polyphosphate kinase 1 (PPK1; polyphosphate:ADP phosphotransferase, EC 2.7.4.1), which catalyzes the reversible transfer of the energy-rich γ-phosphate from ATP to enlongate the polyP chain.

$$PolyP_n + ATP \leftrightarrow PolyP_{n+1} + ADP$$
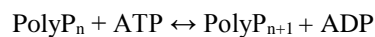
PPK1 (Pfam PF02503) is a member of the phosphotransferases superfamily, and exhibits other enzymatic activities including ATP synthesis from polyP, nucleoside-diphosphate kinase, guanosine 5′-tetraphosphate synthesis and autophosphorylation (Tzeng and Kornberg 2000). A genomes screening using the BLAST engine revealed *ppk1* homologs in more than 354 prokaryotes (Tzeng and Kornberg 1998). However, no *ppk1* homologs have been identified so far in higher eukaryotes, both higher plants and animals. Moreover, various studies have proved the importance of *ppk1*, and PPK1 has been shown to be an essential enzyme. Lack of PPK1 severely compromised cell viability of many bacteria under stationary-growth phase and their effective responses to a wide range of stress factors, such as heat, UV light, pH, antibiotics, etc. Similarly, bacterial mutants lacking PPK1 are defective in cell motility, quorum sensing, biofilm formation and virulence, and show ultrastructural defects (Brown and Kornberg 2008; Fraley et al. 2007; Rashid et al. 2000b; Sanyal et al. 2013). As a result, *ppk1* has been proposed as a novel target for next generation antibiotics.

Nonetheless, PPK1 is not the sole enzyme responsible for polyP synthesis. In particular, high M_m polyP were identified in *ppk1*-lacking null mutants of *Pseudomonas aeruginosa* (Ishige et al. 2002). The alternative enzyme was called PPK2 (Pfam PF03976). Similarly to *ppk1*, *ppk2* is absent in plants and metazoans and has been claimed to have a role in virulence of bacterial pathogens, in connection with alginate synthesis and biofilm formation, being then considered as an attractive target for antibiotics. However, PPK2 is frequently a polyP-degrading enzyme since its capacity to use polyP for GTP synthesis is 75-fold greater than its Poly P synthetic activity from GTP (Ishige et al. 2002). PPK2 can also serve as a PolyP:AMP phosphotransferase (EC 2.7.4.B2) and PolyP:ADP phosphotransferase (Ishige and Noguchi 2000):

$$PolyP_n + AMP \leftrightarrow PolyP_{n-1} + ADP$$
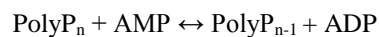
Conversely to PPK1, PPK2 is no strictly specific for ATP and it is able to efficiently use either GTP or ATP. Many microbial genomes encode multiple *ppk2* paralogs (Zhang et al. 2002). In fact, there are probably three subfamilies of PPK2 enzymes containing a single or two homologous PPK2 domains. Thus, whereas class I PPK2 is monodomain and catalyzes NTP synthesis from NDP, classes II and III are bi-domain PPK2 enzymes which catalyse the synthesis of NMP, or both NMP and NDP, respectively (Motomura et al. 2014). Likewise *ppk1*, *ppk2* widely occur among prokaryotic microorganisms, and hundreds of *ppk2* homologs have been identified to date. However, many bacteria should synthesize polyP by unknown enzymes, since one-third of bacterial species known so far lack both *ppk1* and *ppk2* (Whitehead et al. 2014).

*Synthesis of PolyP in Protists: Arp and VTC Proteins*

In the slime mold *Dictyostellium discoideum* a new type of PPK, named DdPPK2, was identified. This enzyme is a complex of three actin-related proteins (Arp), which can polymerize into an actin-like filament concurrently with the reversible synthesis of polyP chain from ATP (Gomez-Garcia and Kornberg 2004; Spudich 2004).

In yeast and trypanosomes (Lander et al. 2013) an alternative pathway responsible for polyP synthesis that involves VTC4, a subunit of the vacuolar transport chaperone (VTC) complex, has been described. VTC4 is a member of the Conserved Protein Domain family VTC (Pfam PF09359), which belongs to the CYTH-like phosphatases superfamily (cl11964). *S. cerevisiae* VTC complex is also involved in several other cellular

processes, like vacuolar-membrane fusion (Hothorn et al. 2009; Ogawa et al. 2000a; Uttenweiler et al. 2007), microautophagy (Cohen et al. 1999; Hothorn et al. 2009; Muller et al. 2002; Ogawa et al. 2000a; Uttenweiler et al. 2007). Homologs of VTC4 have been inferred in the genomes of apicomplexan protists, fungi and microalgae (Aksoy et al. 2014). *Chlamydomonas reinhardtii* VTC1 is required for polyP synthesis and polyP granule accumulation in acidocalcisomes. A deficient acidocalcisome formation in protistan cells deprived of N, P, or mainly S, may impact various function associated with energetics, trafficking of periplasmic proteins and regulation of cellular processes (Aksoy et al. 2014; Moreno and Docampo 2013).

Other Enzymes for PolyP Synthesis

The dolichyl diphosphate:polyphosphate phosphotransferase (EC 2.7.4.20) was related to the synthesis of the small fraction of polyP associated with the vacuolar membrane of *Saccharomyces cerevisiae* (Schomburg and Stephan 1997), and performs the following reaction:

$$\text{Dolichyl diphosphate} + \text{PolyP}_n \rightarrow \text{Dolichyl phosphate} + \text{PolyP}_{n-1}$$
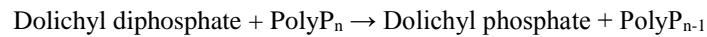
Lastly, an 3-phospho-D-glycerol-phosphate:polyphosphate phosphotransferase (EC 2.7.4.17) was found in the fungus *Neurospora crassa* (Kukaev et al. 1971). The enzyme, which has not been purified and needs further investigations, catalyzes the following reaction:

$$\text{3-Phospho-D-glycerol-1-phosphate} + \text{PolyP}_n \rightarrow \text{3-Phosphoglycerate} + \text{PolyP}_{n+1}$$

4. Enzymes that Degrade PolyP

Exopolyphosphatase and Guanosine Pentaphosphate Hydrolase

The main enzyme responsible for polyP usage in microorganisms is the exopolyphosphatase (PPX; Polyphosphate phosphohydrolase, EC 3.6.1.11). PPX hydrolyzes and processively splits Pi from the end of the polyP chain:

$$\text{PolyP}_n + H_2O \rightarrow \text{PolyP}_{n-1} + \text{Pi}$$
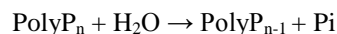
Two major non-homologous classes of PPX are defined based on their primary structure. A first PPX class is established by the archetypical exopolyphosphatase PPX1, first identified in *Saccharomyces cerevisiae*, and

their orthologues later described in yeasts, other fungi and protists. PPX1 belongs to the superfamily of DHH-DHHA2 phosphoesterases (Pfam PF02833), which also includes the prokaryotic family II pyrophosphatases (Young et al. 1998) and the Nudix hydrolase family (Lonetti et al. 2011). Some of these Nudix proteins, such as the human protein h-prune, a binding protein of the metastasis suppressor nm23-H1, have been proved to efficiently hydrolyze polyP (Tammenkoski et al. 2008). The human protein h-prune and the yeast PPX1 proteins share a high sequence identity (27%). PPX1 is an extremely active phoshohydrolase which can hydrolyze polyP, adenosine tetraphosphate and GPT; but does not hydrolyze PPi or NTPs.

A second exopolyphosphatase class includes the Ppx-GppA polyphosphatases (Pfam PF02541) which belong to the sugar kinase/actin/hsp 70 superfamily. Ppx-GppA exopolyphosphatases are widely distributed among bacteria and archaea, processively hydrolyse linear polyP of 3 up to thousands of Pi residues, and also have nucleoside triphosphatase (NTPase) activity (Albi and Serrano 2014). Thus, prokaryotic PPXs and eukaryotic (fungal/protistan) PPXs belong to different families of polyphosphatases and do not have structural similarity. In addition, bacteria posses another Ppx-GppA exopolyphosphatase sharing ca 40% sequence similarity with its archetypical prokaryotic paralog and catalytically less efficient than the latter, the guanosine pentaphosphate phosphohydrolase (GppA, EC 3.6.1.40) which also catalyzes the following reaction:

Guanosine 5'-triphosphate, 3'-diphosphate → Guanosine 5'-diphosphate,3'-diphosphate + Pi
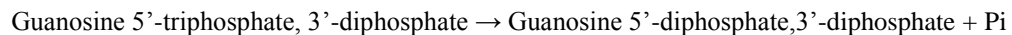
Endopolyphosphatase

Besides PPX1 exopolyphosphatase, yeasts, fungi and protists also posses an endopolyphosphatase enzyme. The archetypical endopolyphosphatase of *S. cerevisiae* (PPN1; Polyphosphate depolymerase, EC 3.6.1.10) is a transmembrane bitopic protein which belong to the Calcineurin-like phosphoesterase superfamily (Pfam PF00149), and cleaves long polyP into shorter polyP molecules without releasing Pi (Sethuraman et al. 2001):

$$PolyP_n + H_2O \rightarrow oligopolyphosphates$$
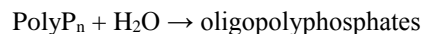
In rich growth conditions, yeast PPN1 acts as an endopolyphosphatase in the presence of Mg^{2+}. However, under certain stress conditions, such as toxic heavy-metals, PPN1 shifts to an Co^{2+}-dependent exopolyphosphatase activity (Andreeva et al. 2015).

Functions of PolyP in Microorganisms

1. PolyP as a Structural Component

One of the most exciting chemical features of polyP is its strong ability to interact with a variety of inorganic and organic compounds resulting in ternary complexes. Thus, polyP (with and average length of 150 Pi residues) can form complexes with poly-β-hydroxybutyrate (mean size of 170) an Ca^{2+} ions. In the proposed structure, poly-β-hydroxybutyrate (PHB) corresponds to the outer layer which is directly in contact with the lipid membrane. Inside, PHB ester groups are bonded to polyP by ionic interactions and to Ca^{2+} by ion dipoles (Reusch and Sadoff 1988). The polyP/Ca^{2+}/PHB complex has been detected in the plasma membrane of many naturally competent bacteria, and in various subcellular compartments of eukaryotes (Reusch 1989). This ternary complex constitutes a transient channel increasing membrane permeability - selective for Ca^{2+} ions - which probably plays an important physiological role in competence for DNA entry and transformation (Castuma et al. 1995).

Further researches in mammals support this assumption (Dedkova and Blatter 2014). It has been recently review that polyP may be a strong activator of the mitochondrial permeability transition pore in cardiomycetes, heart muscle cells, playing a structural role in their mitochondria membrane systems (Dedkova and Blatter 2014; Seidlmayer et al. 2012a; Seidlmayer et al. 2012b).

2. PolyP as a Substitute for ATP

As phosphorylated compound with a Gibbs free energy of hydrolysis similar to ATP (-30.5 kJ mol^{-1}), polyP may act as a substitute for ATP in diverse enzymatic reactions (Kornberg et al. 1999).

*Donor for Sugars: PolyP-gluco(manno)kinase*

Polyphosphate gluco(manno)kinase (PPGK; Polyphosphate-glucose phosphotransferase, EC 2.7.1.63), a member of the ROK (Repressor-ORF-Kinase) superfamily (Pfam PF00480), catalyzes the phosphorylation of monosaccharides –glucose, mannose, and in some cases fructose– using polyP or ATP as a phosphoryl donor.

$$PolyP_n + D\text{-glucose} \rightarrow PolyP_{n-1} + D\text{-glucose-6-phosphate}$$

This enzymatic activity has been reported in a variety of phylogenetically different bacteria, including important pathogens (Szymona and Ostrowski 1964; Szymona and Szymona 1978), bacteria of activated sludge (Tanaka et al. 2003), and N_2-fixing cyanobacteria (Albi and Serrano 2015).

Donor for Adenylate Kinases: NAD Kinase

NAD kinase ((polyP)/ATP:NAD 2'-phosphotransferase, EC 2.7.1.23; Pfam PF01513) catalyzes the phosphorylation of NAD to yield NADP. In some prokaryotes, NAD kinases use either ATP or polyP as phosphoryl donors:

$$ATP + NAD \rightarrow ADP + NADP$$

$$PolyP_n + NAD \rightarrow PolyP_{n-1} + NADP$$
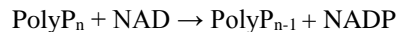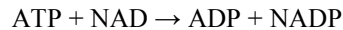
PolyP have been postulated as the precursor of ATP and the primitive energy donor in the origin of life (Kornberg 1995; Lipmann 1965). Similarly to ATP, polyP is composed by high-energy phosphate groups and it was likely present on prebiotic earth. Moreover, polyP formed by high pressure and desiccation (phosphate condensation) might have been naturally abundant (sedimentary rocks, hydrothermal vents, volcanic exudates) in the early earth (Lohrmann and Orgel 1968; Miller and Parris 1964). Based on their comparable chemical properties and its ubiquity, we should not exclude a role for polyP in energy protometabolism of primordial cells (Achbergerova and Nahalka 2011; Brown and Kornberg 2004). Furthermore, subsequent biochemical studies on bacterial NAD kinases and glucokinases revealed that some of them are active with polyP. In particular, polyP can really substitute for ATP, and some enzymes are even strictly dependent on polyP. This hypothesis is substantiated by several later observations from the biochemical properties of NAD kinases and glucokinases: most bacterial enzymes are able to use both phosphoryl donors with an observed progressive decrease in the preference for polyP in the phylogenetically newer taxa (Albi and Serrano 2015).

Interestingly, human mitochondrial NAD kinase uses both phosphoryl donors, ATP and polyP (Ohashi et al. 2012). Finally, no eukaryotic polyP-dependent NAD kinases or glucokinases have been reported so far. Given the polyP substitution for ATP together with the presence of polyP-dependent enzymes in a variety of ancient microorganisms, it is conceivable that polyP may have been the ancestor of ATP on the early stages of life evolution.

3. PolyP Role in Cell Acclimation to Nutrient-limiting Conditions

Bacteria tend to accumulate polyP under growth in unbalanced media. For instance, *Escherichia coli* or *Acetobacter xylinum* cells (Rao et al. 1998) concentrate the excess of extracellular Pi as PolyP granules under amino acid or nitrogen deficiency, respectively (Kuroda and Ohtake 2000). Such limitations may lead to polyP accumulation, with fluctuation in polyP levels up to 10^3-fold. This reserve of polyP may be rapidly mobilized to serve as a significant energy source. In this regard, many marine organisms tend to accumulate polyP in surface water layers or when Pi is abundant, for a future use of polyP as a source of energy and Pi in case of anaerobic conditions (Diaz et al. 2008). In a similar way, some mycorrhizal fungi accumulate large amounts of polyP by dissolving mineral phosphorous from soil. The reserves of polyP are later locally hydrolyzed in fungal cells to supply phosphate to the symbiotic plant (Mensah et al. 2015). In addition, the chlorophyte microalga *Chlamydomonas reinhardtii* accumulates polyP in acidocalcisomes in response to sulfur, phosphorous or nitrogen deprivation (Aksoy et al. 2014). The fungi *Cryptococcus humicola* and *S. cerevisiae* accumulate polyP in absence of nitrogen (Breus et al. 2012). Another example of polyP sequestration by symbiotic microorganisms has been recently reported in cyanobacterial symbionts of marine sponges (Zhang et al. 2015). The importance of polyP in the environmental adaptation was evidenced by the fact that *Chlamydomonas* null-mutants in the polyP synthetase VTC1 formed less acidocalcisomes and with structural abnormalities, which impacts trafficking of periplasmic proteins. These data suggest that polyP (and acidocalcisomes) are essential for adaptation of algal cells to nutrient deficiency (Aksoy et al. 2014). Overall, these data corroborate the notion that polyP actually serves as a reservoir of Pi and energy that could be efficiently mobilized in case of further requirements. However, even in phosphorous-depleted medium, Pi-starved yeast cells maintain a reduced but significant level of polyP (Vagabov et al. 2000). This effect was

also observed in phytoplankton under phosphorous stress. In particular, the ratio polyP to total phosphorous was five-fold higher in phytoplankton of ultra-low-phosphorous waters than in the one of normal waters. However, cells undergoing P stress had activated their typical transcriptional and metabolic machineries in response to starvation, namely an enhanced of alkaline phosphatase activity and substitution of sulfolipids for phospholipids (Van Mooy et al. 2009). Therefore, these studies revealed that polyP serve many functions besides P homeostasis and energy source. It seems that there are multiple polyP cellular pools with different roles and dynamics in eukaryotic microorganisms: most polyP performs the function of phosphorous reserve (in the vacuole/acidocalcisome), while another smaller but critical fractions of polyP (nucleus, cytoplasm) are responsible for several regulatory processes challenging the classical view of polyP as a luxury phosphorous storage molecule.

4. PolyP as a Phosphorous Reservoir. PolyP Reserves

In most microorganisms the main phosphorous reserve is polyP. Although some archaea, bacteria and fungi accumulate other kinds of P reserves like insoluble magnesium phosphates or sugar complexes (phosphomanan), PolyP has several advantages. It has no effect on the osmotic pressure and can be easily mobilized by exopolyphosphatases, NAD kinases or glucokinases.

Some bacteria from activated sludge –especially from the taxa *Mycobacteria*, *Corynebacteria*, and *Eubacteria*– in addition to yeasts are able to accumulate considerable amounts of polyP (up to 30% of dry weight). Highest rates of polyP accumulation are achieved under the called "phosphate overplus" condition, consisting in the transfer of cells previously adapted to Pi deficiency to a Pi rich medium.

In a diverse range of organisms, from bacteria to mammals, polyP accumulates in acidic, calcium-storage compartments, called acidocalcisomes. Acidocalcisomes are acidic single-membrane organelles especially adapted for accumulation of large amounts of polyP containing divalent cations and pyrophosphate as well. Essential proteins not only for Ca^{2+} signaling and phosphate homeostasis but also for membrane transport (primary pumps, channels and transporters) and growth are localized in acidocalcisomes. A number or recent

studies have revealed the importance of acidocalcisomes as evolutionary conserved organelles present in many organisms, from bacteria to mammals (Docampo et al. 2005b).

5. PolyP as a Metal-ion Chelator

Heavy-metal Detoxification

Polyphosphates are inorganic polyanions and can bind or act as a potent chelator of metal cations. It is well-known the ability of polyP to sequestrate very diverse cations (many of them biohazardous), including Al^{3+} (Pettersson 1985), Mn^{2+}(Andreeva et al. 2013), Ni^{2+} (Gonzalez and Jensen 1998), Cu^{2+}(Alvarez and Jerez 2004; Grillo-Puertas et al. 2014; Remonsellez et al. 2006), Cd^{2+}(Keasling 1997; Keasling and Hupf 1996), Zn^{2+}(Baxter and Jensen 1980), Hg^{2+} (Pan-Hou et al. 2002), Pb^{2+} (Keasling et al. 1998), Ca^{2+}(Dunn et al. 1994), Ba^{2+}(Baxter and Jensen 1980), La^{3+} (Andreeva et al. 2014) and U^{6+} (Renninger et al. 2004), as well as other heavy-metal ions (Mg^{2+}, Fe^{2+}, Co^{2+}). The capacity of polyP to efficiently chelate heavy metals has been straightly associated by numerous studies with an intensification in the tolerance of microorganisms to toxic metals. Thus, there is a straight relationship between the cell reserves of polyP and the resistance to poisonous heavy metals. This feature is particularly evident in those bacterial strains capable to accumulate elevated polyP levels, either naturally or by genetic manipulation, used for bioremediation. In addition, bacterial phenotypes of polyP-dependent enzymes null mutants, such as *ppk-*and *ppx-*, have provided further evidences in this respect. For instance, mutants unable to accumulate or degrade polyP were highly sensitive to heavy metals even in high phosphate media. Likewise, the inclusion of certain metals, such as Mn^{2+} or Co^{2+} may alter the polyP content of yeast cells, by way of an increase of polyP synthesis by VTC4 protein or through modulation of exopolyphosphatase activities. The metal sensitivity of bacterial *ppx* single mutants represents an evidence that not only polyP presence, but also its hydrolysis to Pi is required for heavy metal detoxification. Particularly, metal addition to bacterial cultures induced polyP degradation via exopolyphosphatase and Pi efflux as well. Most bacteria own two phosphate transporters: Pit and Pst. Pit is a constitutive low-affinity Pi transporter which has been also described as a metal-phosphate:H^+ symporter with the capacity to co-export metal-phosphate complexes ($MeHPO_4$) from cells. Consequently, the important role of polyP in heavy metal resistance and detoxification has been proposed in a model described by Keasling

(Keasling 1997) where metals are initially chelated by polyP, then polyP is degraded by exo/endopolyphosphatases yielding metal-phosphate complexes that are removed from cells via the Pit transport system.

Antioxidative Protection Involving Complexation with PolyP

PolyP can protect cells from oxidative stress caused by highly toxic superoxide anion radicals (O_2^-) through their efficiency to coordinate metal cations, such as Mn^{2+} and Fe^{3+}. Some bacteria accumulate high concentrations of Mn^{2+}, which when coordinated with various metabolites is able to carry out O_2^- dismutation. In fact, Mn^{2+} is first chelated by polyP, and later the Mn^{2+}-polyP complexes formed are hydrolyzed by exopolyphosphatases yielding $MnHPO_4$, which efficiently detoxifies O_2^- ions. Consistent with this role, it was recently described that _S. cerevisiae_ superoxide dismutase (_Sod_)-_VTC4_ double mutants cannot be rescued by addition of Mn^{2+}, contrary to the superoxide dismutase _Sod_ single mutant (Hothorn et al. 2009; Reddi et al. 2009). Therefore, it is evident that polyP presence is relevant for O_2^- detoxification in yeast.

PolyP also protects against the Fenton reaction (Gray and Jakob 2015). In such process, the highly reactive hydroxyl radical (OH$^{\bullet}$) is generated by the redox reaction of Fe^{2+} or Cu^{2+} with oxidants like H_2O_2 or HOCl. As a polyanion, polyP easily form Cu^{2+}-phosphate complexes that are exported, and stabilize Fe^{3+} ion, an intermediate necessary for the cyclic reaction which produces OH$^{\bullet}$.

6. PolyP Roles in Cell Motility and Pathogen Virulence and Persistence

PolyP is required for bacterial biofilm formation, motility, and sporulation in _Bacillus cereus (Shi et al. 2004)_, _Pseudomonas aeruginosa_ (Rashid and Kornberg 2000) and _Escherichia coli_ (Grillo-Puertas et al. 2012). _ppk_-mutants of several enteropathogenic strains are defective in motility (swimming, swarming and twitching) and surface attachment, all features linked to virulence (Ogawa et al. 2000b; Rashid and Kornberg 2000; Rashid et al. 2000a).

PolyP accumulation in _Mycobacterium tuberculosis ppx_-null mutants reduces pathogenicity and survival in the host (Chuang et al. 2015). The importance of polyP for stationary-phase survival and virulence is widely

conserved among many bacterial pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp. (Kim et al. 2002) and *Salmonella* spp. (Kim et al. 2002; McMeechan et al. 2007). *Campylobacter jejuni* *ppk1*-null mutants exhibit low levels of poly-P and are defective for survival during osmotic shock, low-nutrient stress and for virulence into intraepithelial cells (Candon et al. 2007). The role of acidocalcisomal polyP in osmotic stress has been studied in parasitic protists. According to the model proposed, hyposmotic stress triggered acidocalcisome polyP hydrolysis in the haemoparasite *T. cruzi* (Montalvetti et al. 2004). Conversely, hyperosmotic stress resulted in increased synthesis of acidocalcisomal polyP (Jimenez and Docampo 2012).

PolyP contributes to the cell envelope formation and consequently has a strong impact in virulence. *Neisseria mutants* lacking capsular polyP exhibits reduced pathogenicity in comparison with the wild-type strain (Tinsley and Gotschlich 1995).

Many bacteria are able to enter in a high stress-tolerant state of dormancy. Persistent cells accumulate the alarmone (p)ppGpp, which modulates several physiological changes in response to stress known as a whole as the stringent response. Bacterial responses to stress conditions, such as nutrient deprivation (amino-acid starvation, fatty acid limitation, iron limitation), heat shock, and other stresses, are termed as the stringent response or stringent control, which is signed by the alarmone (p)ppGpp. This secondary-messenger exerts a control on general metabolism, modulating growth rate and inhibiting production of ribosomal RNA. The stringent response also exerts a central role in regulatory circuits controlling bacterial virulence, survival during host invasion, antibiotic resistance and persistence. During the stringent response, (p)ppGpp produces a direct effect on polyP metabolism through PPK targeting, which leads to persistence. The enzymes synthesizing and degrading (p)ppGpp are highly conserved in bacteria. One of them is the guanosine pentaphosphate phosphohydrolase GppA above described, which also posses exopolyphosphatase activity and is inhibited by high concentrations of polyP. In turn, elevated concentrations of (p)ppGpp competitively inhibits polyP degradation by PPX, increasing polyP levels. As a result, (p)ppGpp and polyP contents increase significantly during the stringent response. On the other hand, polyP forms a complex with the ATP-dependent protease Lon which degrades most ribosomal proteins (Kuroda et al. 2001; Nomura et al. 2004), and the antitoxin components of type II toxin–antitoxin systems (Gerdes and Maisonneuve 2012). As a result,

free toxins dramatically accumulate, which inhibits cell metabolism and growth rate, thus inducing persistence.

7. PolyP Role in Cell Energy

Some studies in the 90's have established a relationship between the turnover of polyP and cell metabolism via mitochondrial activity in mammalian cells (Kumble and Kornberg 1995). In addition, it was later reported a plausible feedback mechanism involving polyP levels and cell energy (Pavlov et al. 2010). In particular, while substrates of the mitochondrial respiratory chain promote polyP synthesis, its levels were reduced by mitochondrial inhibitors or respiratory chain uncouplers. Moreover, polyP depletion by mitochondrial-targeted PPX1 overexpression impairs respiratory chain activity and reduced ATP production (Pavlov et al. 2010). Based on the somewhat similitude between mitochondria and bacteria, a similar role for polyP appears plausible in microbial cell energy metabolism. However, further research is needed to elucidate the exact role of polyP in bioenergetics of prokaryotic and eukaryotic microorganisms.

8. Cell Signaling and Stress Response Network

Recently, experimental evidence has been presented that polyP may play an important role in signaling of some nuclear proteins (Azevedo et al. 2015). Thus, polyP can trigger through a non-enzymatic mechanism a novel post-translational process called polyphosphorylation. This modification consists on the covalent attachment of inorganic polyP to lysine residues of proteins containing a polyacidic serine and lysine-rich cluster. Polyphosphorylation is essentially similar to other protein modifications previously known as phosphorylation or pyrophosphorylation (Bhandari et al. 2007).

Moreover, there are direct evidences of polyP involvement in regulation of general stress response network with small differences among bacterial species (Shiba et al. 1997). The general outline includes the stationary-phase gene *rpoS*, encoding the σ-factor σ^{38}. PolyP and PPK are required for the transcription of *rpoS*. In turn, factor σ^{38} controls a regulon including *ppk* and numerous genes involved in osmotic and nitrogen starvation

stress responses (Maciag et al. 2011). σ^{38} also controls the alginate production as well as swimming and twitching motility in *Pseudomonas aeruginosa*. Similarly, the σ^E regulon in *Mycobacterium* spp. determines the response to oxidative and phosphate-limiting stresses and is also required for virulence and persistence of this pathogen (Manganelli et al. 2001).

9. Stress Protection

Microbial strategies in response to oxidative and other stresses comprise several mechanisms on post-translational control, redox-regulated adjustment of cellular metabolism, and the activation of specific molecular chaperones. Recently, it was reported that polyP exerts as a functional protein-protective chemical chaperone at physiological levels (Gray et al. 2014). Thus, polyP is able to stabilize a broad range of proteins maintaining their competent conformations, preventing them from unfolding and aggregation. In addition, bacterial *ppk*-null mutants suffer from higher protein damage than the wild-type strain upon a similar proteotoxic stress. Besides, polyP may have some advantages compared to other chaperones since it does not react with oxidants, such as HClO, and does not require ATP hydrolysis for its protective activity (Kampinga 2014).

On the other hand, polyP enhance *Vibrio cholerae* cells tolerance to environmental stresses in Pi-limiting conditions (Jahid et al. 2006), and *ppk*-null mutants of *Lactobacillus* spp. (Alcantara et al. 2014) and *E. coli* (Gray et al. 2014), which are not able to produce polyP, show reduced growth or are more sensitive to acidic pH, ethanol, heat, high-salt and oxidative stresses. Furthermore, it has been reported that polyP production by bacteria of the human gastrointestinal tract protects the intestinal epithelia from oxidative stress (Segawa et al. 2011). To summarize, these results corroborate the important contribution of polyP to the regulation of growth, cell survival and stress tolerance of many microorganisms.

PROSPECT

Despite the identification of polyP at the end of the nineteenth century and its extensive occurrence covering the whole evolutionary lineages, polyP was largely dismissed and forgotten during decades. Recently, there is an increasing interest in this polymer, which goes hand-in-hand with the revelation of its unexpected and intriguing involvement in critical cellular functions in prokaryotes and eukaryotes. Thanks to these studies, it has become evident an active association between polyP and many physiological processes of paramount importance for microorganisms, such as multilayer metabolic regulation, stress responses, pathogen resistance, etc. In a broader context, polyP was also recently reported to be involved in a variety of biological processes related to human health, such as cardiac ischaemia, blood coagulation, apoptosis and stress-induced cell death (Dedkova and Blatter 2014; Hernandez-Ruiz et al. 2006; Seidlmayer et al. 2012b), suggesting its therapeutic use. The recognized importance of such recent advances, as well as the growing number of researchers engaged in the study of polyP, shall foster original and fascinating progresses and applications related to this ancient biopolymer in the next future.

REFERENCES

Achbergerova L, Nahalka J (2011) Polyphosphate--an ancient energy source and active metabolic regulator. Microb Cell Fact 10:63 doi:10.1186/1475-2859-10-63

Aksoy M, Pootakham W, Grossman AR (2014) Critical function of a Chlamydomonas reinhardtii putative polyphosphate polymerase subunit during nutrient deprivation. Plant Cell 26(10):4214-29 doi:10.1105/tpc.114.129270

Albi T, Serrano A (2014) Two exopolyphosphatases with distinct molecular architectures and substrate specificities from the thermophilic green-sulfur bacterium Chlorobium tepidum TLS. Microbiology 160(Pt 9):2067-78 doi:10.1099/mic.0.080952-0

Albi T, Serrano A (2015) Two strictly polyphosphate-dependent gluco(manno)kinases from diazotrophic Cyanobacteria with potential to phosphorylate hexoses from polyphosphates. Appl Microbiol Biotechnol 99(9):3887-900 doi:10.1007/s00253-014-6184-7

Alcantara C, Blasco A, Zuniga M, Monedero V (2014) Accumulation of polyphosphate in Lactobacillus spp. and its involvement in stress resistance. Appl Environ Microbiol 80(5):1650-9 doi:10.1128/aem.03997-13

Alvarez S, Jerez CA (2004) Copper ions stimulate polyphosphate degradation and phosphate efflux in Acidithiobacillus ferrooxidans. Appl Environ Microbiol 70(9):5177-82 doi:10.1128/aem.70.9.5177-5182.2004

Andreeva N, Ryazanova L, Dmitriev V, Kulakovskaya T, Kulaev I (2013) Adaptation of Saccharomyces cerevisiae to toxic manganese concentration triggers changes in inorganic polyphosphates. FEMS Yeast Res 13(5):463-70 doi:10.1111/1567-1364.12049

Andreeva N, Ryazanova L, Dmitriev V, Kulakovskaya T, Kulaev I (2014) Cytoplasmic inorganic polyphosphate participates in the heavy metal tolerance of Cryptococcus humicola. Folia Microbiol (Praha) 59(5):381-9 doi:10.1007/s12223-014-0310-x

Andreeva N, Trilisenko L, Eldarov M, Kulakovskaya T (2015) Polyphosphatase PPN1 of Saccharomyces cerevisiae: switching of exopolyphosphatase and endopolyphosphatase activities. PLoS One 10(3):e0119594 doi:10.1371/journal.pone.0119594

Angelova PR, Agrawalla BK, Elustondo PA, Gordon J, Shiba T, Abramov AY, Chang YT, Pavlov EV (2014) In situ investigation of mammalian inorganic polyphosphate localization using novel selective fluorescent probes JC-D7 and JC-D8. ACS Chem Biol 9(9):2101-10 doi:10.1021/cb5000696

Azevedo C, Livermore T, Saiardi A (2015) Protein polyphosphorylation of lysine residues by inorganic polyphosphate. Mol Cell 58(1):71-82 doi:10.1016/j.molcel.2015.02.010

Babes V (1895) Beobachtungen über die metachromatischen Körperchen, Sporenbildung, Verzweigung, Kolben-und Kapselbildung pathogener Bakterien. Zeitschr f Hygiene 20(1):412-437 doi:10.1007/bf02216664

Baxter M, Jensen T (1980) Uptake of magnesium, strontium, barium, and manganese byPlectonema boryanum (Cyanophyceae) with special reference to polyphosphate bodies. Protoplasma 104(1-2):81-89 doi:10.1007/bf01279371

Bhandari R, Saiardi A, Ahmadibeni Y, Snowman AM, Resnick AC, Kristiansen TZ, Molina H, Pandey A, Werner JK, Jr., Juluri KR, Xu Y, Prestwich GD, Parang K, Snyder SH (2007) Protein pyrophosphorylation by inositol pyrophosphates is a posttranslational event. Proc Natl Acad Sci U S A 104(39):15305-10 doi:10.1073/pnas.0707338104

Breus NA, Ryazanova LP, Dmitriev VV, Kulakovskaya TV, Kulaev IS (2012) Accumulation of phosphate and polyphosphate by Cryptococcus humicola and Saccharomyces cerevisiae in the absence of nitrogen. FEMS Yeast Res 12(6):617-24 doi:10.1111/j.1567-1364.2012.00812.x

Brown MR, Kornberg A (2004) Inorganic polyphosphate in the origin and survival of species. Proc Natl Acad Sci U S A 101(46):16085-7 doi:10.1073/pnas.0406909101

Brown MR, Kornberg A (2008) The long and short of it - polyphosphate, PPK and bacterial survival. Trends Biochem Sci 33(6):284-90 doi:10.1016/j.tibs.2008.04.005

Candon HL, Allan BJ, Fraley CD, Gaynor EC (2007) Polyphosphate kinase 1 is a pathogenesis determinant in Campylobacter jejuni. J Bacteriol 189(22):8099-108 doi:10.1128/jb.01037-07

Castuma CE, Huang R, Kornberg A, Reusch RN (1995) Inorganic polyphosphates in the acquisition of competence in Escherichia coli. J Biol Chem 270(22):12980-3

Cohen A, Perzov N, Nelson H, Nelson N (1999) A novel family of yeast chaperons involved in the distribution of V-ATPase and other membrane proteins. J Biol Chem 274(38):26885-93

Chuang YM, Bandyopadhyay N, Rifat D, Rubin H, Bader JS, Karakousis PC (2015) Deficiency of the novel exopolyphosphatase Rv1026/PPX2 leads to metabolic downshift and altered cell wall permeability in Mycobacterium tuberculosis. MBio 6(2):e02428 doi:10.1128/mBio.02428-14

Dedkova EN, Blatter LA (2014) Role of beta-hydroxybutyrate, its polymer poly-beta-hydroxybutyrate and inorganic polyphosphate in mammalian health and disease. Front Physiol 5:260 doi:10.3389/fphys.2014.00260

Diaz J, Ingall E, Benitez-Nelson C, Paterson D, de Jonge MD, McNulty I, Brandes JA (2008) Marine polyphosphate: a key player in geologic phosphorus sequestration. Science 320(5876):652-5 doi:10.1126/science.1151751

Docampo R, de Souza W, Miranda K, Rohloff P, Moreno SN (2005a) Acidocalcisomes - conserved from bacteria to man. Nat Rev Microbiol 3(3):251-61 doi:10.1038/nrmicro1097

Docampo R, de Souza W, Miranda K, Rohloff P, Moreno SNJ (2005b) Acidocalcisomes ? conserved from bacteria to man. Nat Rev Micro 3(3):251-261

Dunn T, Gable K, Beeler T (1994) Regulation of cellular Ca2+ by yeast vacuoles. J Biol Chem 269(10):7273-8

Fraley CD, Rashid MH, Lee SS, Gottschalk R, Harrison J, Wood PJ, Brown MR, Kornberg A (2007) A polyphosphate kinase 1 (ppk1) mutant of Pseudomonas aeruginosa exhibits multiple ultrastructural and functional defects. Proc Natl Acad Sci U S A 104(9):3526-31 doi:10.1073/pnas.0609733104

Gerdes K, Maisonneuve E (2012) Bacterial persistence and toxin-antitoxin loci. Annu Rev Microbiol 66:103-23 doi:10.1146/annurev-micro-092611-150159

Gomez-Garcia MR, 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(45):15876-80 doi:10.1073/pnas.0406923101

Gonzalez H, Jensen TE (1998) Nickel sequestering by polyphosphate bodies in Staphylococcus aureus. Microbios 93(376):179-85

Gray MJ, Jakob U (2015) Oxidative stress protection by polyphosphate--new roles for an old player. Curr Opin Microbiol 24:1-6 doi:10.1016/j.mib.2014.12.004

Gray MJ, Wholey WY, Wagner NO, Cremers CM, Mueller-Schickert A, Hock NT, Krieger AG, Smith EM, Bender RA, Bardwell JC, Jakob U (2014) Polyphosphate is a primordial chaperone. Mol Cell 53(5):689-99 doi:10.1016/j.molcel.2014.01.012

Grillo-Puertas M, Schurig-Briccio LA, Rodriguez-Montelongo L, Rintoul MR, Rapisarda VA (2014) Copper tolerance mediated by polyphosphate degradation and low-affinity inorganic phosphate transport system in Escherichia coli. BMC Microbiol 14:72 doi:10.1186/1471-2180-14-72

Grillo-Puertas M, Villegas JM, Rintoul MR, Rapisarda VA (2012) Polyphosphate degradation in stationary phase triggers biofilm formation via LuxS quorum sensing system in Escherichia coli. PLoS One 7(11):e50368 doi:10.1371/journal.pone.0050368

Hernandez-Ruiz L, Gonzalez-Garcia I, Castro C, Brieva JA, Ruiz FA (2006) Inorganic polyphosphate and specific induction of apoptosis in human plasma cells. Haematologica 91(9):1180-6

Hothorn M, Neumann H, Lenherr ED, Wehner M, Rybin V, Hassa PO, Uttenweiler A, Reinhardt M, Schmidt A, Seiler J, Ladurner AG, Herrmann C, Scheffzek K, Mayer A (2009) Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. Science 324(5926):513-6 doi:10.1126/science.1168120

Ishige K, Noguchi T (2000) Inorganic polyphosphate kinase and adenylate kinase participate in the polyphosphate:AMP phosphotransferase activity of Escherichia coli. Proc Natl Acad Sci U S A 97(26):14168-71 doi:10.1073/pnas.011518098

Ishige K, Zhang H, Kornberg A (2002) Polyphosphate kinase (PPK2), a potent, polyphosphate-driven generator of GTP. Proc Natl Acad Sci U S A 99(26):16684-8 doi:10.1073/pnas.262655299

Jahid IK, Silva AJ, Benitez JA (2006) Polyphosphate stores enhance the ability of Vibrio cholerae to overcome environmental stresses in a low-phosphate environment. Appl Environ Microbiol 72(11):7043-9 doi:10.1128/aem.00924-06

Jimenez V, Docampo R (2012) Molecular and electrophysiological characterization of a novel cation channel of Trypanosoma cruzi. PLoS Pathog 8(6):e1002750 doi:10.1371/journal.ppat.1002750

Kampinga HH (2014) Chaperoned by prebiotic inorganic polyphosphate molecules: an ancient transcription-independent mechanism to restore protein homeostasis. Mol Cell 53(5):685-7 doi:10.1016/j.molcel.2014.02.023

Keasling JD (1997) Regulation of intracellular toxic metals and other cations by hydrolysis of polyphosphate. Ann N Y Acad Sci 829:242-9

Keasling JD, Hupf GA (1996) Genetic manipulation of polyphosphate metabolism affects cadmium tolerance in Escherichia coli. Appl Environ Microbiol 62(2):743-6

Keasling JD, Van Dien SJ, Pramanik J (1998) Engineering polyphosphate metabolism in Escherichia coli: implications for bioremediation of inorganic contaminants. Biotechnol Bioeng 58(2-3):231-9

Kim KS, Rao NN, Fraley CD, Kornberg A (2002) Inorganic polyphosphate is essential for long-term survival and virulence factors in Shigella and Salmonella spp. Proc Natl Acad Sci U S A 99(11):7675-80 doi:10.1073/pnas.112210499

Kornberg A (1995) Inorganic polyphosphate: toward making a forgotten polymer unforgettable. J Bacteriol 177(3):491-6

Kornberg A (1999) Inorganic Polyphosphate: A Molecule of Many Functions. In: Schröder H, Müller WG (eds) Inorganic Polyphosphates. Progress in Molecular and Subcellular Biology, vol 23. Springer Berlin Heidelberg, pp 1-18

Kornberg A, Rao NN, Ault-Riche D (1999) Inorganic polyphosphate: a molecule of many functions. Annu Rev Biochem 68:89-125 doi:10.1146/annurev.biochem.68.1.89

Kukaev IS, Bobyk MA, Nikolaev NN, Sergeev NS, Uryson SO (1971) [Polyphosphate-synthesizing enzymes of some fungi and bacteria]. Biokhimiia 36(5):943-9

Kulaev IS, Vagabov VM, Kulakovskaya TV (2005) The Chemical Structures and Properties of Condensed Inorganic Phosphates The Biochemistry of Inorganic Polyphosphates. John Wiley & Sons, Ltd, pp 3-13

Kumble KD, Kornberg A (1995) Inorganic polyphosphate in mammalian cells and tissues. J Biol Chem 270(11):5818-22

Kuroda A, Nomura K, Ohtomo R, Kato J, Ikeda T, Takiguchi N, Ohtake H, Kornberg A (2001) Role of inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in E. coli. Science 293(5530):705-8 doi:10.1126/science.1061315

Kuroda A, Ohtake H (2000) Molecular analysis of polyphosphate accumulation in bacteria. Biochemistry (Mosc) 65(3):304-8

Lander N, Ulrich PN, Docampo R (2013) Trypanosoma brucei vacuolar transporter chaperone 4 (TbVtc4) is an acidocalcisome polyphosphate kinase required for in vivo infection. J Biol Chem 288(47):34205-16 doi:10.1074/jbc.M113.518993

Lipmann F (1965) Projecting Backward from the Present Stage of Evolution of Biosynthesis. In: Fox SW (ed) The Origins of Prebiological Systems and of their Molecular Matrices. Academic Press, pp 259-280

Lohrmann R, Orgel LE (1968) Prebiotic synthesis: phosphorylation in aqueous solution. Science 161(3836):64-6

Lonetti A, Szijgyarto Z, Bosch D, Loss O, Azevedo C, Saiardi A (2011) Identification of an evolutionarily conserved family of inorganic polyphosphate endopolyphosphatases. J Biol Chem 286(37):31966-74 doi:10.1074/jbc.M111.266320

Maciag A, Peano C, Pietrelli A, Egli T, De Bellis G, Landini P (2011) In vitro transcription profiling of the sigmaS subunit of bacterial RNA polymerase: re-definition of the sigmaS regulon and identification of sigmaS-specific promoter sequence elements. Nucleic Acids Res 39(13):5338-55 doi:10.1093/nar/gkr129

Manganelli R, Voskuil MI, Schoolnik GK, Smith I (2001) The Mycobacterium tuberculosis ECF sigma factor σE: role in global gene expression and survival in macrophages†. Molecular Microbiology 41(2):423-437 doi:10.1046/j.1365-2958.2001.02525.x

McMeechan A, Lovell MA, Cogan TA, Marston KL, Humphrey TJ, Barrow PA (2007) Inactivation of ppk differentially affects virulence and disrupts ATP homeostasis in Salmonella enterica serovars Typhimurium and Gallinarum. Res Microbiol 158(1):79-85 doi:10.1016/j.resmic.2006.10.008

Mensah JA, Koch AM, Antunes PM, Kiers ET, Hart M, Bucking H (2015) High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. Mycorrhiza 25(7):533-46 doi:10.1007/s00572-015-0631-x

Miller SL, Parris M (1964) Synthesis of Pyrophosphate Under Primitive Earth Conditions. Nature 204(4965):1248-1250

Montalvetti A, Rohloff P, Docampo R (2004) A functional aquaporin co-localizes with the vacuolar proton pyrophosphatase to acidocalcisomes and the contractile vacuole complex of Trypanosoma cruzi. J Biol Chem 279(37):38673-82 doi:10.1074/jbc.M406304200

Moreno SN, Docampo R (2013) Polyphosphate and its diverse functions in host cells and pathogens. PLoS Pathog 9(5):e1003230 doi:10.1371/journal.ppat.1003230

Motomura K, Hirota R, Okada M, Ikeda T, Ishida T, Kuroda A (2014) A new subfamily of polyphosphate kinase 2 (class III PPK2) catalyzes both nucleoside monophosphate phosphorylation and nucleoside diphosphate phosphorylation. Appl Environ Microbiol 80(8):2602-8 doi:10.1128/aem.03971-13

Muller O, Bayer MJ, Peters C, Andersen JS, Mann M, Mayer A (2002) The Vtc proteins in vacuole fusion: coupling NSF activity to V(0) trans-complex formation. EMBO J 21(3):259-69 doi:10.1093/emboj/21.3.259

Nomura K, Kato J, Takiguchi N, Ohtake H, Kuroda A (2004) Effects of inorganic polyphosphate on the proteolytic and DNA-binding activities of Lon in Escherichia coli. J Biol Chem 279(33):34406-10 doi:10.1074/jbc.M404725200

Ogawa N, DeRisi J, Brown PO (2000a) New components of a system for phosphate accumulation and polyphosphate metabolism in Saccharomyces cerevisiae revealed by genomic expression analysis. Mol Biol Cell 11(12):4309-21

Ogawa N, Tzeng CM, Fraley CD, Kornberg A (2000b) Inorganic polyphosphate in Vibrio cholerae: genetic, biochemical, and physiologic features. J Bacteriol 182(23):6687-93

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

Pan-Hou H, Kiyono M, Omura H, Omura T, Endo G (2002) Polyphosphate produced in recombinant Escherichia coli confers mercury resistance. FEMS Microbiol Lett 207(2):159-64

Pavlov E, Aschar-Sobbi R, Campanella M, Turner RJ, Gomez-Garcia MR, Abramov AY (2010) Inorganic polyphosphate and energy metabolism in mammalian cells. J Biol Chem 285(13):9420-8 doi:10.1074/jbc.M109.013011

Pettersson A, Kunst, Ljerka, Bergman, Birgitta, Roomans, Godfried M. (1985) Accumulation of Aluminium by Anabaena cylindrica into Polyphosphate Granules and Cell Walls: an X-ray Energy-dispersive Microanalysis Study. Microbiology 131(10):2545-2548 doi:doi:10.1099/00221287-131-10-2545

Rao NN, Gomez-Garcia MR, Kornberg A (2009) Inorganic polyphosphate: essential for growth and survival. Annu Rev Biochem 78:605-47 doi:10.1146/annurev.biochem.77.083007.093039

Rao NN, Liu S, Kornberg A (1998) Inorganic polyphosphate in Escherichia coli: the phosphate regulon and the stringent response. J Bacteriol 180(8):2186-93

Rashid MH, Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 97(9):4885-90 doi:10.1073/pnas.060030097

Rashid MH, Rao NN, Kornberg A (2000a) Inorganic polyphosphate is required for motility of bacterial pathogens. J Bacteriol 182(1):225-7

Rashid MH, Rumbaugh K, Passador L, Davies DG, Hamood AN, Iglewski BH, Kornberg A (2000b) Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 97(17):9636-41 doi:10.1073/pnas.170283397

Reddi AR, Jensen LT, Naranuntarat A, Rosenfeld L, Leung E, Shah R, Culotta VC (2009) The overlapping roles of manganese and Cu/Zn SOD in oxidative stress protection. Free Radic Biol Med 46(2):154-62 doi:10.1016/j.freeradbiomed.2008.09.032

Remonsellez F, Orell A, Jerez CA (2006) Copper tolerance of the thermoacidophilic archaeon Sulfolobus metallicus: possible role of polyphosphate metabolism. Microbiology 152(Pt 1):59-66 doi:10.1099/mic.0.28241-0

Renninger N, Knopp R, Nitsche H, Clark DS, Keasling JD (2004) Uranyl precipitation by Pseudomonas aeruginosa via controlled polyphosphate metabolism. Appl Environ Microbiol 70(12):7404-12 doi:10.1128/aem.70.12.7404-7412.2004

Reusch RN (1989) Poly-beta-hydroxybutyrate/calcium polyphosphate complexes in eukaryotic membranes. Proc Soc Exp Biol Med 191(4):377-81

Reusch RN, Sadoff HL (1988) Putative structure and functions of a poly-beta-hydroxybutyrate/calcium polyphosphate channel in bacterial plasma membranes. Proc Natl Acad Sci U S A 85(12):4176-80

Sanyal S, Banerjee SK, Banerjee R, Mukhopadhyay J, Kundu M (2013) Polyphosphate kinase 1, a central node in the stress response network of Mycobacterium tuberculosis, connects the two-component systems MprAB and SenX3-RegX3 and the extracytoplasmic function sigma factor, sigma E. Microbiology 159(Pt 10):2074-86 doi:10.1099/mic.0.068452-0

Schomburg D, Stephan D (1997) Dolichyl-diphosphate-polyphosphate phosphotransferase. In: Schomburg D, Stephan D (eds) Enzyme Handbook. Springer Berlin Heidelberg, pp 417-419

Segawa S, Fujiya M, Konishi H, Ueno N, Kobayashi N, Shigyo T, Kohgo Y (2011) Probiotic-derived polyphosphate enhances the epithelial barrier function and maintains intestinal homeostasis through integrin-p38 MAPK pathway. PLoS One 6(8):e23278 doi:10.1371/journal.pone.0023278

Seidlmayer LK, Blatter LA, Pavlov E, Dedkova EN (2012a) Inorganic polyphosphate--an unusual suspect of the mitochondrial permeability transition mystery. Channels (Austin) 6(6):463-7 doi:10.4161/chan.21939

Seidlmayer LK, Gomez-Garcia MR, Blatter LA, Pavlov E, Dedkova EN (2012b) Inorganic polyphosphate is a potent activator of the mitochondrial permeability transition pore in cardiac myocytes. J Gen Physiol 139(5):321-31 doi:10.1085/jgp.201210788

Sethuraman A, Rao NN, Kornberg A (2001) The endopolyphosphatase gene: essential in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 98(15):8542-7 doi:10.1073/pnas.151269398

Shi X, Rao NN, Kornberg A (2004) Inorganic polyphosphate in Bacillus cereus: motility, biofilm formation, and sporulation. Proc Natl Acad Sci U S A 101(49):17061-5 doi:10.1073/pnas.0407787101

Shiba T, Tsutsumi K, Yano H, Ihara Y, Kameda A, Tanaka K, Takahashi H, Munekata M, Rao NN, Kornberg A (1997) Inorganic polyphosphate and the induction of rpoS expression. Proceedings of the National Academy of Sciences 94(21):11210-11215

Spudich JA (2004) Two important polymers cross paths. Proc Natl Acad Sci U S A 101(45):15825-6 doi:10.1073/pnas.0406932101

Szymona M, Ostrowski W (1964) Inorganic polyphosphate glucokinase of Mycobacterium phlei. Biochimica et Biophysica Acta (BBA) - Specialized Section on Enzymological Subjects 85(2):283-295 doi:http://dx.doi.org/10.1016/0926-6569(64)90249-4

Szymona O, Szymona M (1978) Multiple forms of polyphosphate-glucose phosphotransferase in various Mycobacterium strains. Acta Microbiol Pol 27(1):73-6

Tammenkoski M, Koivula K, Cusanelli E, Zollo M, Steegborn C, Baykov AA, Lahti R (2008) Human metastasis regulator protein H-prune is a short-chain exopolyphosphatase. Biochemistry 47(36):9707-13 doi:10.1021/bi8010847

Tanaka S, Lee SO, Hamaoka K, Kato J, Takiguchi N, Nakamura K, Ohtake H, Kuroda A (2003) Strictly polyphosphate-dependent glucokinase in a polyphosphate-accumulating bacterium, Microlunatus phosphovorus. J Bacteriol 185(18):5654-6

Tinsley CR, Gotschlich EC (1995) Cloning and characterization of the meningococcal polyphosphate kinase gene: production of polyphosphate synthesis mutants. Infect Immun 63(5):1624-30

Tzeng CM, Kornberg A (1998) Polyphosphate kinase is highly conserved in many bacterial pathogens. Mol Microbiol 29(1):381-2

Tzeng CM, Kornberg A (2000) The multiple activities of polyphosphate kinase of Escherichia coli and their subunit structure determined by radiation target analysis. J Biol Chem 275(6):3977-83

Uttenweiler A, Schwarz H, Neumann H, Mayer A (2007) The vacuolar transporter chaperone (VTC) complex is required for microautophagy. Mol Biol Cell 18(1):166-75 doi:10.1091/mbc.E06-08-0664

Vagabov VM, Trilisenko LV, Kulaev IS (2000) Dependence of inorganic polyphosphate chain length on the orthophosphate content in the culture medium of the yeast Saccharomyces cerevisiae. Biochemistry (Mosc) 65(3):349-54

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblizek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS, Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458(7234):69-72 doi:http://www.nature.com/nature/journal/v458/n7234/suppinfo/nature07659_S1.html

Whitehead MP, Eagles L, Hooley P, Brown MR (2014) Most bacteria synthesize polyphosphate by unknown mechanisms. Microbiology 160(Pt 5):829-31 doi:10.1099/mic.0.075366-0

Young TW, Kuhn NJ, Wadeson A, Ward S, Burges D, Cooke GD (1998) Bacillus subtilis ORF yybQ encodes a manganese-dependent inorganic pyrophosphatase with distinctive properties: the first of a new class of soluble pyrophosphatase? Microbiology 144 (Pt 9):2563-71

Zhang F, Blasiak LC, Karolin JO, Powell RJ, Geddes CD, Hill RT (2015) Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. Proc Natl Acad Sci U S A 112(14):4381-6 doi:10.1073/pnas.1423768112

Zhang H, Gomez-Garcia MR, Shi X, Rao NN, Kornberg A (2007) Polyphosphate kinase 1, a conserved bacterial enzyme, in a eukaryote, Dictyostelium discoideum, with a role in cytokinesis. Proc Natl Acad Sci U S A 104(42):16486-91 doi:10.1073/pnas.0706847104

Zhang H, Ishige K, Kornberg A (2002) A polyphosphate kinase (PPK2) widely conserved in bacteria. Proc Natl Acad Sci U S A 99(26):16678-83 doi:10.1073/pnas.262655199

FIGURE CAPTIONS

Figure 1. Representative schematic structures of the three main polyPs structural classes. (a) Linear polyphosphates, (b) cyclic polyphosphates (also named metaphosphates), and (c) branched polyphosphates (also named ultraphosphates).

Figure 2. Functions of polyPs in prokaryotic and eukaryotic microorganisms. In most cases the indicated functions have been reported for both prokaryotes (bacteria, archaea) and eukaryotes (fungi, microalgae and parasitic and free-living heterotrophic protists).
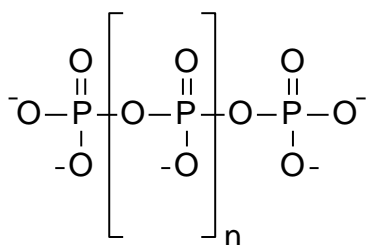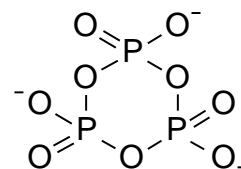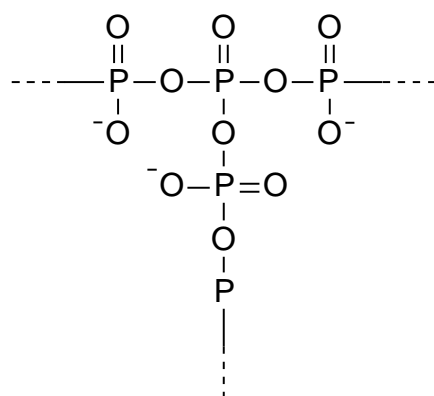
(a)

(b)

Linear polyphosphate

Cyclic tripolyphosphate
(metaphosphate)

(c)

Branched polyphosphate
(ultraphosphate)

Fig. 1

Figure 2

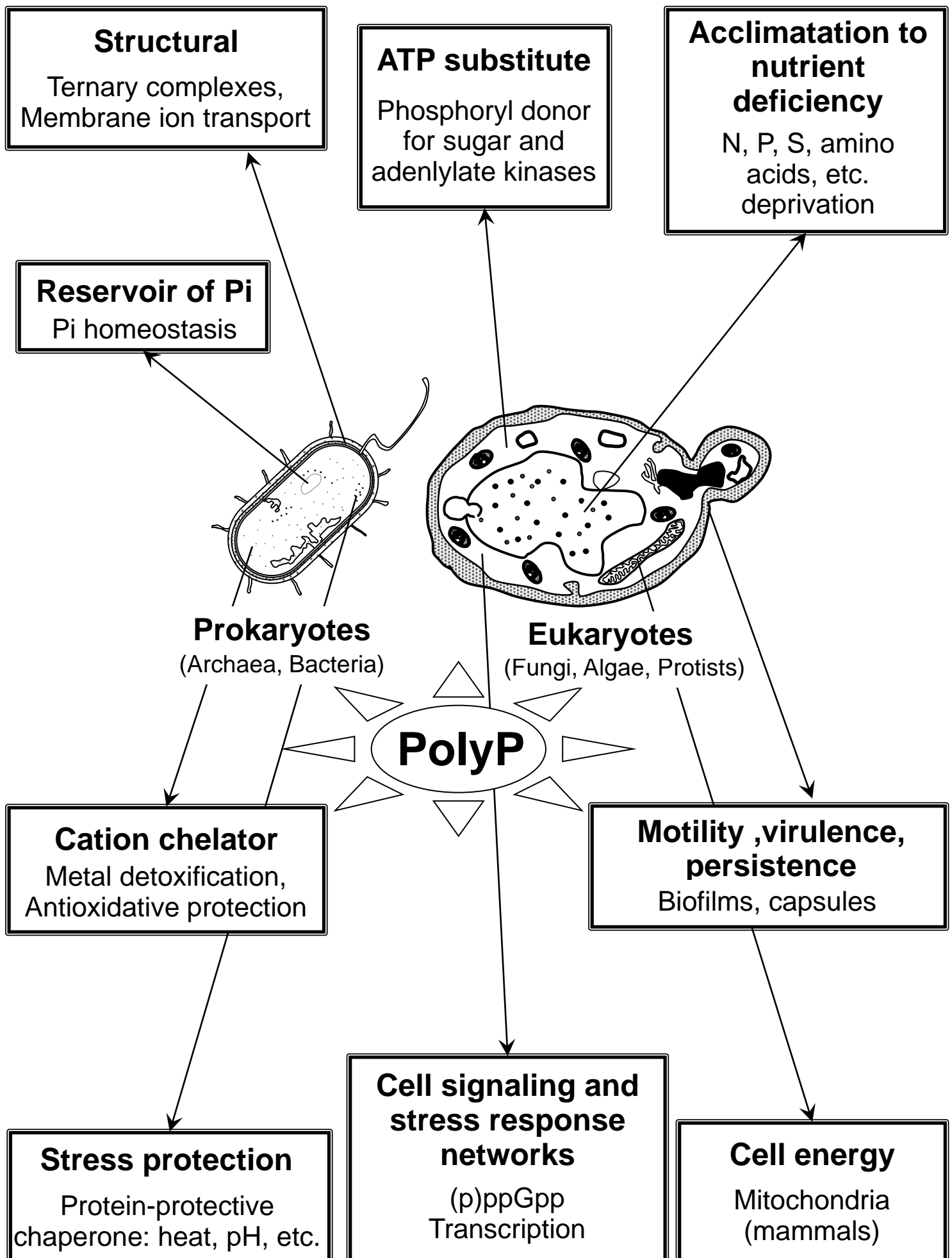**Structural**

Ternary complexes,
Membrane ion transport

ATP substitute

Phosphoryl donor
for sugar and
adenlylate kinases

Acclimatation to nutrient deficiency

N, P, S, amino
acids, etc.
deprivation

Reservoir of Pi
Pi homeostasis

Prokaryotes
(Archaea, Bacteria)

Eukaryotes
(Fungi, Algae, Protists)

PolyP

Cation chelator
Metal detoxification,
Antioxidative protection

Motility ,virulence, persistence
Biofilms, capsules

Stress protection

Protein-protective
chaperone: heat, pH, etc.

Cell signaling and stress response networks

(p)ppGpp
Transcription

Cell energy

Mitochondria
(mammals)

Fig. 2