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REVIEW AND EXPERIMANTAL ARTICLES

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Specific Features of Metabolism and Functions of High-Molecular Inorganic Polyphosphates in Yeasts as Representatives of Lower Eukaryotes

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Abstract—This review considers recent data demonstrating an important role of high-molecular-weight inorganic polyphosphates (polyPs) in regulatory processes in yeasts. PolyPs occur in various compartments of the cell and are metabolized by compartment-specific sets of enzymes. Evidence is provided for the multiplicity of polyP functions in the cells. Data on the pleiotropic effects of mutations of genes coding for polyP-metabolizing enzymes are summarized.

Key words: inorganic polyphosphate, exopolyphosphatase, endopolyphosphatase, metabolism, yeast

INTRODUCTION

Andrei Nikolaevich Belozersky is renowned for his classical works in the fields of nucleic acids and molecular biology. In addition, he started research into the biochemistry of high-molecular inorganic polyphosphates (polyPs) in Russia. He and his colleagues performed many important studies on polyP metabolism in yeasts and other fungi.

Recent studies of these compounds in yeasts have given us a new horizon, providing for a better understanding of the important role of polyPs in the living activity of yeasts and elucidating the specific features of metabolism and functions of polyPs in individual organelles of the yeast cell. This work is dedicated to the 100th anniversary of our teacher A.N. Belozersky.

PolyPs, linear polymers of orthophosphoric acid, perform a variety of functions that are important for adaptation to continuously changing environmental conditions. In particular, polyPs are involved in phosphate reservation, bioenergetics, transport, and complexation with cations. PolyPs play an important role in the formation and function of the cell wall, regulation of enzyme activities and gene expression, adaptation to stress, and switching of genetic programs in the course of ontogeny.

PolyPs were first found in yeasts among all living organisms [1], and it is in yeasts, among all eukaryotes, that their metabolism is best studied [2–14]. The polyP content in yeasts strongly depends on the culture conditions and the growth phase. Under certain conditions, polyPs account for 10–20% of the cell dry biomass, with chain length varying from 3 to 300 phosphate residues. Several specific features are characteristic of polyP metabolism in yeasts, as well as in other lower eukaryotes, fungi, algae, and protozoa. These features include accumulation of polyP to a considerable amount under certain conditions, localization of polyP in various organelles and cell compartments, compartment specificity of enzymes involved in polyP metabolism, multiplicity of polyP functions, and their dependence on the polyP location in the cell. All these features are considered in detail below.

LOCALIZATION AND FUNCTIONS OF POLYPHOSPHATES IN THE YEAST CELL

When yeasts grow under certain conditions, especially when orthophosphate (P_i) is in excess in the culture medium, the cell cytoplasm contains metachromatic, or volutin, granules, which are specific inclusions long known as Babes–Ernst bodies [15, 16]. Volutin granules isolated from *Saccharomyces cerevisiae* cells harbor about 14% of the total cell polyP, metal ions, and four specific proteins [17]. It seems that volutin granules are not just polyP aggregates, but rather cell structures that are organized in a certain manner and contain polyPs in complex with metal ions and specific proteins. It is also possible that there are complexes of polyPs with RNA in the cytosol; their role needs further investigation [6, 7].

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The amount of cytosolic polyPs varies from 10 [18] to 70% [19] of the total polyP content in the cell in *S. cerevisiae*. The cytosolic contents of polyPs and volutin granules increases in a medium containing excessive P_i and dramatically decreases in a P_i-deficient medium. It was assumed on this evidence that cytoplasmic polyPs serve first and foremost as a phosphate reserve in the yeast cell [2–14].

Recent studies have shown that polyPs can exist in the living cell in a new form, as complexes with Ca^{2+} and poly-β-hydroxybutyrate, which surround polyP molecules and ensure their incorporation into membranes [20]. Such complexes play a role in the formation of ionic channels, regulate the permeability of membranes, and occur both in bacteria and in eukaryotes [21].

The presence of polyPs in the yeast cell envelope is evident from the fact that cells lose from one-fourth to one-third of the total polyP upon spheroplast isolation [6, 7]. PolyPs were detected in the cell envelope of some yeast species by staining with the specific dyes 4',6'-diamino-2-phenylindole [22] and 9-aminoacridine [23], cell exposure to osmotic shock [24], ^{32}P-NMR spectroscopy of cells treated with UO_2^{2+} (a decrease in signal intensity) [25], X-ray microanalysis, and electron microscopy [26, 27]. The yeast cell envelope harbors up to 20% of the total cell polyP [23, 28, 29]. There is evidence that polyPs play an important role in regulating the negative charge of the cell surface [23, 28, 29]. The polyP contribution to this charge was estimated with the fluorescent probe 9-aminoacridine, the binding of which depends on the charge of the cell envelope [23]. Cells growing in the absence of P_i and having a low polyP content in the cell envelope absorbed a lower amount of 9-aminoacridine as compared with cells growing in a medium with a sufficient concentration of P_i. The polyP content affects the sensitivity of yeast cells to the damaging effect of the cationic detergent cetylmethylammonium bromide [29]. The higher the polyP content in the cell envelope, the greater the amount of detergent interacting with the cell surface and the greater its damaging effect. It is thought that PolyPs contained in the cell envelope provide the primary barrier that prevents penetration of heavy metal ions into the cell.

PolyPs are involved in the formation of the cell envelope in yeasts. A direct correlation was observed between $polyP_4$ accumulation and biosynthesis of cell-wall polysaccharides [30–32] and was due to a specific enzyme, dolichyl pyrophosphate–polyP phosphotransferase [EC 2.7.4.20]. Concerted synthesis of polysaccharides and polyPs occurs on membranes of the endoplasmic reticulum. On the cytoplasmic side of the endoplasmic reticulum, GDP-mannose interacts with the phosphate group of dolichyl phosphate. Dolichyl pyrophosphate mannose crosses the membrane so that the phosphomannose residue finds its way into the lumen of the endoplasmic reticulum. The mannosyltransferase and dolichyl pyrophosphate–polyP phosphotransferase reactions take place in the lumen. Dolichyl phosphate is released, crosses the membrane, and again reacts with phosphomannose. Mannoproteins and polyPs remain in the lumen and are transported in special vesicles to the cell envelope [28].

Vacuoles, which store various hydrolytic enzymes, amino acids, nucleosides [33, 34], and metal ions [18], contain polyPs as well [34, 35]. Cytochemical analysis and electron microscopy revealed volutin granules in vacuoles [27]. As shown by ^{31}P-NMR spectroscopy, there are two polyP fractions in vacuoles, with mean chain lengths of 5 and 20 phosphate residues [19].

The assumption that a major part of the total polyP is contained in vacuoles in the yeast cell is widely discussed in the literature [35–37] and is supported by the fact that ^{31}P-NMR spectroscopy did not detect polyPs in cells of a mutant strain defective in vacuole formation [37]. However, this method detects only free polyPs, not those bound with other compounds. Moreover, the contribution of the vacuolar polyP to the total polyP pool of the yeast cell depends to a great extent on the culture conditions. In *S. cerevisiae,* vacuoles contained most of the total polyP pool when cells were grown in a deficient mineral medium containing arginine as the only source of nitrogen and only 15% of the total polyP pool when cells were grown on the Rider medium, enriched in phosphorus and nitrogen [19]. *Candida utilis* vacuoles contained no more than 30% of the total polyP pool, and this portion decreased depending on the culture growth rate and the source of nitrogen [38]. When *S. carlsbergensis* cells grow in a P_i-deficient medium, the polyP content in vacuoles decreases almost sevenfold, suggesting a role of vacuolar polyPs in generating an osmotically inert reserve of phosphate in the cell [18].

Yeast and fungal vacuoles contain polyPs in complexes with arginine, lysine, spermidine, Mg^{2+}, Ca^{2+}, Mn^{2+}, and K^+ [18, 33, 39]. The polyP content in *S. carlsbergensis* vacuoles increased sevenfold and tenfold upon accumulation of K^+ or Mn^{2+}, respectively [39].

There is evidence that polyPs are involved in overcoming alkaline stress in a medium containing an excess of ammonium ions: their rapid degradation increases the P_i content in the cytoplasm and vacuoles [40, 41]. When amino acids or ammonium ions are added to a cell culture growing in a medium deficient in nitrogen sources, the $polyP_3$ content rapidly increases as a result of degradation of longer polyP chains [42]. It was assumed that polyP degradation contributes to the maintenance of intracellular pH, including acidic pH in vacuoles. Thus, vacuolar polyPs ensure the storage of positively charged components in an osmotically inert form and contribute to pH homeostasis and phosphate reservation.

Table 1. Yeast polyPs: localization, function, and complexation with other components of the cell

Localization	Main functions	Components producing complexes with polyPs
Volutin granules, cytoplasm	Reservation of P_i and cations in the form of osmotically inert complexes, regulation of enzyme activities	Mg^{2+}, Ca^{2+}, other cations, RNA (through cations), proteins
Vacuoles	Reservation of P_i and cations in the form of osmotically inert complexes, pH homeostasis	Mg^{2+}, Ca^{2+}, other divalent metal cations, lysine, arginine, amines
Nucleus	Regulation of gene expression	DNA (through cations or proteins), proteins, RNA
Membranes	Formation of transport channels	Ca^{2+}, polyhydroxybutyrate
Cell envelope	Contribution to the cell surface charge, barrier to heavy metal ions, role in regulation of glycoprotein synthesis	Polysaccharides, cations, proteins
Mitochondria	Role in bioenergetics, P_i reserve	

Many studies have revealed polyPs in cell nuclei of various organisms from fungi and yeasts to mammals [6, 7]. Although occurring in minor amounts, nuclear polyPs probably play an important role in gene expression regulation [6–14].

In *S. cerevisiae* mitochondria, polyPs with the mean chain length of 14–25 phosphate residues were detected by ^{31}P-NMR spectroscopy [43], chemical extraction, chromatography, and electrophoresis [44]. Mitochondria harbor 7–10% of the total polyP and accumulate polyPs predominantly in the intermembrane space. The polyP content decreases almost tenfold in P_i deficiency and increases twofold after subsequent cell transfer onto a P_i-containing medium as compared with the content observed without preliminary phosphorus starvation [44]. The content and the chain length of mitochondrial polyPs increase not only when P_i is in excess, but also in glucose suppression [45]. It is possible to assume that polyPs serve as a phosphate reserve and play a role in bioenergetics, especially under conditions unfavorable for the function of yeast mitochondria.

Many studies of intact cells and subcellular fractions by chemical extraction, electrophoresis, cytochemical methods, ^{31}P-NMR spectroscopy, and electron microscopy have testified to multiple locations of polyPs in cells of yeasts and other lower eukaryotes [6–14]. PolyPs form complexes with various biologically active compounds and cations and occur in all main compartments of the cell, playing a variety of roles (Table 1).

ENZYMES OF POLYPHOSPHATE METABOLISM

The set of enzymes involved in polyP metabolism considerably differs between yeasts and bacteria. The main enzyme of polyP synthesis in bacteria is polyphosphate kinase (polyphosphate–ADP phosphotransferase [EC 2.7.4.1]), which catalyzes the transfer of the energy-rich phosphate residue from ATP to the terminal phosphate group of a polyP chain and the reverse transfer of the phosphate residue from polyP to ADP [46]:

$$\text{polyP}_n + \text{ATP} \rightleftharpoons \text{polyP}_{n+1} + \text{ADP}.$$

Most bacterial genomes contain *ppk1* and *ppk2*, which code for polyphosphate kinases, but similar eukaryotic genes are unknown [47]. In eukaryotes, ADP phosphorylation with the use of polyP prevails over polyP synthesis [48]. Moreover, this reaction is performed by diadenosine-5',5'''-P^1, P^4-tetraphosphate α,β-phosphorylase (A-P4-A phosphorylase [EC 2.7.7.53]) acting together with exopolyphosphatase and adenylate kinase [49]. The transfer of a phosphate residue from polyP to ADP and the reverse reaction were also observed in isolated yeast vacuoles [50]. Yet a combined effect of several enzymes cannot be excluded in this case, because attempts to isolate the corresponding enzyme have failed. Thus, yeasts lack polyphosphate kinases characteristic of bacteria. The presence of other polyphosphatases and their role in polyP metabolism are still an open question.

At the same time, yeasts have polyP-metabolizing enzymes that were not found in bacteria. As mentioned above, preparations of endoplasmic reticulum membranes contain dolichyl pyrophosphate–polyP phosphotransferase, which synthesizes polyP from β-phosphate groups of dolichyl pyrophosphate [32]:

$$\text{dolichyl pyrophosphate} + \text{polyP}_n \longrightarrow \text{dolichyl phosphate} + \text{polyP}_{n+1}.$$

The gene for this enzyme is still unidentified, necessitating further work in the field. A similar enzymatic activity was not detected in bacteria. However, bacteria possibly have undecaprenyl–polyP phosphotransferase with a similar function.

Endopolyphosphatase (polyP depolymerase [EC 3.6.1.10]), which was also found only in eukaryotes, cleaves extended polyP chains into shorter polyPs:

$$\text{polyP}_n + \text{H}_2\text{O} \longrightarrow \text{oligophosphates}.$$

Table 2. Properties of exopolyphosphatases isolated from various compartments of *S. cerevisiae* cells

Property	Cell envelope	Cytosol, PPX1	Cytosol, high-molecular-weight enzyme	Vacuole	Mitochondria, soluble fraction	Mitochondria, membrane fraction*	Nucleus
Molecular weight, kDa	40–45	40	>500	245	40	120 and 76	200
Specific activity**, units per mg protein							
$PolyP_3$	320	420	17	5	3.8	0.04	0.50
$PolyP_9$	220	320	–	15	2.5	0.045	–
$PolyP_{15}$	200	300	114	60	2.0	0.085	0.10
$PolyP_{208}$	180	270	135	70	1.8	0.11	0.95
K_m, μM							
$PolyP_{15}$	15	11	75	93	18	23	133
$PolyP_{208}$	0.9	1.2	3.5	2.4	0.25	1	25
Relative activity***, rel. units							
2.5 mM Mg^{2+}	10	39	2	2	27	0.95	15
0.1 mM Co^{2+}	14	66	6	6	24	0.7	31

* Data for a mitochondrial membrane preparation; (–), no data.
** In the presence of divalent metal cations.
*** In the presence of divalent metal cations (activity in the absence of cations was taken as unity).

The enzyme was purified from *S. cerevisiae* cells, and its gene was sequenced [51].

Enzymes common for prokaryotes and eukaryotes still differ genetically and in physicochemical properties. An example is provided by exopolyphosphatases (polyP phosphohydrolase [EC 3.6.1.11]), which removes P_i from the end of a polyP chain:

$$polyP_n + H_2O \longrightarrow polyP_{n-1} + P_i.$$

The major *Escherichia coli* exopolyphosphatase is encoded by *ppx* [52]. The enzyme cleaves $polyP_3$ and other short polyPs with a low efficiency and requires divalent cations and K^+ for its activity.

Guanosine pentaphosphate phosphorylase, which is encoded by *gppA*, is another bacterial enzyme that possesses exopolyphosphatase activity [53]. This enzyme is involved in regulating the level of guanosine pentaphosphate (pppGpp) and tetraphosphate (ppGpp), which are important signal molecules in bacteria. PolyPs compete with its major substrates and suppress their hydrolysis [10, 11]. The *ppx* and *gppA* genes show ~39% identity and each contain five conserved regions characteristic of the superfamily of genes coding for saccharide kinases, actin, and heat shock proteins [54].

The best studied yeast phosphatase is encoded by *PPX1*, which lacks homology to *ppx* [55]. This enzyme was purified first from the cell envelope [56] and then from a homogenate [57] and the cytosol [58] and is far more active (200–400 units per mg protein) than bacterial exophosphatases (0.02–0.04 units per mg protein) [52]. This explains the difference in exopolyphosphatase activity between *S. cerevisiae* (0.10–0.13 units per mg protein) and bacterial (0.02–0.04 units per mg protein) homogenates [52, 56, 57]. In contrast to the bacterial enzyme, the PPX1 exopolyphosphatase hydrolyzes $polyP_3$ 1.5 more efficiently than more extended polyPs (Table 2) and requires only divalent metal ions, but not K^+, for its activity. PPX1 efficiently hydrolyzes adenosine tetraphosphate and guanosine tetraphosphate, which possibly act as signal molecules or their precursors [59, 60].

Yeast cells with PPX1 deficiency preserve the exophosphatase activity, suggesting the existence of other exopolyphosphatases [55, 59]. Our studies aimed at purification and characterization of exopolyphosphatases from various cell compartments showed that enzymes of this class are many in the yeast cell and vary not only in location, but also in combinations of properties (Table 2) [59, 61, 62].

Exopolyphosphatase found in the soluble mitochondrial fraction is similar to PPX1 in substrate specificity, molecular weight, and dependence on divalent cations (Table 2) and differs from it in showing a higher affinity for polyPs, no activation in the presence of EDTA, and no interaction with antibodies against PPX1 [59]. The enzyme is absent from mitochondria of cells with mutant *PPX1*, which suggests that it is encoded by the same gene as PPX1 and that the above difference in properties is due to posttranslational modification [59].

The mitochondrial membrane contains exopolyphosphatase that more efficiently hydrolyzes high-molecular-weight polyPs and is inhibited by divalent metal cations (Table 2). Inactivation of *PPX1* has no effect on the activity and properties of exopolyphosphatases from mitochondrial membranes, nuclei, and

Table 3. Prokaryotic and eukaryotic enzymes of polyP metabolism

Enzyme	Prokaryotes	Eukaryotes
PolyP kinase	+	–
1,3-Diphosphoglycerate-polyP phosphotransferase	+	+
Dolichyl pyrophosphate-polyP phosphotransferase	–	+
polyP glucokinase	+	–
Exopolyphosphatase	+	+
Endopolyphosphatase	–	+
PolyP-dependent NAD^+ kinase	+	–
PolyP-AMP phosphotransferase	+	–

Note: (–), the enzyme was not found.

vacuoles of *S. cerevisiae* cells, testifying that these enzymes are encoded by other genes [59].

In addition to PPX1, we found another, larger cytosolic exopolyphosphatase, which becomes detectable upon the transition from the stationary phase to active growth in a P_i-rich medium [61] and upon *PPX1* inactivation [59]. Like exopolyphosphatase found in vacuoles, this enzyme is low-active with $polyP_3$, fails to cleave adenosine tetraphosphate and guanosine tetraphosphate, most efficiently hydrolyzes highly polymeric polyPs, and is activated by divalent metal cations to a lower extent as compared with PPX1 (Table 2). High-molecular-weight cytosolic and vacuolar exopolyphosphatases are similar to bacterial exopolyphosphatases in substrate specificity, but have a higher activity and do not require K^+.

Thus, *S. cerevisiae* exopolyphosphatases provide an illustrative example of compartment-specific enzymes (Table 2) and differ in properties from known bacterial exopolyphosphatases.

Considering the enzymes of polyP metabolism, it should be noted that Belozersky was the first who assumed that, in primitive organisms, polyPs performed the energy-related functions that are performed mostly by ATP in modern organisms. This hypothesis is supported by the fact that so-called fossil biochemical reactions are characteristic of some recent microorganisms. For instance, polyPs, rather than ATP, mediate phosphorylation of glucose and NAD in bacteria representing the most ancient taxonomic groups [6, 64–66]. Glucokinase and NAD^+ kinase [EC 2.7.1.23] of these bacteria each possess two active centers, one utilizing polyP and the other using ATP as a phosphate donor. The polyP-binding center is absent in phylogenetically younger bacteria, such as *E. coli* [64–66]. The above reactions were not detected in eukaryotes. At the same time, eukaryotes have another fossil reaction [67]:

$$\text{1,3-diphopshoglycerate} + \text{polyP}_n \longrightarrow \text{3-phosphoglycerate} + \text{polyP}_{n+1}.$$

The corresponding activity was observed first in mutant *Neurospora crassa* with a reduced intracellular concentration of ATP, ADP, and AMP and then in bacteria [6]. Although not isolated so far, 1,3-diphosphoglycerate–polyP phosphotransferase is thought to play an important role in polyP biosynthesis in yeasts [68].

The known polyP-metabolizing enzymes include those that are specific either for prokaryotes or for eukaryotes (Table 3). First and foremost, polyphosphate kinase is such an enzyme. It is still obscure why this key enzyme of polyP metabolism in bacteria was lost during evolution from prokaryotes to eukaryotes. A possible explanation is that polyPs ceased to play an important role in energy metabolism. The absence of polyP-dependent glucokinase and polyP-dependent NAD^+ kinase in eukaryotes seems more natural: these enzymes simply lost the polyP-binding center and preserved the ATP-binding center. The evolution of exopolyphosphatases is still an open question. In view of the compartment-specificity of these enzymes, its solution may provide for a better understanding of the evolution of organelles of the eukaryotic cell. It should be noted that almost all polyP-metabolizing enzymes are multifunctional to a certain extent and utilize not only polyPs, but also nucleoside phosphates as substrates. This fact possibly reflects the evolution of these enzymes from forms utilizing simpler and less specific polyPs to those utilizing nucleoside phosphates. It is also clear that metabolic pathways involving organic and inorganic phosphorus compounds are tightly associated with each other. This association is still preserved in recent organisms and plays an important role in the regulation of energy metabolism.

GROWTH-RELATED CHANGES IN THE CONTENT OF INDIVIDUAL POLYPHOSPHATE FRACTIONS IN YEASTS

Chemical extraction of polyPs from whole cells is still among the most efficient and widespread methods in polyP studies, because polyPs degrade during isolation of individual subcellular fractions [6, 68, 69]. The following polyP fractions are commonly isolated from yeast cells in the cold: acid-soluble fraction polyP1 (extraction with 0.5 M $HClO_4$ or 10% trichloroacetic acid), salt-soluble fraction polyP2 (extraction with a saturated solution of $NaClO_4$), and alkali-soluble fractions polyP3 (extraction with a NaOH solution, pH 9–10) and polyP4 (extraction with 0.05 M NaOH). The content of the polyP5 fraction is inferred from the amount of P_i released upon treatment of the residual biomass with 0.5 M $HClO_4$ at 90°C for 40 min.

The contents of these fractions dramatically change during the growth of *S. cerevisiae* cultures.

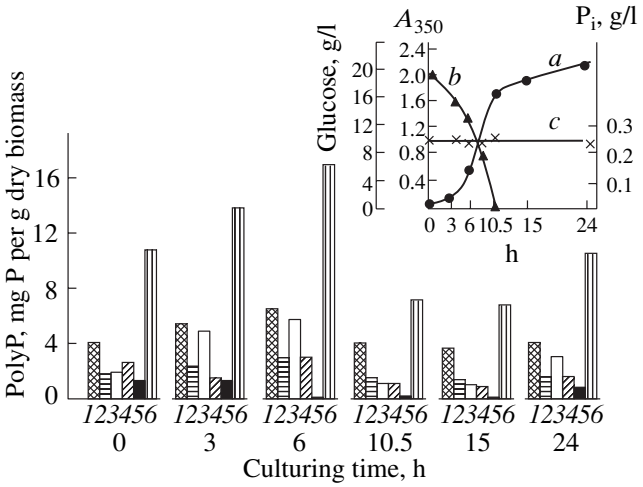

Fig. 1. Contents of individual polyP fractions in *S. cerevisiae* cells growing in the Rider medium [69]. Fractions: *1,* polyP1; *2*, polyP2; *3*, polyP3; *4*, polyP4; *5*, polyP5; and *6*, total polyP. The inset shows the time courses of (*a*) culture growth and concentrations of (*b*) glucose and (*c*) P_i in the medium.

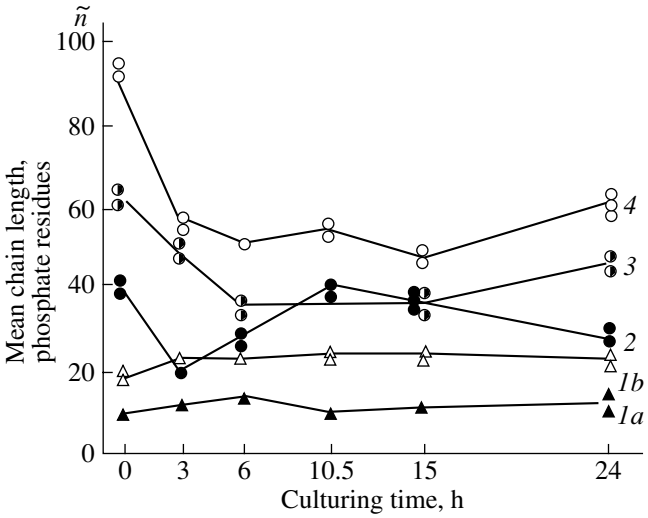

Fig. 2. Degree of polymerization ($\tilde{n}$) of individual polyP fractions in *S. cerevisiae* cells growing in the Rider medium [69]. Fractions: *1a* and *1b*, polyP1 precipitated with barium salts at pH 8.2 or 4.5, respectively; *2*, polyP2; *3*, polyP3; and *4*, polyP4.

The most detailed data were obtained by ^{31}P-NMR spectroscopy for cells cultured in the Rider medium, which contains glucose and has a relatively high (10–20 mM) concentration of P_i [69]. Yeast cells are not starved of P_i in this medium, and the observed changes in polyP content are determined by changes in cell state during culture development. The total polyP content in the cell increases in parallel with biomass until extracellular glucose is exhausted, then decreases dramatically, and increases again by the stationary phase. The individual polyP fractions change differently (Fig. 1). It is important to note that the polyP chain length also changes, decreasing considerably in the first three hours of culturing and increasing at the stationary phase in the polyP3 and polyP4 fractions (Fig. 2) [69].

In total, the changes in the content and chain length observed for individual polyP fractions during the growth and development of cell cultures make it possible to assume that at least some fractions act as an energy reserve when the extracellular P_i concentration is sufficient. It is still unclear how this energy is utilized. PolyP hydrolysis by exopolyphosphatases or endopolyphosphatase cannot convert energy stored in polyPs into other forms. We cannot exclude, however, that these or other enzymes perform the function of phosphotransferases. It is also possible that there are some enzyme systems that create a gradient of H^+, K^+, or other ions on membranes as a result of polyP hydrolysis, as is the case with ATPases and pyrophosphatases.

EFFECT OF EXTRACELLULAR P_i ON POLYPHOSPHATE CONTENT AND ACTIVITIES OF CYTOSOLIC EXOPOLYPHOSPHATASES

PolyPs serve as the major reserve of phosphorus in yeasts. The polyP content in the cell decreases considerably in P_i deficiency [3–8]. Yeast cells grown in a P_i-deficient medium and transferred into a P_i-rich medium accumulate polyPs to a greater amount than cells cultured without preliminary phosphorus starvation. This phenomenon is termed overcompensation (phosphate overplus) [3].

We studied the contents of individual polyP fractions (Fig. 3) and the degree of their polymerization (Fig. 4) in *S. cerevisiae* cells growing in a P_i-free medium and under overcompensation conditions [70]. After 7-h phosphorus starvation, cells almost completely exhausted the polyP reserve to sustain their growth (Fig. 3), which was associated with a decrease in polyP chain length (Fig. 4). When such cells were transferred into a medium containing 10 mM P_i and glucose, we observed accumulation of polyP1, polyP2, and polyP3, while the polyP4 content was only restored to the normal level. The polyP chain length did not increase in the first two hours of overcompensation and then increased as cells gradually ceased accumulating polyP (Fig. 4, D). This was especially clear with the polyP3 and polyP4 fractions. Individual polyP fractions differ in the time course of accumulation during overcompensation. The polyP accumulation and the increase in polyP chain length are temporally separate, suggesting different pathways for their regulation.

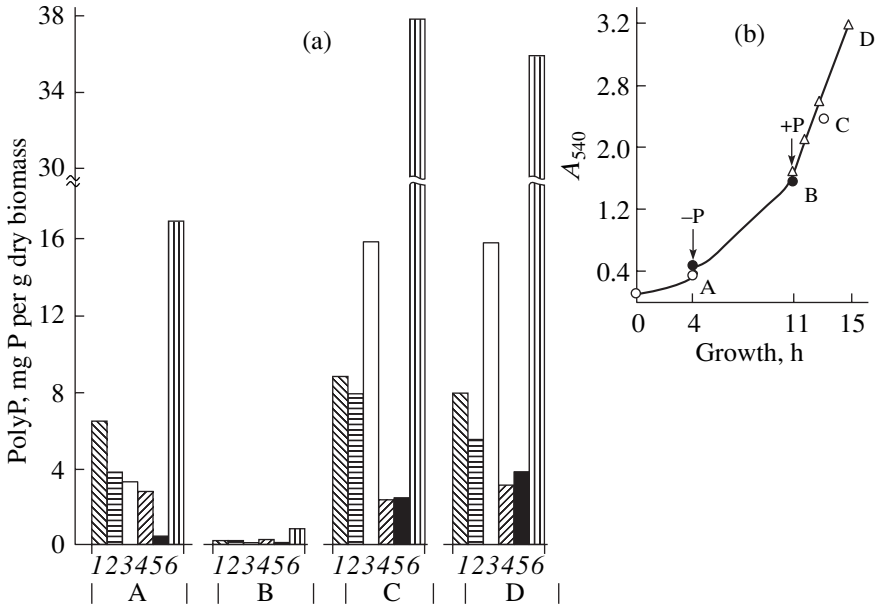

Fig. 3. PolyP content (a) and *S. cerevisiae* cell growth (b) as dependent on the P_i concentration in the medium [69]. Fractions: *1*, polyP1; *2*, polyP2; *3*, polyP3; *4*, polyP4; *5*, polyP5; and *6*, total polyP. Here and in Fig. 4: Cells were (A) transferred from a P_i -complete into a P_i -free medium, (B) transferred from a P_i -free into a P_i -complete medium, and grown in the complete medium for (C) 2 or (D) 4 h after transfer.

Overcompensation was also observed when *S. cerevisiae* cells were grown in the presence of 1 mM P_i until the late logarithmic phase and then transferred into a medium containing 20 mM P_i. The polyP pool was exhausted in these cells [71]. At an earlier growth phase, the polyP content was sufficiently high and overcompensation was not observed after cell transfer. The protein synthesis inhibitor cycloheximide did not disturb overcompensation [71]. Therefore, enzymes responsible for polyP accumulation were already synthesized during phosphorus starvation, and exhaustion of the polyP pool was an essential prerequisite to their synthesis. To induce subsequent mass accumulation of polyPs, it was sufficient to supplement the culture with glucose and a high concentration of P_i.

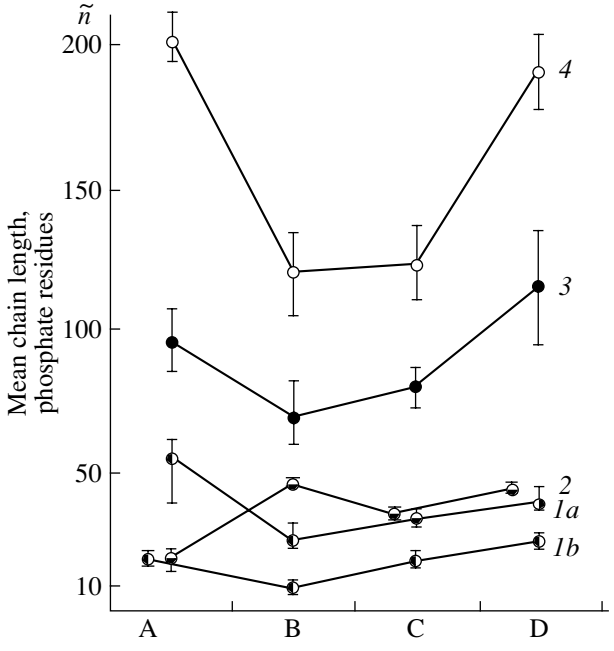

Fig. 4. Degree of polymerization ($\tilde{n}$) of individual polyP fractions in *S. cerevisiae* cells as dependent on the P_i concentration in the medium [69]. Fractions: *1a* and *1b*, polyP1 precipitated with barium salts at pH 8.2 or 4.5, respectively; *2*, polyP2; *3*, polyP3; and *4*, polyP4.

Overcompensation was accompanied by the appearance of high-molecular-weight cytosolic exopolyphosphatase (Table 2; Fig. 5a, curve *2*), which differs in some physicochemical properties from the PPX1 exopolyphosphatase, characteristic of the cytosol under normal conditions [61, 71]. The PPX1 activity decreased in overcompensation (Fig. 5a, curve *1*). However, the high-molecular-weight enzyme was also observed in the absence of overcompensation, when cells were grown in a P_i-containing medium until the late logarithmic phase and then transferred into the fresh medium (Fig. 5b, curve *2*). The increase in the activity of this enzyme was prevented by cycloheximide; i.e., high-molecular-weight exopolyphosphatase was synthesized *de novo* after cell transfer, in contrast to the enzymes responsible for polyP accumulation. Synthesis of the enzyme was not observed when cells grown in the presence of 1 mM P_i were transferred into the same medium at the stage of decelerated

growth or into a medium containing an excess of P_i at the early logarithmic phase. Thus, the appearance of high-molecular-weight exopolyphosphatase in the cytosol is associated with cell transition from decelerated growth to active proliferation provided that extracellular P_i is in excess. PolyP accumulation and production of high-molecular-weight exopolyphosphatase are independent of each other.

POLYPHOSPHATES AND ENERGY PROCESSES

The polyP content depends on cell energetics in yeasts, because polyP synthesis is energy-consuming and depends, in particular, on the source of carbon. Although this problem has not been studied systematically, it is known that polyP accumulation in *S. cerevisiae* and *Kluyveromyces marxianus* growing in an ethanol-containing medium is slower than in a glucose-containing medium [68]. The polyP content decreases under anaerobic conditions, and polyP synthesis starts only when the culture is supplemented with glucose [41, 72]. Yeasts adapted to hydrocarbon utilization accumulate polyP in the presence of hydrocarbons to a two- to threefold higher amount than in the presence of glucose [6, 7].

The polyP content in *S. cerevisiae* mitochondria increases upon glucose suppression and decreases when extracellular glucose is exhausted [45]. The mitochondrial polyP pool is even lower when yeast cells are grown on lactate; moreover, the degree of polyP polymerization is decreased [44, 45].

The association between the polyP level and cell energetics is commonly studied using inhibitors of energy metabolism, ionophores, and other substances disrupting transmembrane ion gradients. The main difficulty in such studies is that polyP synthesis is impossible in the absence of P_i uptake. All effectors reducing the P_i uptake change the polyP level to some extent. For instance, P_i uptake at pH 5.5 is 4.5 times more efficient than at pH 7.5 in the yeast *Candida humicola*, which correlates with a tenfold increase in polyP content at a lower pH [73]. It is clear that, to correctly estimate the effect of energy metabolism inhibitors on polyP accumulation, it is essential to consider their capability of suppressing polyP uptake.

Notwithstanding, the effects of energy metabolism inhibitors on the polyP content in yeasts under various conditions are of interest and provide for a better understanding of the mechanisms of polyP synthesis and the role of polyPs in energy processes. For instance, ^{31}P-NMR spectroscopy of intact cells showed that polyPs are rapidly degraded into short fragments when *S. cerevisiae* cells are exposed at a high (5%) concentration of ethanol, which causes disintegration of the plasma and vacuolar membranes [74]. Deoxyglucose is a potent inhibitor of polyP accumulation in *S. cerevisiae*. The polyP content in the cell decreases fivefold in the presence of 5 mM deoxyglucose [75]. Used at 10 mM, deoxyglucose halves the polyP accumulation upon overcompensation [71].

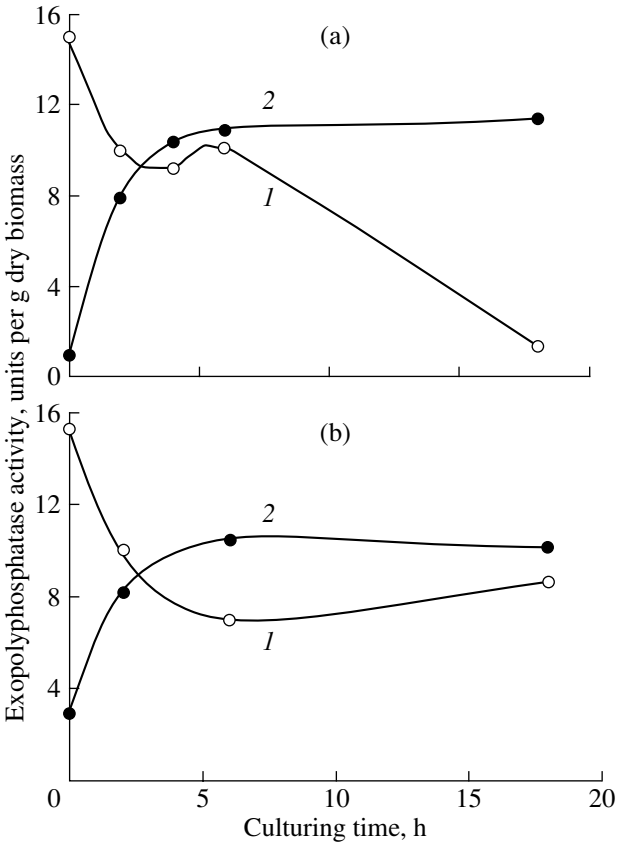

Fig. 5. Activities of (*1*) exopolyphosphatase encoded by *PPX1* and (*2*) high-molecular-weight exopolyphosphatase encoded by another gene in the cytosol of *S. cerevisiae* cells transferred at the late logarithmic phase from a medium containing (a) 1 mM P_i (overcompensation) or (b) 20 mM P_i into a medium containing 20 mM P_i. The enzymes were separated by gel filtration of the cytosolic fraction on Sephacryl S-300.

Syringomycin, which impairs the integrity of the plasma membrane, causes P_i extrusion from *Rhodotorula pillimanae* cells, which is accompanied with a decrease in polyP content and acidification of the cytoplasm [76]. As demonstrated by ^{31}P-NMR spectroscopy of intact *C. tropicalis* cells grown on xylose, sodium azide and the protonophore carbonyl cyanide trichlorophenylhydrazone (CCCP) reduce intracellular pH, suppress P_i transport, and decrease polyP synthesis [77].

Antimycin A suppresses P_i transport into the cell and polyP synthesis in *S. cerevisiae* growing in the presence of ethanol, when oxidative phosphorylation is the main source of energy [68]. Such effects were not observed in cells growing on glucose, when glycolysis mostly supplies energy for various processes,

Table 4. Effect of mutations in *PPX1* and *PPN1* on the exopolyphosphatase activity in compartments of *S. cerevisiae* cells

Strain	Cytosol	Nucleus	Vacuole	Mitochondria, soluble fraction	Mitochondria, membrane fraction
Activity, microunits per mg protein					
Parental	130	100	370	135	100
Knocked-out in *PPX1*	80	80	350	35	80
Knocked-out in *PPN1*	45	15	30	15	0
Knocked-out in *PPX1* and *PPN1*	5	0	80	0	0

including the maintenance of ionic transmembrane gradients [68]. The polyP content in *S. cerevisiae* cells decreases in the presence of CCCP or bafilomycin A, a specific inhibitor of vacuolar membrane ATPase [78]. The protonophore carbonyl cyanide trifluorophenylhydrazone (FCCP) suppresses, to a varying extent, the accumulation of all polyP fractions upon overcompensation [79] and completely prevents polyP accumulation in mitochondria [44]. The results suggest a tight association between the polyP content and electrochemical gradients of ions on membranes of various organelles. Although the association is probably indirect and is mediated by the effect on P_i transport in many cases, there are grounds for believing that polyP synthesis is at least partly due to the $\Delta\mu H^+$ energy in yeasts [44, 78–80]. Transmembrane complexes of polyPs with poly-β-hydroxybutyrate are probably one of the components involved in transformation of polyP energy into the energy of ionic transmembrane gradients [21].

The association between the polyP content and cell energetics suggests that polyPs provide not only a phosphorus, but also an energy reserve, which is expended in the cases of a high growth rate, intense transport and phosphorylation of saccharides, anaerobic growth, and radiation repair [14].

MUTATIONS AFFECTING POLYPHOSPHATE METABOLISM IN *S. CEREVISIAE*

Kornberg and colleagues [10, 11, 51, 55] made a major contribution to the identification of *S. cerevisiae* genes involved in polyP metabolism: they cloned and sequenced the *PPX1* exopolyphosphatase and *PPN1* endopolyphosphatase genes and constructed mutants with *PPX1* and *PPN1* inactivated by modern methods of site-directed mutagenesis.

We observed that a *PPX1* mutant lacks the PPX1 exopolyphosphatase in the cytosol, cell envelope, and mitochondria, while exopolyphosphatase activities of the mitochondrial membrane fraction, vacuoles, and the nucleus are maintained at the same level and preserve the properties characteristic of the parental strain [59]. The cytosol of the mutant contained high-molecular-weight exopolyphosphatase, which was earlier detected in overcompensation [59].

Inactivation of *PPN1* produces a more intricate picture [81]. A mutant with inactivated *PPN1* has a dramatically low exopolyphosphatase activity in the cytosol, soluble mitochondrial fraction, vacuoles, and the nucleus and completely lacks the membrane-associated exopolyphosphatase activity in mitochondria (Table 4). A double mutant defective in both genes has an exopolyphosphatase activity only in vacuoles (Table 4). These findings suggest an association between expression of *PPN1, PPX1*, and, possibly, other genes coding for polyP-metabolizing enzymes.

We observed that inactivation of *PPX1* and *PPN1* results in a higher total polyP content in yeast cells, especially in the double mutant. The mutations differently affect the contents of individual polyP fractions (Fig. 6). The polyP1 content increases by a factor of about 1.4 or 2.0 upon inactivation of *PPX1* or *PPN1*, respectively, while the polyP2 content changes only slightly. The polyP3 content increases only in the double mutant. According to published data, disruption of *PPN1* (*PHM5*) considerably increases the polyP chain length and has no effect on the polyP content [82].

We studied the effects of the above mutations on polyP metabolism in mitochondria [45]. Inactivation of *PPX1* does not affect the state of mitochondria, the mitochondrial polyP content, or the capacity to grow on nonfermentable substrates. By contrast, *PPN1* inactivation leads to a near complete loss of exopolyphosphatase activity (Table 4) and increases the content and the chain length of mitochondrial polyPs (Fig. 7). Moreover, these mutants show a dramatic impairment of the respiratory control and the succinate dehydrogenase and ATPase activities in mitochondria [45]. Yeast strains with inactivated *PPN1* cannot grow on lactate or ethanol. Taken together, the above data demonstrate that *PPN1* inactivation distorts both polyP metabolism and the development of functional mitochondria from promitochondria when extracellular glucose is exhausted. It is possible to assume that polyPs play an important role in regulating the functional state of mitochondria.

Again, the dependence of polyP metabolism on P_i uptake is of importance for interpreting the effects of

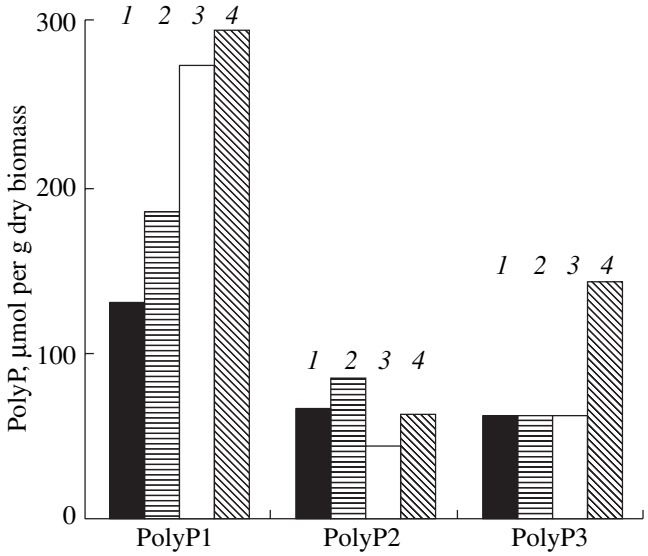

Fig. 6. Contents of polyP fractions in *S. cerevisiae* cells of (*1*) the parental strain and strains knocked-out in (*2*) the *PPX1* exopolyphosphatase gene, (*3*) the *PPN1* endopolyphosphatase gene, or (*4*) both *PPX1* and *PPN1* (double mutant) at the stationary growth phase.

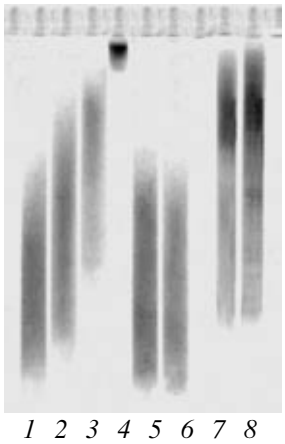

Fig. 7. Electrophoresis of *S. cerevisiae* mitochondrial polyPs in 20% PAG in the presence of 7 M urea. Gel was stained with Toluidine Blue. PolyP markers: (*1*) $polyP_{15}$, (*2*) $polyP_{25}$, (*3*) $polyP_{45}$, and (*4*) $polyP_{188}$. Acid-soluble mitochondrial polyPs were isolated from *S. cerevisiae* cells of (*5*) the parental strain and strains knocked-out in (*6*) *PPX1*, (*7*) *PPN1*, or (*8*) both *PPX1* and *PPN1* (double mutant). Cells were grown in a glucose–peptone medium until the stationary growth phase.

mutations. The *S. cerevisiae* system of P_i transport includes many genes [83], and it is quite natural that their mutations exert a considerable effect on polyP metabolism. In total, 22 relevant genes were identified. Some of these—such as *PHM1*, *PHM2, PHM3*, and *PHM4*—have 32–56% homology [82]. The *PHM3* and *PHM4* mutants and the *PHM1*/*PHM2* double mutant are defective in P_i transport and accumulate polyPs to a lower content [82]. The proteins encoded by these genes are thought to play a role in transporting low-molecular-weight substances into vacuoles [82].

A lack of polyPs was reported for mutants defective in vacuole functioning [36, 37, 78]. It should be noted, however, that these results were obtained for ^{31}P-NMR-detectable polyPs [37, 78], which are only a fraction of the total polyP pool, or for yeast cells grown under such conditions that vacuolar polyPs accounted for a major part of the total polyP pool [36]. A mutation of *SPT7* increases polyP accumulation in vacuoles and makes yeast cells less sensitive to nickel ions, owing to their more efficient binding in complexes with polyPs [84].

In addition, effects on the polyP content were observed for some other mutations that do not directly change polyP metabolism or P_i transport. For instance, a mutant defective in the respiratory chain has a lower polyP content upon aerobic growth on glucose and galactose [85]. Disruption of *YOL002c* induces several genes involved in fatty acid metabolism, determines nistatin resistance, and increases polyP accumulation [86].

Thus, studies with modern genetic techniques yielded new data on polyP metabolism and functions in yeasts. Some mutations of polyP metabolism genes, in particular, *PPN1*, have a pleiotropic effect, testifying again to the multiplicity of polyP functions.

CONCLUSIONS

The accumulation and utilization of polyPs in yeasts depends to a great extent on the culture conditions and the growth phase. Individual polyP fractions differ first and foremost in polyP localization and state and in the specifics of their metabolism, rather than in polyP chain length. Studies of polyP metabolism in yeasts must take account of the fact that each cell compartment contains its own exopolyphosphatases and, possibly, other enzymes of polyP metabolism.

The available data on polyP metabolism in yeasts pose some important problems, which concern other lower eukaryotes as well. First, the mechanisms of polyP synthesis are still obscure. Although the polyphosphate kinase activity was observed in yeasts [48–50, 73], the corresponding enzyme was not isolated nor its gene identified. The role of this activity in polyP accumulation is still unclear in yeasts as opposed to bacteria, whose polyphosphate kinase is the major enzyme responsible for polyP synthesis [10, 11]. The activities of other polyP-synthesizing enzymes, 1,3-diphosphoglycerate phosphotransferase and dolichyl–polyP phosphotransferase, are too low to account for the high polyP content in yeast cells. Second, although polyP–RNA complexes were observed rather long ago [6, 7], their role in regulation of bio-

chemical processes is poorly understood. Little is known of the polyP function in the nucleus, although the presence of polyPs [6, 7] and exopolyphosphatase [59, 62] in the nucleus is well documented. Third, the available data suggest additional pathways for the role of polyPs in regulatory processes in yeasts and other lower eukaryotes. One such pathway possibly depends on the interplay of polyP metabolism and the function of some signaling molecules acting as secondary messengers. Metabolic pathways involving pppGpp and polyPs are tightly associated in bacteria [10, 11]. Phosphoinositides and diadenosine tetra-, penta-, and hexaphosphates are candidates for such an interaction in eukaryotes [87]. It should be noted that, in the yeast cell, the PPX1 exopolyphosphatase efficiently hydrolyzes adenosine 5'-tetraphosphate and guanosine 5'-tetraphosphate [59] and diadenosine 5',5'''-P^1,P^4-tetraphosphate α,α-phosphorylase displays a polyphosphate kinase activity in combination with other enzymes [49].

The data considered in this review testify that the regulatory role of polyPs in the yeast cell should be a subject of further studies. This role is probably no less important than in bacteria [10, 11].

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