

Inorganic polyphosphate, a multifunctional polyanionic protein scaffold

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Polyphosphate (polyP) consists of a linear arrangement of inorganic phosphates and defies its structural simplicity with an astounding number of different activities in the cell. Already well known for its ability to partake in phosphate, calcium, and energy metabolism, polyP recently gained a new functional dimension with the discovery that it serves as a stabilizing scaffold for protein-folding intermediates. In this review, we summarize and discuss the recent studies that have identified polyP not only as a potent protein-like chaperone that protects cells against stress-induced protein aggregation, but also as a robust modifier of amyloidogenic processes that shields neuronal cells from amyloid toxicity. We consider some of the most pressing questions in the field, the obstacles faced, and the potential avenues to take to provide a complete picture about the working mechanism and physiological relevance of this intriguing biopolymer.

Polyphosphate (polyP)² consists of a minimum of three up to 1000 inorganic phosphates, connected via high-energy phosphoanhydride bonds (Fig. 1). PolyP originally emerged during volcanic eruptions and is thought to have served as one of the first energy-rich molecules on earth (1). Early work revealed that some microorganisms accumulate polyP in dense, so-called metachromatic granules, which stain red with toluidine blue and are easily visible with a light microscope (2). However, few studies reported on the potential physiological role(s) of polyP in bacteria or any other organism, causing the polyP field to lay dormant for much of the 20th century. This situation changed, however, once polyP caught the attention of Sylvie Kornberg and later that of her husband Arthur Kornberg, who spent the last 15 years of his career investigating the “forgotten polymer” (3). Kornberg and his team developed many of the currently available polyP detection and quantification methods (4, 5) and, most importantly, identified the enzyme responsible for polyP synthesis in bacteria (6, 7). This breakthrough discov-

ery enabled them, for the first time, to genetically manipulate polyP synthesis in a living organism and hence to systematically evaluate the physiological consequences of polyP depletion. It turned out that polyP fulfills a number of different functions in bacteria: polyP production was found to increase stress resistance, motility, and biofilm formation (5, 8–12) as well as to contribute to sporulation, quorum-sensing, and virulence (11–13). In eukaryotes, polyP appears to play an equally large number of diverse roles, ranging from stimulating blood clotting through the activation of factor XII to chelating calcium for bone mineralization, activating mTOR, and triggering apoptosis (14–17). This amalgam of seemingly unrelated functions (Fig. 1) poses new and even more intriguing questions as to how such a simple polyanion like polyP can fulfill all these different roles (Table 1). We will review the many known facets of polyP activity in pro- and eukaryotic organisms and explore whether polyP’s ability to function as a protein-stabilizing scaffold might serve as one of the unifying principles of polyP action.

PolyP biosynthesis and regulation in bacteria

Many bacteria, including numerous pathogens, encode the nonessential enzyme polyphosphate kinase (PPK1), which catalyzes the reversible transfer of the terminal γ -phosphate of ATP to polyP (the *in vivo* starting molecule is not yet known) (6). In addition to PPK1, some microorganisms encode a second, structurally unrelated kinase, PPK2, which catalyzes the transfer of terminal P_i from polyP to GDP to form GTP (7, 18, 19). Most, if not all, of these bacteria also encode the exopolyphosphatase PPX, which hydrolyzes polyP into individual phosphates, thereby indirectly utilizing the cellular ATP pool to maintain phosphate homeostasis (20).

Steady-state concentrations of polyP in the bacterial cytosol are typically in the low micromolar range, even in mutant strains that lack the polyphosphatase PPX (21). These results suggest that the levels and/or activity of PPK must be tightly regulated, a conclusion that makes energetic sense given that polyP synthesis draws from the cellular ATP pool. Yet, upon nutrient shift (4, 22) or exposure to osmotic changes (4), acidic pH (23), oxidants such as hypochlorous acid (24), or very high temperatures (25), bacteria rapidly accumulate high levels of polyP. In fact, under some of these stress conditions, bacteria have been shown to convert millimolar amounts of ATP into extremely ($>1000 P_i$) long chains of polyP, decreasing their cellular ATP pool by up to 30–50% in the process (24). Not surprisingly, preventing bacteria from synthesizing polyP

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² The abbreviations used are: polyP, polyphosphate; mTOR, mechanistic target of rapamycin; PPX, polyphosphatase; PPK, polyphosphate kinase; mPTP, mitochondrial permeability transition pore.

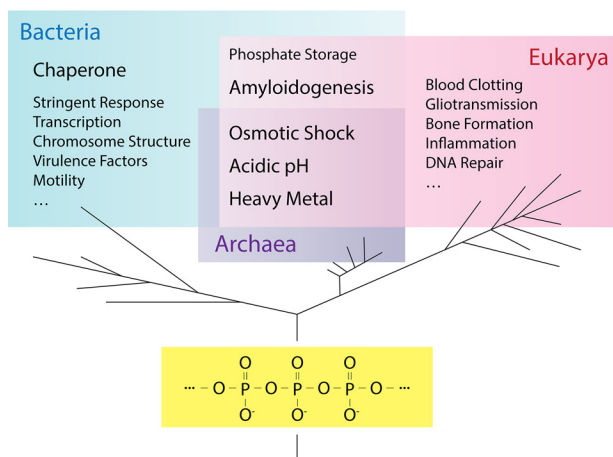


Figure 1. PolyP, a simple, universal, and highly multifunctional polyanion. The linear polyP chain consists of minimally three and maximally up to about 1000 orthophosphates linked by high-energy phosphoanhydride bonds. PolyP has been identified in all organisms tested so far, where it fulfills an astonishing array of different functions.

(i.e. by deleting *ppk*) makes them exquisitely sensitive to nutrient deprivation, oxidants, or high temperature (24–28). In addition, deletion of the *ppk* gene has been shown to decrease bacterial pathogenicity in a comprehensive manner, i.e. by reducing motility, colonization, virulence factor production, and biofilm formation (Table 1) (28, 29).

Much of Kornberg's research focused on how bacterial polyP synthesis is regulated and which potential transcriptional regulators might control *ppk* gene expression (4, 5, 22). These studies revealed that the *ppk* gene is a direct target of σ^{38} , the master transcriptional regulator for late stationary phase genes (30). PolyP, in turn, induces transcription of *rpoS* (the gene encoding σ^{38}) and further amplifies its own synthesis (22, 28, 31). Although these results nicely explained the increased PPK and polyP levels observed in stationary phase bacteria, they failed to reveal how polyP synthesis is regulated during *rpoS*-independent stress conditions, such as oxidative or heat stress. As it turns out, in contrast to many stress-induced transcriptional responses, polyP synthesis appears to be primarily regulated on a post-transcriptional and/or post-translational level. In *Pseudomonas fluorescens*, for instance, mRNA transcripts of the *ppk* gene have been shown to be targeted by antisense RNA, which fine-tunes PPK synthesis and hence regulates polyP abundance (32). In *Escherichia coli* and other tested Gram-negative bacteria, nutrient shift-induced up-regulation of polyP has been proposed to be directly mediated by ppGpp, a secondary metabolite that quickly accumulates upon nutrient deprivation (4, 5, 33). This metabolite is thought to directly inhibit PPX, thereby preventing polyP hydrolysis (34). Similarly, our studies in HOCl-treated *E. coli* cells revealed that polyP accumulation is, at least in part, mediated by the reversible inactivation of PPX. In this case, reversible oxidation of a critical cysteine, located in the polyP-binding site, directly inactivates PPX until reducing conditions are restored (24). Despite these insights, it is clear that inactivation of PPX is only part of the polyP accumulation story. Because *ppx* deletion strains do not accumulate polyP in the absence of stress and PPK levels do not seem to significantly increase upon nutrient shift or other stress

conditions in *E. coli* (21, 35, 36), it is highly likely for PPK to be post-translationally regulated, either directly or through stress-sensitive regulators. Once PPK is activated and polyP is synthesized, however, transient inactivation of PPX will guarantee that polyP levels remain high until nonstress conditions are restored.

PolyP in eukaryotic organisms

Intriguingly, the polyP-synthesizing machinery is not universally conserved, and for most eukaryotic organisms, the mechanism by which polyP is synthesized remains unknown (37). One exception is the baker's yeast *Saccharomyces cerevisiae*, where the vacuolar transporter chaperone 4 (VTC4) was found to use ATP to generate polyP and concomitantly transport the polyanion into the vacuole (38, 39). Vacuolar polyP serves a central role in maintaining phosphate homeostasis, sequestering phosphate during growth in phosphate-rich environments (38), and releasing phosphate during the cell cycle to provide the building blocks for DNA replication (40). Moreover, polyP chelates metals, such as manganese and cadmium, thereby preventing metal-induced cellular damage (41, 42), and sequesters arginine to increase nitrogen storage without affecting the osmotic pressure (43). One other eukaryotic polyP-generating system has been proposed to exist in *Dictyostelium discoideum*, which, in addition to a bacterial PPK homologue, contains a tripartite complex of actin-related proteins that was found to synthesize polyP *ex vivo* (44). Unfortunately, however, the protein(s) responsible for the polyP-synthesizing activity have not yet been identified. No sequence or structural homology exists between the known polyP-synthesizing enzymes PPK1, PPK2, or Vtc4, and respective homologues have not been found in higher eukaryotic species, leaving the field for new discoveries wide open.

The earliest studies about the distribution of polyP in mammalian cells used subcellular fractionation and biochemical quantification assays. Kumble and Kornberg (45) reported that polyP is present in the nucleus, plasma membrane, cytoplasm, and intracellular organelles. Staining of cells and tissues for polyP using 4',6-diamidino-2-phenylindole (DAPI), whose excitation and emission spectra shift significantly when bound to polyP instead of DNA (46) as well as recently developed polyP-specific probes, including fusion proteins with the polyP-binding domain of PPX (47, 48) or JC-D7/8 (49), supported these results. Moreover, they demonstrated that polyP is particularly enriched in the nucleolus, acidocalcisomes (organelles rich in protons, calcium, and phosphorus), and mitochondria. Moreover, polyP was found to be secreted from astrocytes and subsequently taken up by neurons, suggesting that it is present both inside and outside of the cell (50, 51). Apart from certain cell types such as thrombocytes and mast cells, which store up to 130 mM P_i in the form of medium-sized polyP chains in dense granules (14, 48, 52), eukaryotic polyP levels are generally in the nanomolar range and the chain lengths range between 50 and 800 P_i (45). Brain tissue appears to contain some of the highest concentrations of polyP ($\sim 100 \mu\text{M}$) based on tissue-wide studies in rats and mice (45, 53). So far, no trigger for increased polyP synthesis has been reported in mammalian cells. However, brain polyP levels do seem to decline with age and disease

Table 1
The physiological functions of polyP

Species	Function	Subcellular localization (if described)	Refs.
<i>E. coli</i>	Stringent response		4, 5
	Stationary phase survival		8, 26
	Stress resistance (heat, oxidants, osmotic shock, acidic pH, and heavy metal)		8, 24–26, 28, 79
	Biofilm formation		28, 54
	Persistence		28
	Transcription		22, 28, 31
	Translation	Ribosome	77
	DNA entry	Membrane	118
<i>Pseudomonas aeruginosa</i>	Cellular structures	Nucleoid	29
	Motility		9, 10, 29
	Stress resistance (oxidants, desiccation)		27, 29
	Biofilm formation		12, 29
	Virulence		12
	Quorum sensing		12
	Colonization		28
<i>S. cerevisiae</i>	DNA synthesis and repair	Nucleus	40, 119
	Post-translational modification (polyphosphorylation)	Nucleus/nucleolus	58, 120
	Phosphate homeostasis	Vacuole	38
	Arginine sequestration	Vacuole	43
	Stress resistance (heavy metal)	Cytoplasm	41, 42
<i>D. discoideum</i>	Development and germination		121, 122
	Predation		121
	Cytokinesis		123
<i>Trypanosoma brucei</i> <i>Trypanosoma cruzi</i>	Stress resistance (osmotic shock)		124–126
	Cytokinesis		124
	Infection		125, 127
<i>Homo sapiens</i>	Ion channel activation	Plasma membrane	59, 128
	Signaling (mTOR, FGF2, integrin β 1, P2Y ₁)	Plasma membrane	16, 50, 60, 61
	Mitochondrial activity (mPTP opening, Ca ²⁺ homeostasis, energy metabolism)	Mitochondria	17, 57
	Nucleolar function (rRNA transcription)	Nucleolus	47
	DNA repair	Nucleus	120
	Blood clotting	Secreted from polyP granules	14
	Inflammation	Secreted from polyP granules	14, 48, 129–132
	Bone mineralization	Secreted from polyP granules	15

state (*i.e.* Alzheimer's disease), suggesting that physiological events might affect polyP synthesis in mammalian species (54, 55).

Despite the challenges in elucidating the function of a molecule, whose abundance and/or activity cannot be directly manipulated via genetic approaches, we know a surprising amount about the processes that eukaryotic polyP appears to be involved in (Table 1). Much of this knowledge comes again from the Kornberg group, who generated the first mammalian cell line that stably expressed yeast PPX to degrade endogenous polyP (16). Pavlov and co-workers (17) applied a similar strategy to test the role of polyP in mitochondria, and Morrissey and co-workers (56) developed end-labeled polyP to monitor its dynamics and interactions. These studies produced ample evidence that polyP is either directly or indirectly involved in a myriad of different processes, including opening of the mitochondrial permeability transition pore (mPTP) and Ca²⁺ signaling (17, 57), activating plasma protease factor XII and forming fibrin fibers at pro-coagulant sites upon release from thrombocytes (14), and exerting pro-inflammatory responses upon release from mast cells (48). PolyP has also been proposed to function as a gliotransmitter in the autonomic nervous system (50) and to be involved in maintaining mitochondrial membrane potential (17). Finally, a variety of other proteins have been identified to stably or transiently interact with polyP, including mTOR, Nsr1, TRPM8, FGF-2, integrin β 1, as well as several ribosomal proteins and glycosomal enzymes,

resulting in altered activities and profound influences on cell growth and survival (16, 58–63). The underlying mechanism by which polyP interacts with these structurally unrelated proteins remains unknown.

PolyP—an inorganic polyanion with protein-like chaperone qualities

Molecular chaperones serve as the guardians of the proteome (64). Their primary functions are to support the *de novo* folding of newly translated polypeptides and prevent irreparable stress-induced misfolding of native proteins within the crowded and metastable cellular environment (65). Most chaperones are proteins, grouped into individual families that differ in structure, complexity, and regulation (66). The chaperone families range from energy-independent small proteins that function as monomeric (*e.g.* HdeA), dimeric (*e.g.* Hsp33), or high-molecular-weight oligomers (*e.g.* sHsps) to complex multichaperone machineries (*e.g.* TRIC, Hsp70, Hsp90) that use ATP binding and hydrolysis to regulate co-chaperone and substrate protein binding and release (67, 68). The structural and functional diversification among the individual chaperone families appears to contribute to their ability to cater to a wide variety of different client proteins that are present in vastly diverse folding environments (69). Given the highly sophisticated mechanisms that many of these chaperones employ, it came as a significant surprise when our lab discovered that the simple polyanion polyP exerts protein-like chaperone qualities

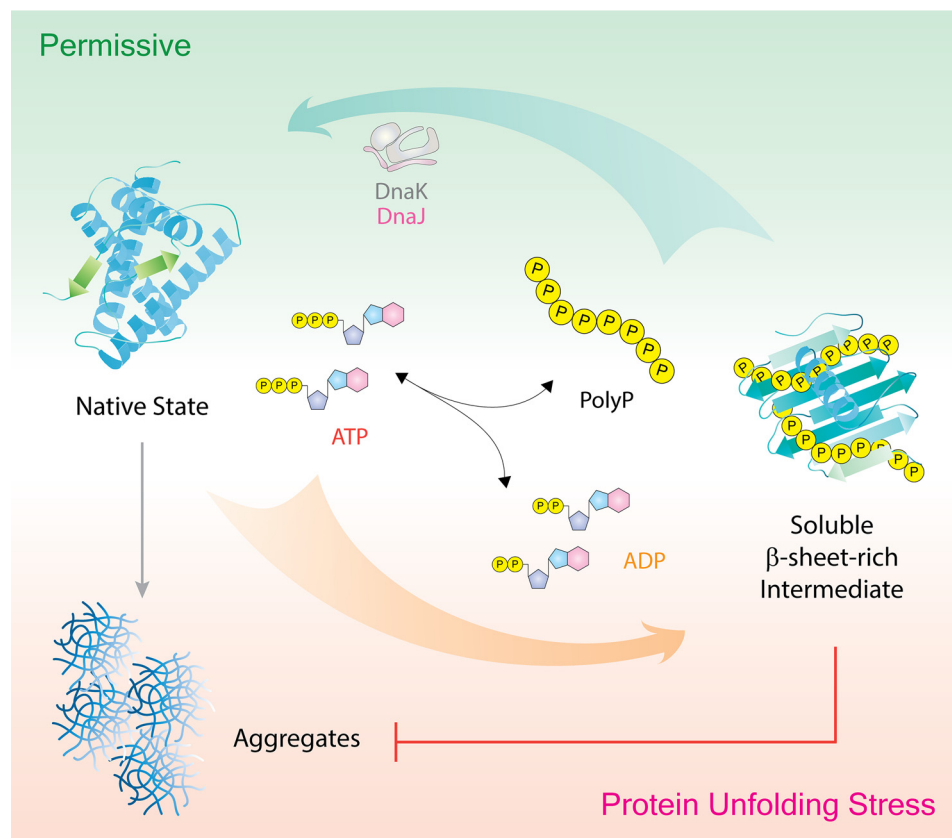


Figure 2. PolyP, an inorganic molecule with protein-like chaperone qualities. Under proteotoxic stress conditions, such as oxidative stress or heat shock, bacteria convert significant quantities of cellular ATP into long chains of polyP, which functions as a protein-stabilizing scaffold that binds protein unfolding intermediates and stabilizes them in a soluble β -sheet-rich conformation. This stable interaction effectively prevents protein aggregation both *in vitro* and *in vivo*. Once stress conditions are alleviated, chaperone foldases such as the DnaK system are able to refold polyP-bound client proteins and restore their native structure and function.

(24). Like classical protein chaperones, we found that micromolar concentrations of polyP effectively prevented protein aggregation during a variety of different proteotoxic stress conditions, including oxidative stress (24) and high temperature (Fig. 2) (25). Deletion of the PPK gene led to the accumulation of aggregated proteins *in vivo* despite the bacteria's attempts to compensate for the lack of polyP with increased heat shock gene expression (24, 25). These results helped to explain previous reports about the exquisite sensitivity of *ppk* deletion strains toward proteotoxic stress conditions and suggested that the chaperone function of polyP plays a physiologically relevant role in stress protection (24, 27, 28). In addition, some of the stress-protective effects of polyP *in vivo* might also be due to polyP's ability to serve as a reservoir for high-energy phosphoanhydride bonds or its metal-chelating activity (24, 70). However, dissecting which of these functions might play the predominant cytoprotective role will be extremely challenging.

The real potential of polyP as a protein-stabilizing scaffold became evident when we heated known thermolabile proteins, such as luciferase or lactate dehydrogenase in the presence of polyP, and found that the proteins remained fully soluble even upon heating to near-boiling temperatures (*i.e.* 85 °C) (24, 25). The effective polyP concentration was in the low millimolar range (per P_i) and hence at least 3 orders of magnitude below the working concentration of chemical chaperones (*e.g.* glycerol or trehalose), which are thought to exert their protein-

stabilizing effects through nonspecific solvation effects (71, 72). Moreover, we found that the chaperone function of polyP is chain length-dependent, with longer polyP chains being disproportionately more effective in preventing protein aggregation *in vitro* than short-chain polyPs (<16-mer), excluding a "simple" charge effect (24, 25). In summary, these results suggested that polyP, which itself is redox-inert and thermostable, might have served as the primordial chaperone and now functions in the first line of defense under extreme stress conditions that might inactivate protein-based chaperones (Fig. 2) (24, 25).

Analysis of the fate of the bound client proteins revealed that polyP not only preserves their solubility but also maintains the client proteins in a folding-competent conformation, even upon incubation at near-boiling temperatures (25). Subsequent incubation of polyP–client complexes under nonstress conditions restored the enzymatic activity of the clients provided that the DnaK-refolding system was present (Fig. 2) (24, 25). In contrast, hydrolysis of polyP in the absence of other chaperone systems caused the rapid aggregation of the client proteins (24, 25). At this point, we do not know whether substrate release is simply triggered by the presence of chaperone foldases or whether polyP directly affects and regulates the activity of other chaperones as well. More research is clearly needed to gain detailed insights into the precise roles that polyP plays within the cellular proteostasis network.

For many years, it has been a well-accepted dogma that molecular chaperones bind client proteins through hydrophobic contact sites, thereby shielding aggregation-sensitive surfaces of clients against nonspecific interactions (73–75). This mechanism is clearly not compatible with polyP, which is one of the most highly negatively charged molecules known (54). Instead, it strongly suggests that the main driving force behind polyP–client interactions is of an electrostatic nature. Yet, how polyP is able to recognize and bind a wide range of different unfolding intermediates while discriminating against their native counterparts is not known. So far, no correlation has been observed between the preference of polyP and the isoelectric points (pI) of the potential clients, although a comprehensive analysis still needs to be performed. Moreover, polyP appears indiscriminative toward aggregation-sensitive endogenous *E. coli* proteins, which appear to be all similarly protected by polyP (24). Of course, it is unclear whether polyP interacts individually with all of the different proteins or broadens its effect by manipulating the activity of other chaperones that are present in these lysates. Finally, the chaperone activity of polyP appears to be chain length–dependent (24, 25), which suggests that certain structural elements in polyP rather than simply the presence of negative charges are responsible for the disproportionately higher activity of longer polyP chains. Our recent studies showed that polyP stabilizes a range of different thermally unfolded proteins in a β -sheet–rich, amyloid-like yet soluble conformation (24, 25). This is despite the fact that the proteins that we tested so far are predominantly α -helical in their native conformation. Because polyP does not affect the conformation of these proteins in their native state, we propose that polyP chains bind and stabilize β -sheet structures as they transiently populate during the unfolding process. More studies are clearly needed to investigate this idea (Fig. 2). It is of note that other polyanions, such as DNA and RNA, have now been shown to also moonlight as chaperones *in vitro*, with an efficiency that in some cases even exceeds that of polyP (76). We are now awaiting high-resolution structures, which will hopefully reveal how these polyanions interact with and stabilize proteins.

PolyP's other potential roles in cellular proteostasis

In addition to the chaperone function, polyP has several other features and functional activities that might contribute to maintaining proteostasis in the cell. For example, it has been proposed that polyP oversees the fidelity of protein translation by refining the magnesium-sensitive codon–anticodon pairing and/or by modifying the structure of the ribosome (77). By preventing the overproduction of error-prone polypeptides, polyP might thereby mitigate the burden on the quality control network. Moreover, it has been known for years that polyP is able to chelate heavy metals, such as cadmium, copper, and manganese, that are known to target protein folding (41, 78–82). Yet, another strategy to protect the proteome might utilize the osmotic properties of polyP to attain an optimal folding environment. For instance, the extremophile *Methanosarcina mazei* deliberately up-regulates polyP to maintain its normal osmolarity at a concentration of salt that would otherwise render the cytoplasm nearly desiccated (83). In summary, it appears that polyP, one of the simplest yet most versatile mol-

ecule known, is able to protect the proteome under vastly different and constantly changing conditions.

PolyP—a modifier of amyloidogenic processes *in vitro*

Inspired by the observation that protein-folding intermediates, once in complex with polyP, exert β -sheet–rich features that are reminiscent of soluble amyloid-like microaggregates (24, 25), we explored the role of polyP in amyloidogenic processes. All of the known amyloidogenic proteins start life as soluble proteins, typically with an intrinsically disordered or α -helix–rich structure (84). Using mechanisms and following *in vivo* kinetics that have yet to be defined for the majority of amyloids, the monomers eventually undergo conformational rearrangements into association-competent β -sheet structures, which form oligomers, proto-fibrils, and finally the mature fibrils (84, 85). Irrespective of the sequence and structure of the amyloidogenic monomers, the overall morphology and characteristics of the mature fibrils appear to be surprisingly similar: cross- β -sheet repeat structures, which are protease-resistant, SDS-insoluble, and thioflavin T-positive (86–88). It is important to note that the mature fibrils are not inert end products but are known to undergo shedding events that increase the number of oligomers and proto-fibrils, which will nucleate additional oligomerization processes (89–91).

Functional amyloid fibrils are found in bacteria where they constitute the structural framework of biofilms (92, 93), as well as in mammals, where they serve as a reservoir of pituitary hormones (94) or generate pigmentation in melanocytes (95). However, amyloidogenic proteins are even better known for their roles in protein-folding diseases (84, 85), particularly A β , tau, α -synuclein, and huntingtin, whose fibril depositions in the brain have been associated with Alzheimer's, Parkinson's, and Huntington's disease, respectively (96–98). Because age is the greatest risk factor for developing these neurodegenerative diseases (99), amyloid pathology has become exceedingly prevalent, and effective therapeutics are desperately needed to target these devastating diseases (100).

It is generally agreed upon that the rate-determining step in most amyloidogenic processes is the initial conformational change that converts soluble monomers into association-competent β -sheet–rich proteins (101). Hence, our finding that polyP stabilizes β -sheet–containing folding intermediates (24, 25) fueled the idea that polyP might increase the rate of fibril formation. At a minimum, this activity would explain the previous observation that polyP accelerates biofilm formation in bacteria (11, 12). Indeed, experiments with the bacterial amyloid CsgA revealed that polyP accelerates CsgA fibril (*i.e.* Curli) formation both *in vitro* and *in vivo* (54). Subsequent analysis with different disease-associated amyloids confirmed this activity and revealed, in some cases, an extraordinary acceleration of *in vitro* fibril formation through polyP (Fig. 3) (54). One extreme example is the human tau protein, whose lag phase is reduced from many months (102) to around 30 h simply by adding polyP at a 1:5 (polyP/tau) ratio (54). Another example is the tau fragment TauK19, which takes 24–48 h to form fibrils in the absence of polyP yet fibrillates within minutes in its presence (54). Depending on the amyloidogenic protein tested, polyP shortens the initial lag phase and/or expedites fibril elon-

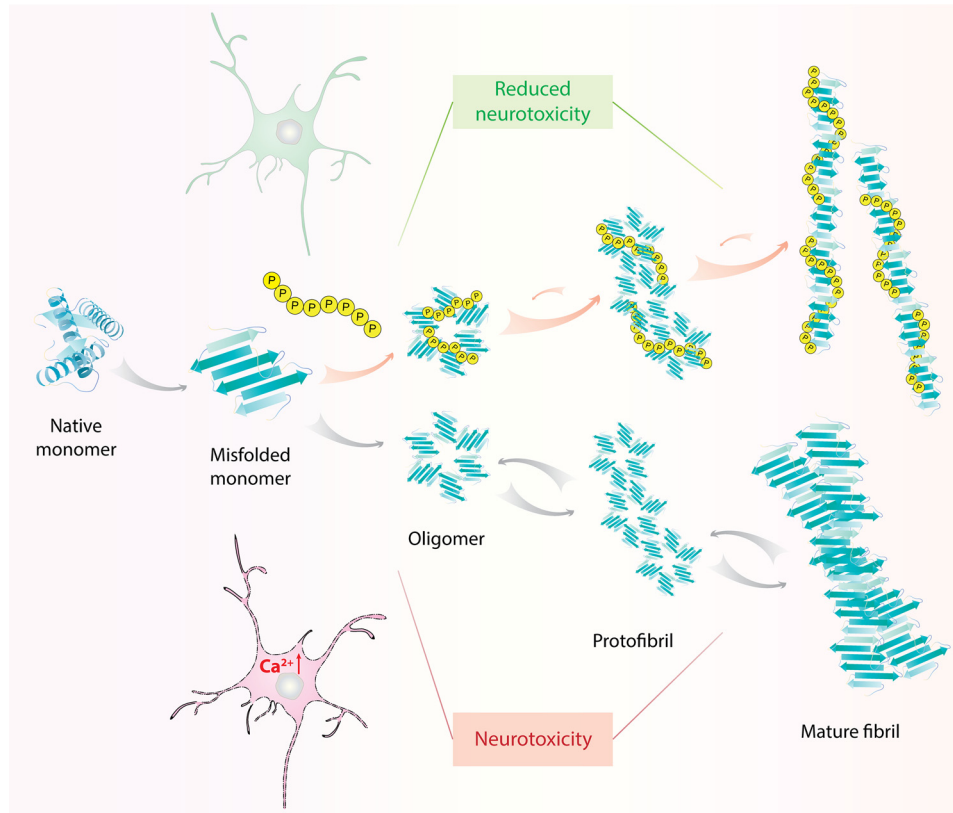


Figure 3. PolyP, a modifier of amyloidogenic processes. Amyloidogenic proteins undergo conformational changes from their native monomeric state into a misfolded, β -sheet-rich aggregation-prone state, which can form insoluble oligomers, protofibrils, and mature fibrils with characteristic cross- β -sheet conformation. Shedding of mature fibrils into protofibrils and/or oligomers promotes further fibril formation. The prevalent model of amyloid toxicity identifies the oligomers and protofibrils as the major cytotoxic species, which disrupts the integrity of cellular membranes and destroys calcium homeostasis. PolyP accelerates the fibrillation of both functional amyloids (e.g. bacterial CsgA) as well as disease-associated amyloids, including A β -peptides, α -synuclein, and tau, and triggers morphological changes in mature fibrils. Cytotoxicity assays using neuronal cell lines revealed that the fibrils, formed in the presence of polyP, are no longer cytotoxic, suggesting that polyP might be a physiologically relevant modifier in amyloidogenic processes.

gation. Similar to the anti-aggregation function of polyP, the pro-aggregation function of polyP is also chain length-dependent, with longer chains being disproportionately more effective in nucleating fibril formation than shorter chains (54). This is a particularly intriguing aspect given that cells in the mammalian brain contain extremely long polyP chains (>800 P_i), and it makes one wonder whether or how fibril formation is avoided in cells that contain both amyloids and polyP. In addition to affecting nucleation and elongation, polyP binding also causes substantial structural changes in the mature fibrils (Fig. 3) (54). These structural rearrangements likely contribute to the fact that fibrils formed in the presence of polyP are less proteolytically stable, less able to shed, and less effective in seeding (54). Although the data appear convincing that polyP accelerates amyloid fibril formation, a better temporal resolution is needed to define when polyP binds and to determine which specific steps in the fibril formation are accelerated. Moreover, we need higher-resolution structural information to visualize how polyP interacts with fibrils. The most important aspect, however, is to understand the extent to which polyP contributes to *in vivo* fibril formation and how premature polyP-mediated fibril formation might be prevented in the human brain. With the advancement of super-resolution microscopy and in combination with novel labeling techniques, answers for these crucial questions will hopefully be found soon.

PolyP—a potential novel player in neurodegenerative diseases

Studies with several different neuronal cell cultures, including differentiated human neuroblastoma cells and PC12 cells, revealed that the presence of polyP during the fibril formation abrogated amyloid cytotoxicity. A similar cytoprotective effect was elicited by simply adding polyP to cells shortly before adding pre-formed amyloid fibrils (54, 103). Because polyP is both secreted and taken up by neuronal cells (50, 104), these results suggest that the presence of polyP in mammalian brains might influence amyloid toxicity, propagation, and/or disease progression.

At this point, it is unclear how polyP protects cells against the toxic effects of amyloids. The current model of amyloid toxicity proposes that the process of amyloid formation but not the mature fibrils elicits toxicity (105, 106). Toxic oligomers and protofibrils are thought to cause loss of membrane integrity and disruption of calcium homeostasis that ultimately leads to aberrant Ca²⁺ signaling and eventually cell death (107, 108). Hence, it is possible that by simply speeding up fibril formation, suppressing shedding, and preventing seeding, polyP decreases the effective concentration of toxic oligomers and protofibrils. It is also possible that polyP-associated fibrils are no longer recognized by receptor proteins responsible for the uptake of amyloid fibrils (109) or that the association with polyP increases the

turnover of fibrils or triggers sequestration of the fibrils into inert structures. Finally, it has been shown that depletion of mitochondrial polyP blocks mPTP opening and the subsequent initiation of apoptosis induced by β -amyloid peptide (17). Therefore, polyP might serve as an indirect regulator of amyloid toxicity. More research is clearly needed to investigate these effects and to test whether the age-related decline in brain polyP levels that has previously been observed (55) might correlate with or potentially even contribute to disease onset and progression.

Summary and outlook

The goal of this review is to invigorate interest into a molecule, whose simple structure clearly defies its stunning functional versatility (Table 1). In addition to its fundamental roles as a P_i donor and energy source, the ability of polyP to serve as a stabilizing scaffold for countless soluble and amyloidogenic proteins has added a new dimension to its activities in the cell (24, 54, 70). It is intriguing that polyP can serve both as an anti-aggregation molecule to keep proteins soluble during stress-induced unfolding, as well as a pro-aggregation molecule that accelerates amyloid fibril formation. Based on some recent studies, it appears that the fundamental mechanism for both activities is the same: polyP binds and stabilizes proteins in a β -sheet-rich, amyloid-like conformation; however, the functional consequences appear to be determined by the intrinsic properties of the clients (24, 54). For nonamyloidogenic proteins such as luciferase and citrate synthase, association with polyP means solubility and the ability to refold if conditions permit. For amyloidogenic proteins exemplified by CsgA, A β , tau, and α -synuclein, association with polyP stimulates fibril formation and alters fibril morphology. Our findings that polyP aids bacteria in forming protective biofilms and shields mammalian cells against amyloid toxicity support the idea that polyP's stimulatory effects on fibril formation is indeed a desired cellular event. However, it now remains to be determined whether and how polyP alters and affects the *in vivo* fate of disease-associated amyloid fibrils, and whether age-mediated losses in cellular polyP levels contribute to the observation that age is one of the largest risk factors of amyloid-related neurodegenerative diseases.

We also want to point the readers to one of the biggest remaining puzzles in the field: the question of how polyP is synthesized in mammals. Unlike many other conserved molecules that are known in biology, polyP is not synthesized via the same conserved pathway but is derived from seemingly unrelated ATP-fueled engines that show no sequence or structural homology and have no homologues in higher eukaryotes! Even if one focuses only on the prokaryotic branch of the phylogenetic tree, clear homologues of *E. coli* PPK are absent in a large number of polyP-synthesizing species (110). During the search for alternative polyP-synthesizing machineries, a few enzymes have been recognized for their ability to use polyP as a phosphate donor (110, 111). Presumably, these reactions can be reversed by the presence of excess substrate; however, it is unknown whether such conditions can ever be achieved *in vivo*. Another hypothesis is that polyP may be generated by the proton-motive force without a designated enzyme (37). In mito-

chondria, complete depolarization of the membrane results in decreased polyP production, which suggests that a robust proton gradient is involved (112). This result also explains reports from several labs that found that cell lysis robustly kills polyP-synthesizing activity (45, 113). Yet, there is no direct evidence that polyP synthesis is indeed a spontaneous process that does not need catalysis. Finally, another group of phosphate species, inositol phosphates, has been implicated in the metabolism of polyP. Yeast, trypanosoma, and mouse strains that lack the enzyme to synthesize highly phosphorylated inositols show significantly reduced levels of polyP (114–117). Because the inositol phosphate pathway is well characterized, it is compelling to resolve the connection between inositol phosphates and polyP. This might serve as the entry point to gain a more detailed picture about polyP regulation. Finding the enzyme(s) that synthesize and regulate polyP synthesis in mammals is key; once this is achieved, many of the remaining doors will open and hopefully reveal how this ancient biomolecule works today.

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