Polyphosphate and Its Diverse Functions in Host Cells and Pathogens

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Polyphosphate (polyP) is a linear polymer of a few to many hundreds of phosphate (P) residues linked by high-energy phosphoanhydride bonds (Figure 1A). This ubiquitous polymer is found in bacteria, protists, and mammalian cells, and it was likely present prebiotically [1]. In bacteria, polyP accumulates in the vacuolar transporter chaperone 4, or VTC4 [16]. VTC4 is part of a complex of VTC proteins that are present in fungi, algae, trypanosomatids [17], and apicomplexans [18], but is absent in mammalian cells. Another potential pathway for polyP synthesis in yeast is through the metabolism of inositol pyrophosphates (InsPP) [19], but it is not known whether this pathway is operative in other organisms. Yeast deficient in phosophoinositide phospholipase C (PI-PLC) are depleted of polyP and a pathway for polyP synthesis via InsPP (also known as diphosphoinositol polyphosphates) was postulated. Diphosphoinositol tetraakisphosphate (PP-IP4) is the precursor proposed for polyP

Acidocalcisomes and PolyP

Acidocalcisomes were first described in trypanosomes and later found in Apicomplexan parasites, algae, slime molds, fungi, eggs of different origins, and human cells [3]. These organelles were originally described as acidic compartments storing high concentrations of calcium, and later work found that they are highly enriched in polyP [2]. As the description of acidocalcisomes progressed over the years, it was found that they are similar to the vacuolar or metachromatic granules described in bacteria and are now considered to be the only organelles enriched in polyP and the endo- (PPNs) and exopolyphosphatases (PPXs), respectively. Bacteria express one or two polyP kinases: PPK1, which catalyzes the reversible transfer of P, from ATP to polyP and from polyP to ADP, and PPK2, which catalyzes the synthesis of polyP from GTP or ATP [7]. Bacteria also have PPXs but no PPNs [7]. Genes encoding eukaryotic PPNs [8] and PPXs [9] were initially reported in Saccharomyces cerevisiae. Recombinant PPXs from Leishmania major [10], Trypanosoma cruzi [11], and human cells (H-prune) [12] have also been characterized. A role for polyP in cancer has been proposed based on the role of the human PPX on tumor metastasis [12]. Another interesting finding in this regard is that polyP could activate mTOR kinase, a key step in the proliferation of mammalian cancer cells [13]. A putative polyP kinase gene of bacterial origin (DdPPK1) was found in Dictyostelium discoideum [14] together with a second distinct polyP kinase (DdPPK2), which is apparently localized to the acidocalcisome [15]. DdPPK2 has a similar sequence and shares characteristics of actin-related proteins, which in turn are similar to muscle actins. Actin inhibitors such as phalloidin and DNase I also inhibit DdPPK2-mediated synthesis of polyP. Thus, this particular actin-related protein complex is an enzyme that can polymerize into an actin-like filament concurrent with its synthesis of a polyP chain in a fully reversible reaction [15]. Recent work in yeast identified the first eukaryotic enzyme involved in the synthesis and translocation of polyP to a vacuolar compartment: the vacuolar transporter chaperone 4, or VTC4 [16]. VTC4 is part of a complex of VTC proteins that are present in fungi, algae, trypanosomatids [17], and apicomplexans [18], but is absent in mammalian cells. Another potential pathway for polyP synthesis in yeast is through the metabolism of inositol pyrophosphates (InsPP) [19], but it is not known whether this pathway is operative in other organisms. Yeast deficient in phosphoinositide phospholipase C (PI-PLC) are depleted of polyP and a pathway for polyP synthesis via InsPP (also known as diphosphoinositol polyphosphates) was postulated. Diphosphoinositol tetraakisphosphate (PP-IP4) is the precursor proposed for polyP

Enzymes Involved in PolyP Metabolism

The concentration of polyP in cells is the result of the action of enzymes that catalyze the synthesis and degradation of this polymer—namely, the polyP kinases and the endo- (PPNs) and exopolyphosphatases (PPXs), respectively. Bacteria express one or two polyP kinases: PPK1, which catalyzes the reversible transfer of P, from ATP to polyP and from polyP to ADP, and PPK2, which catalyzes the synthesis of polyP from GTP or ATP [7]. Bacteria also have PPXs but no PPNs [7]. Genes encoding eukaryotic PPNs [8] and PPXs [9] were initially reported in Saccharomyces cerevisiae. Recombinant PPXs from Leishmania major [10], Trypanosoma cruzi [11], and human cells (H-prune) [12] have also been characterized. A role for polyP in cancer has been proposed based on the role of the human PPX on tumor metastasis [12]. Another interesting finding in this regard is that polyP could activate mTOR kinase, a key step in the proliferation of mammalian cancer cells [13]. A putative polyP kinase gene of bacterial origin (DdPPK1) was found in Dictyostelium discoideum [14] together with a second distinct polyP kinase (DdPPK2), which is apparently localized to the acidocalcisome [15]. DdPPK2 has a similar sequence and shares characteristics of actin-related proteins, which in turn are similar to muscle actins. Actin inhibitors such as phalloidin and DNase I also inhibit DdPPK2-mediated synthesis of polyP. Thus, this particular actin-related protein complex is an enzyme that can polymerize into an actin-like filament concurrent with its synthesis of a polyP chain in a fully reversible reaction [15]. Recent work in yeast identified the first eukaryotic enzyme involved in the synthesis and translocation of polyP to a vacuolar compartment: the vacuolar transporter chaperone 4, or VTC4 [16]. VTC4 is part of a complex of VTC proteins that are present in fungi, algae, trypanosomatids [17], and apicomplexans [18], but is absent in mammalian cells. Another potential pathway for polyP synthesis in yeast is through the metabolism of inositol pyrophosphates (InsPP) [19], but it is not known whether this pathway is operative in other organisms. Yeast deficient in phosphoinositide phospholipase C (PI-PLC) are depleted of polyP and a pathway for polyP synthesis via InsPP (also known as diphosphoinositol polyphosphates) was postulated. Diphosphoinositol tetraakisphosphate (PP-IP4) is the precursor proposed for polyP

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synthesis [19]. The polyP synthesis pathway in mammalian cells is still unknown.

Functions of PolyP

The function of polyP has been studied mainly in prokaryotes: as a P_i store, an energy source to replace ATP, in cation sequestration and storage, in cell membrane formation and function, in gene transcription control, in regulation of enzyme activities, in response to stress and stationary phase, and in the structure of channels and pumps [reviewed in [20]]. Kornberg’s group has also described the roles of polyP in the physiological adjustments of bacteria to growth, development, stress, and deprivation; its role in biofilm development, quorum sensing, and virulence; as well as in long-term survival and expression of virulence factors [reviewed in [1]]. The functions of polyP in eukaryotic cells are not so well defined. Functions in apoptosis, enhancement of mitogenic activity of fibroblast growth factor, and in bone mineralization have been reviewed elsewhere [21]. Some critical discoveries about the function of polyP in mammalian cells have renewed interest in studying them in these cells. It was first demonstrated that polyP is stored in the dense granules of human platelets and in mast cell granules (acidocalcisomes) and released upon their activation [22,23]. It was also shown that polyPs have a potent modulatory activity on blood coagulation [24] and inflammation [25]. Recent studies have demonstrated that polyP acts at four points in the blood-clotting cascade (reviewed by [26]). PolyP initiation of the contact pathway by activating Factor XII to Factor XIIa also leads to bradykinin formation by kallikrein-mediated high molecular weight kininogen cleavage [25]. Bradykinin is the ligand of kinin B2 receptor, which activates various intracellular signaling pathways that lead to inflammatory reactions (reviewed in [27]).

A function for polyP in adaptation to stress and osmoregulation has been assigned in less complex eukaryotic cells such as yeast, fungi, algae, and trypanosomes [reviewed in [28]]. PolyP is particularly abundant in pathogenic fungi and trypanosomes. It accounts for nearly 40% of the total phosphate content of S.
cerevisiae [9] and reaches levels >100 mM in P₃ residues, assuming distribution across the entire volume of the cell, in trypanosomes such as Trypanosoma brucei, T. cruzi, and L. major [2], and there are drastic changes in their levels upon osmotic stress [29].

Role of PolyP in Pathogenesis

PolyP, which in bacteria is mainly of long-chain type (>300 and up to 1,000 P₃ residues), has been reported to be important for virulence of different bacteria, such as Salmonella spp., Shigella flexneri, Vibrio cholerae, Neisseria meningitides, Pseudomonas aeruginosa, and Mycobacterium tuberculosis, but the mechanism involved is not known [30]. It has also been reported that conditions that decrease the levels of polyP in parasites such as T. brucei, T. gondii, or L. major (reviewed in [3]) reduce their pathogenicity. Whether this is due to osmotic fragility of the parasites as a result of changes in polyP levels that impact their ability to grow in vivo, making the immune response against them more successful, or to a role of polyP in modulating the immune response is not yet known.

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Concluding Remarks

The late Prof. Arthur Kornberg once stated [30]: “not only is polyP often absent from texts of biology and chemistry but, even when noticed, tends to be dismissed as a molecular fossil.” Considering the wide distribution of this polymer and the diversity of functions that has been attributed to it, it is expected that future research will reveal new findings about this understudied compound. PolyP has been found in bacterial to human cells and has been reported to be important for virulence of different bacteria and a number of parasites, including those that cause toxoplasmosis, African trypanosomiasis, and leishmaniasis. Even more exciting are the findings about the role of polyP in cancer metastasis, blood coagulation, inflammation, and innate immunity. For example, a significant finding is that enzymes involved in polyP metabolism could be excellent targets for drug design not only against bacteria and parasites but also for regulation of important physiological and pathological processes such as coagulation, inflammation, innate immunity, and thrombosis.