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POLYPHOSPHATE AND PHOSPHATE PUMP

I. Kulaev and T. Kulakovskaya
Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Moscow Region, 142292 Pushchino, Russia; e-mail: kulaev@ibpm.serpukhov.su

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■ **Abstract** In microbial cells, inorganic polyphosphate (polyP) plays a significant role in increasing cell resistance to unfavorable environmental conditions and in regulating different biochemical processes. polyP is a polyfunctional compound. The most important of its functions are the following: phosphate and energy reservation, cation sequestration and storage, membrane channel formation, participation in phosphate transport, involvement in cell envelope formation and function, gene activity control, regulation of enzyme activities, and a vital role in stress response and stationary-phase adaptation. The functions of polyP have changed greatly during the evolution of living organisms. In prokaryotes, the most important functions are as an energy source and a phosphate reserve. In eukaryotic microorganisms, the regulatory functions predominate. Therefore, a great difference is observed between prokaryotes and eukaryotes in their polyP-metabolizing enzymes. Some key prokaryotic enzymes are not present in eukaryotes, and conversely, eukaryotes have developed new polyP-metabolizing enzymes that are not present in prokaryotes. The synthesis and degradation of polyP in each specialized organelle and compartment of eukaryotic cells are mediated by different sets of enzymes. This is consistent with the endosymbiotic hypothesis of eukaryotic cell origin.

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INTRODUCTION

Microorganisms are highly dependent on the environment for their viability and metabolism, as well as for the functioning of their cell machinery. As a result, they have evolved the ability to store a number of important metabolites in the form of osmotically inert polymers. Inorganic phosphate is one such metabolite; it is stored within the cell as a high-molecular-weight inorganic polyphosphate (polyP$_n$, where n is the approximate number of phosphate residues in the polyP molecule). This compound, first identified more than 100 years ago (65), is a linear polymer containing from a few to several hundred residues of orthophosphate that are linked by energy-rich phosphoanhydride bonds. polyPs are widespread in living organisms and are found in the cells of microorganisms, animals, and plants (52, 56, 68). Yet, their greatest quantities are found in microbial cells, where they play a significant role in increasing cell resistance to unfavorable environmental conditions and in regulating different biochemical processes.

In recent years, a greater interest in polyP and its functions has been observed. On the one hand, the modern molecular biological methods (8, 47, 49) and special techniques (including ^{31}P-NMR) for analyzing polyP allow rapid progress in the study of the structure and metabolism of this compound (20). On the other hand, the study of polyP biochemistry is important in biotechnology for the creation of systems for enhanced biological removal of phosphate from wastewater (119) and for the development of effective control of pathogenic bacteria and viruses (49, 66).

A number of reviews (47, 49, 51), including a special issue of *Progress in Molecular and Subcellular Biology* (97), have recently been added to the known reviews (32, 52, 54, 125) covering the most important aspects of current research into polyP biochemistry. However, little attention has been paid to the extensive difference between prokaryotic and eukaryotic microorganisms in the intracellular localization, metabolism, and function of this compound. This difference emerged during evolution, because eukaryotes have highly developed compartmentation of biochemical processes and specialized cell organelles. One of the new approaches to polyP biochemistry is the use of cell fractionation techniques to study this compound and related enzymes in individual cell compartments. A comparative biochemical study of polyP and enzymes of its metabolism in different cell compartments of eukaryotic and prokaryotic microbial cells seems to be important both

for further understanding of the functions of this polymer and for further progress in the study of the origin of life.

It is worth mentioning that this review does not touch on the metabolism and functions of pyrophosphate, because it has specific functions and metabolic pathways that are different from those of polyP. To mediate the reactions with pyrophosphate, the cell has a set of special enzymes, evolutionarily unrelated to the enzymes catalyzing hydrolysis/synthesis of high-molecular-weight inorganic polyP (9, 69). This review is devoted to comparative analysis of the metabolism of inorganic polyP (with chain lengths of three or more phosphate residues) in prokaryotic and eukaryotic microorganisms.

LOCALIZATION OF POLYPHOSPHATES IN MICROBIAL CELLS

Prokaryotes

The main compartments of a bacterial cell are the cytoplasm, cell surface, periplasm, and plasma membrane; polyP is found in all of these compartments. polyP-containing granules are present in the cytoplasm of many bacteria (52, 54). Under usual growth conditions, *Escherichia coli* has a low content of polyP (76). The ability of *E. coli* to accumulate polyP may be enhanced by manipulating the genes involved in the transport and metabolism of orthophosphate (P_i) and the genes encoding the enzymes involved in polyP metabolism (31, 39). polyP in the cytoplasm of *E. coli* was found in association with different enzymes and multienzyme complexes, including those with RNA polymerase (60) and with degradosomes that perform the function of RNA processing and degradation (13).

polyP granules were observed in the vicinity of the bacterial nucleoid (54). Some amounts of polyP are localized in the periplasmic region of bacteria outside the cytoplasmic membrane (76, 90). polyP is a component of the cell capsule, which, for *Neisseria* spp., is loosely attached to the surface membrane. This capsular polyP represents ~50% of the polyP content in *Neisseria* cells (109, 110). In the *Helicobacter pylori* bacteria colonizing the gastric antrum, polyP was found in at least three different locations: the cytoplasm, the flagellar pole, and the cell membrane (14).

One of the most remarkable recent discoveries in polyP biochemistry was the finding by Reusch et al (92, 93, 95) of polyP complexes with Ca^{2+} and poly-β-hydroxybutyrate in bacterial membranes. The complex is a double helix, in which the outer chain is represented by poly-β-hydroxybutyrate and the inner chain by polyP linked to the outer chain by Ca^{2+} ions.

Eukaryotes

The basic difference between eukaryotes and prokaryotes is a much better developed compartmentation of biochemical processes in eukaryotes, wherein some

of the processes take place in specialized cell organelles. However, eukaryotic microorganisms possess polyP pools in all cell compartments studied in this respect. Their contents vary, depending on cultivation conditions. The quantitative estimation of the polyP content in different compartments of eukaryotic microorganisms may depend on the methods of extraction and assay. At cell fractionation, labile polyP may degrade. For instance, vacuoles isolated from *Saccharomyces cerevisiae* at a lower osmolarity actually have no polyP (LP Lichko, unpublished data). The results obtained by ^{31}P-NMR also have to be interpreted with caution. The chemical shifts of ^{31}P-NMR spectra of many phosphate-containing substances (including the linear polyP) are well known (20). A recent study (50) has shown that the total intensity of the core phosphate groups depends not only on the concentration of each individual polyP, but also on its chain length and on the extent of polyP binding with other structures and compounds of the cell. One of the conventional methods of polyP extraction isolates five polyP fractions from yeast cells (61, 117). These fractions differ to a greater extent in their state or localization in the cell, rather than in the degree of polymerization (51, 117).

The polyP in different cell compartments was best studied in *S. cerevisiae*. The content of polyP in cytosol depends on culture age and cultivation conditions. The cytosol fraction may contain from 10% (82) to 70% (51) of the polyP cell pool in cells of *S. cerevisiae*. In this yeast, the amount of polyP in the cytosol increases about twofold with the so-called "overplus," which occurs when cells are transferred from a medium without phosphate to a medium with phosphate (51). Some part of cytosolic polyP is a component of volutine granules, which also contain four basic proteins and metal ions (38). Volutine granules possess $\sim$14% of the total polyP content in yeast cells (38).

The data on the amount of polyP in yeast vacuoles are quite contradictory, because the authors used different strains and cultivation conditions. Vacuoles are thought to contain polyPs of small chain lengths. When *S. cerevisiae* is grown on a poor-mineral medium with arginine as the only nitrogen source and the culture growth rate is low, vacuoles may contain the major part of the yeast cell polyP pool (24, 114). The amount of polyP in yeast vacuoles sharply increases when this microorganism accumulates metal cations. *S. carlsbergensis* vacuoles accumulated seven-fold more polyP than cytosol under incubation with phosphate, glucose, and K^+, and ten-fold more with Mn^{2+} (64). Under other growth conditions, the vacuolar polyP pool in *S. cerevisiae* was significantly lower. The vacuoles of *S. cerevisiae* growing on the Rider medium contained $\sim$15% of the total amount of polyP in the cell (51). The vacuoles of *Candida utilis* contained $\leq$30% of the total amount of polyP in the cell, depending on the rate of culture growth and the nitrogen source in the medium (79). polyP was absent in vacuoles during the growth of *Candida* spp. in a phosphate-deficient medium (18). polyP was also found in the vacuoles of *Neurospora crassa* (21) and *Dunaliella salina* (84, 85). Regarding the role of vacuoles as the main compartment of reserve compounds in eukaryotic microorganisms, it can be expected that other fungi, yeasts, and algae have a vacuolar pool of polyP.

Considerable amounts of polyP are present in the cell envelopes of the lower eukaryotes. The cell envelopes of yeasts can contain ≥20% of the total polyP content of the cell (37, 115). This high polymeric polyP is extracted mostly by a weak alkali at pH 9.0 (54). polyP complexes with poly-β-hydroxybutyrate, similar to those in bacteria, were identified in eukaryotic membranes (91).

polyP with short chain lengths of 14 phosphate residues was found in yeast mitochondria by ^{31}P-NMR. This polyP corresponds to 10% of the total content of cellular polyP detected by NMR (11).

The formation of polyP complexes with nucleic acids and their presence in the nuclei of eukaryotic cells are well known (52, 54). polyPs were observed in the nuclei of *Physarvum polycephalum* (86) and *Agaricus bisporus* (52, 54). The DNA preparations from filamentous fungal species of *Colletotrichum* possess $polyP_{60}$ (96). The amount of polyP in nuclei may be low; however, this compound, which occurs in the nuclei of both lower eukaryotes and mammals, is conserved there during evolution (52, 54, 56).

COMPARISON OF THE ENZYMES OF POLYPHOSPHATE METABOLISM IN PROKARYOTES AND EUKARYOTES

Some enzymes involved in the biosynthesis and degradation of polyP are observed in prokaryotes but not in eukaryotes. These are polyP:ADP phosphotransferase (polyP kinase, EC 2.7.4.1), polyP:glucose-6-phosphotransferase (EC 2.7.1.63), polyP:adenosine monophosphate phosphotransferase, and polyP:NAD-phosphotransferase. Endopolyphosphatase (polyP polyphosphohydrolase, EC.3. 6.1.10) and dolichol-pyrophosphate:polyP-phosphotransferase were found in eukaryotes. Other enzymes, for example 1,3-diphosphoglycerate:polyP phosphotransferase (EC 2.7.4.17) and exopolyphosphatase (EC 3.6.1.11), are present both in eukaryotes and prokaryotes, but their significance in cell metabolism is different.

Polyphosphate-Synthesizing Enzymes

Prokaryotes The main enzyme mediating polyP biosynthesis in bacteria is polyP kinase, which was found by Kornberg et al (48). Mutations in the polyP kinase-encoding gene (*ppk*) result in significant deficiency in the polyP content in bacterial cells (2, 22, 88, 109). The amount of polyP in the *ppk* mutants of *Neisseria gonorrhoeae* and *N. meningitidis* was reduced to 2% of the wild-type levels (109). The accumulation of polyP in *E. coli* was increased by a higher dosage of the *E. coli* genes encoding polyP kinase (*ppk*), acetate kinase (*acka*), and phosphate-inducible transport systems (*PSTS*, *PSTC*, *PSTA*, and *PSTB*), and by genetic inactivation of *ppx* encoding exopolyphosphatase (31, 39). About 65% of cellular phosphorus was stored as polyP in such recombinant strains (39). The *ppk* genes from *E. coli* (2), *Klebsiella aerogenes* (40), *N. meningitidis* (109), *Pseudomonas aeruginosa* (36), and *Acinetobacter* sp. (27) have been cloned, sequenced, and characterized. The

deduced amino acid sequences of these enzymes show an extensive homology in different bacterial species (113). The polyP kinase of *E. coli* is a membrane-bound homotetramer with a subunit molecular mass of 80 kDa (1, 2). It catalyzes the reactions of reverse transfer of energy-rich phosphate residues from ATP to polyP and from polyP to ADP, thus linking the energy-rich pools (58, 73). This enzyme is responsible for the processive synthesis of long $polyP_{750}$ chains in vivo (1).

polyP kinase can use polyP as a donor in place of ATP, thereby converting GDP and other nucleoside diphosphates to nucleotide triphosphates (58). This reaction was observed with the activity present in crude membrane fractions of *E. coli* and *P. aeruginosa*, as well as with the purified enzyme obtained from *E. coli*. Membrane fractions obtained from the *E. coli* mutants lacking the *ppk* gene have no such activity. The substrate specificity order was as follows: ADP > GDP > UDP, CDP; the activity with ADP was twofold that of GDP (58). It was confirmed that polyP kinase efficiently catalyzed UTP regeneration in the cyclic system of *N*-acetyl lactosamine synthesis. This activity of pure polyP kinase enables the practical synthesis of oligosaccharides (78). Although the transfer of a phosphate from polyP to GDP by polyP kinase to produce GTP was the predominant reaction, the enzyme also transferred a pyrophosphate group to GDP to form a linear guanosine 5′-tetraphosphate (46). polyP kinase may thus be involved in the regulation of the level of ribonucleoside triphosphates and deoxyribonucleoside triphosphates that modulate cell division and survival in the stationary phase (46, 58).

The polyP kinase of bacteria is a component of different multienzyme complexes and participates in the regulation of their activity. The *E. coli* degradosome, a multienzyme complex whose function is RNA processing and degradation, has been shown to possess polyP kinase (13). Purified polyP kinase was shown to bind RNA, and RNA binding was prevented by ATP (13). *Sulfolobus acidocaldarius* possesses a glycogen-bound polyP kinase, which is active only as a native complex with glycogen. Involvement of this activity in the regulation of metabolism of the above polysaccharide has been proposed (107).

Bacteria have other polyP-synthesizing enzymes. In particular, poly-β-hydroxybutyrate–calcium–polyP membrane complexes are known to occur in *E. coli* mutants lacking polyP kinase (19). Therefore, the polyP in these complexes is synthesized by another enzyme (19). Some prokaryotes were shown to have 3-phosphoglyceroylphosphate–polyP-phosphotransferase, which uses 1,3-diphosphoglycerate to form polyP (52, 54).

Eukaryotes In contrast to bacteria, polyP kinase is evidently less significant for polyP biosynthesis in eukaryotic microorganisms. The homology to the bacterial *ppk* gene has not been observed in eukaryotic cells (49). Recent data (17) show that polyP kinase from the yeast cell homogenate purified by Felter & Stahl (26) is actually diadenosine-5′,5‴-P^1, P^4 tetraphosphate a,b-phosphorylase (AP_4 phosphorylase). The production of ^{32}P-labeled ATP in the presence of ^{32}P-labeled polyP and ADP was not provided by polyP kinase but rather by the enzyme diadenosine-5′,5‴-P^1, P^4 tetraphosphate a,b-phosphorylase, acting in concert with one or more

yeast polyphosphatases (17). polyP kinase activity in the yeast vacuolar membrane was more active during the consumption of polyP and formation of ATP, rather than in the synthesis of polyP (103). The enzyme has not been purified, and the presence of polyP kinase in certain membrane fractions in yeast cells is still in question. The level of this activity observed in yeasts is insufficient to explain the synthesis of a large amount of polyP observed in this microorganism.

Some authors (98) believed that the polyP biosynthesis in yeasts is apparently provided by 1,3-diphosphoglucerate:polyP phosphotransferase. However, the activity of this enzyme under usual growth conditions is low. It was found only in the *Neurospora crassa* mutant that is deficient in adenine, in which the concentrations of ATP and other adenyl nucleotides are sharply reduced (53). This enzyme synthesizes polyP during glycolytic phosphorylation with a low ATP content in the cell (53), probably in the cytosol. This enzyme may actually be involved in the biosynthesis of some intracellular polyP fractions, presumably low-molecular-weight polyP.

The surface high-molecular-weight polyP in yeasts is synthesized via another pathway closely related to the biosynthesis of cell wall mannoproteins (100–102). The synthesis occurs in the membrane fraction of the endoplasmic reticulum. The membrane fraction contains an enzyme, dolichylpyrophosphate:polyP phosphotransferase, which synthesizes polyP by using β-phosphate groups of dolichylpyrophosphate (Dol-P-P). It should be emphasized that mannan and polyP synthesized in these reactions in the endoplasmic reticulum are transported by the corresponding vesicles across the plasmalemma and incorporated in the yeast cell wall. This enzyme was solubilized from the membrane fraction using Triton X-100 (100). The specific activity in the preparation of a solubilized enzyme was 20 times higher than that in the protoplast lysate. Dol-P-P:polyP phosphotransferase is a metal-dependent enzyme exhibiting maximum activity in the presence of Mg^{2+} or Ca^{2+}. A putative scheme of coordination of mannan and polyP biosynthesis by the cell wall formation was proposed (101, 102). Dol-phosphates are transmembrane carriers of carbohydrate residues in glycoprotein biosynthesis. GDP-mannose at the cytoplasmic side of the endoplasmic reticulum interacts with the phosphate residue of Dol-phosphates. The Dol-P-P-mannose is transported across the membrane so that the phosphomannose residue enters the lumen, where mannosyl transferase and Dol-P-P:polyP phosphotranferase reactions occur. As a result, Dol-phosphate is formed, which again crosses the membrane and could interact on its cytoplasmic side with a new molecule of GDP-mannose. The mannoproteins and polyP are transported to the cell envelope by special vesicles. This synthetic pathway explains the presence of polyP outside the cytoplasmic membrane.

Some enzymes, when binding with the membrane, are known to change the direction of a catalyzed reaction. The membrane-bound pyrophosphatase of mitochondria, like its ATPase, synthesizes pyrophosphate in native mitochondria and hydrolyzes it after solubilization (69). Because the level of polyphosphatase activity in eukaryotic microorganisms is appreciably higher than that in prokaryotes, we can assume that membrane-bound polyphosphatases may be involved in the polyP

synthesis in eukaryotes. Membrane-bound exopolyphosphatases are revealed in vacuoles and mitochondria (6, 63). The cell-envelope exopolyphosphatase also shows some properties of a membrane-bound enzyme (7). Possible participation of polyphosphatases in this process can be confirmed by the incorporation of labeled orthophosphate directly in the high-molecular polyP of lysosomes observed in animal cells (87). However, these suggestions still await experimental confirmation.

In yeasts, the degree of polymerization of different polyP fractions changes, depending on the growth stage (117). Therefore, we suggest that endopolyphosphatases could participate in the polyP biosynthesis in some yeast organelles. This activity occurs in nuclei and vacuoles (52, 54, 57). Endopolyphosphatases probably promote the appearance of low-molecular-weight polyP, which can leave these organelles and serve as primer for the synthesis of high-polymer polyP in other intracellular structures. In general, clarification of the pathways of polyP synthesis in the lower eukaryotes and the search for the enzymes involved in these pathways require further investigation. The mechanisms of this synthesis may be different in various cell compartments.

Polyphosphate-Utilizing Enzymes

Prokaryotes Prokaryotes possess polyP-utilizing enzymes that are not detected in eukaryotes. There are polyP:glucose-6-phosphotransferase (34, 83, 108), polyP:NAD-phosphotransferase (72), and polyP:adenosine monophosphate phosphotransferase (15), whose properties are well described. One of the important enzymes involved in polyP metabolism is exopolyphosphatase, the enzyme that splits P_i from the end of the polyP chain. Exopolyphosphatases of microorganisms are very diverse. Even prokaryotes show their different forms. The exopolyphosphatase from *E. coli* is a dimer with a subunit molecular mass of ~58 kDa (3). Its affinity for high-molecular polyP was nearly 100-fold higher than that of yeast polyphosphatases (K_m = 9 nM $polyP_{500}$ as a polymer). This enzyme exhibits a high requirement for K^+ (21-fold stimulation by 175 mM of K^+) (3). It has low activity with short-chain polyP. The exopolyphosphatase from *A. johnsonii* is a monomeric protein of 55 kDa (16). The K_m value for polyP with an average chain length of 64 phosphate residues is 5.9 μM. The activity is maximal in the presence of 2.5 mM Mg^{2+} and 0.1 mM K^+. No activity is observed in the absence of cations or in the presence of Mg^{2+} or K^+ alone (16). The enzyme of *A. johnsonii* was active with tri- and tetraphosphate in the presence of 300 mM NH_4 and 10 mM Mg^{2+}, whereas no activity with tripolyphosphate was observed in the presence of 0.1 mM K^+ and 2 mM Mg^{2+}. A specific tripolyphosphatase was purified from *Thermobacterium thermoautotrophicum* (118). This 22-kDa enzyme hydrolyzes tripolyphosphates fivefold more actively than it does $polyP_{15}$ (118). Although the two most-studied bacterial exopolyphosphatases from *E. coli* and *A. johnsonii* differ from each other to a great extent, they exhibit some similarities. They have a low specific activity (1 U/mg protein for the enzyme from *A. johnsonii* and

22 U/mg of protein for the enzyme from *E. coli*) in comparison with the yeast cytosol and cell envelope enzymes (4, 7); have low activity on tripolyphosphates and short-chain polyP; and require K^+ for the maximal activity. These properties also represent the most appreciable difference between most yeast and bacterial polyphosphatases. The gene of yeast polyphosphatase (*PPXs*) has no sufficient similarity to the bacterial *ppx* gene (127). The low activity of bacterial polyphosphatases can probably be explained by the finding that polyP kinase in prokaryotes is able both to synthesize and to hydrolyze polyP (58, 73). The gene *ppx*, encoding the major *E. coli* exopolyphosphatase, has been cloned and sequenced (2). Another enzyme encoded by the gene *gppA* and possessing exopolyphosphatase activity was purified from *E. coli* (42). This enzyme is a dimer with a monomer molecular mass of 50 kDa; the K_m is 0.5 nM ($polyP_{500}$). It has a preference for long-chain polyP, but one of its substrates is guanosine pentaphosphate (pppGpp), an important second messenger in bacteria. No similarities in the *ppx* and *gppA* genes were found (42).

Eukaryotes Endopolyphosphatase has escaped detection in prokaryotes (47, 57). The presence of this enzyme in eukaryotes is supposed to be associated with polyP pools in different compartments and polyP transport between the compartments. The endopolyphosphatase splits long polyP molecules into shorter ones. This enzyme was purified from yeast (57). It is a dimer with subunits of 35 kDa. Its activity requires divalent metal cations. Mn^{2+} is more active than Mg^{2+}, with an optimum concentration of ~2.5 mM. This enzyme hydrolyzes polyP to shorter chains, even to tripolyphosphate. The authors suggest that the endopolyphosphatase is localized in vacuoles (57).

Exopolyphosphatases of eukaryotic microorganisms are still more diverse. The data obtained recently indicate that each compartment of a yeast cell contains not only its own specific pool of polyP but also its own exopolyphosphatases, which differ in properties from the corresponding enzymes in other compartments (4, 5, 7, 62, 63). *S. cerevisiae* exopolyphosphatases exhibit only some similar properties. They hydrolyze polyP with different chain lengths and show the optimal activity at neutral pH. They fail to hydrolyze pyrophosphate, *p*-nitrophenylphosphate, or nucleoside triphosphates (4–7, 62, 63). The polyphosphatase activities of the cell envelope, cytosol, vacuoles, nuclei, and mitochondrial matrix increased in the presence of divalent metal cations. However, the degree of stimulation of these polyphosphatases is different, depending on the nature of cations and their concentration. The cell envelope and cytosol polyphosphatases revealed great similarities in cation stimulation (5, 7). The best activators, Mg^{2+} and Co^{2+}, stimulate these enzymes to the same optimal level (200 U/mg of protein), that is, 10- to 14-fold. The soluble mitochondrial polyphosphatase was less stimulated (63). The vacuolar enzyme was stimulated only twofold by Mg^{2+}. Co^{2+}, the best activator, stimulated its activity sixfold (4). The nuclear polyphosphatase activity was stimulated twofold by divalent cations (62). A distinctive feature of the polyphosphatase activity of mitochondrial membrane is that the addition of divalent cations results

in the inhibition of activity, which also depends on the nature and concentration of cations (63). Purified preparations of exopolyphosphatases differ in their maximal activities. The highest are specific activities of the cytosol and cell envelope polyphosphatases (200–280 U/mg of protein with $polyP_{15}$) (4, 6). Specific activities of the vacuolar (5) and soluble mitochondrial (63) polyphosphatase were lower: ~60 and 2.2 U/mg of protein (with $polyP_{15}$), respectively. Exopolyphosphatases of cell envelope, cytosol, mitochondrial matrix, and nuclei can be called tripolyphosphatases because their activities with tripolyphosphate are 1.5-fold greater than with higher-molecular-weight polyP (4, 7, 62, 63). The polyphosphatase purified from vacuoles (5) exhibits only low activity on tripolyphosphate (7% of the activity with $polyP_{208}$). Although the cytosol polyphosphatase is able to split adenosine-5′-tetraphosphate, the vacuolar polyphosphatase does not hydrolyze this compound. The vacuolar polyphosphatase is able to split polyphosphates only to tripolyphosphates, whereas the cytosol polyphosphatase can split them to pyrophosphates (4, 5). In mitochondrial membranes, tripolyphosphatase activities were somewhat lower than those for $polyP_{15}$ (63). The polyphosphatase activities were nearly the same on substrates ranging from $polyP_9$ to $polyP_{208}$ for polyphosphatases from the cell envelope, the cytosol (4, 7), the nuclei (62), and a soluble mitochondrial preparation (63). On the contrary, the vacuolar and membrane-bound mitochondrial polyphosphatases hydrolyzed $polyP_{208}$ more efficiently than they did $polyP_{15}$ (5, 63). The cell envelope and cytosol polyphosphatases are similar in their K_m values for polyPs with different chain lengths (400 μM, 10 μM, and 1 μM for $polyP_3$, $polyP_{15}$, and $polyP_{208}$, respectively). The apparent K_m values of the soluble mitochondrial enzyme are 300 μM, 18 μM, and 0.25 μM at hydrolysis of polyPs with chain lengths of 3, 15, and 188 phosphate residues, respectively. For vacuolar enzymes, these values are higher: 110 μM and 6 μM for $polyP_{15}$ and $polyP_{208}$, respectively. The affinity of the above enzymes to polyP increases together with the length of chain, which suggests the existence of special sites for substrate length recognition in their molecules. Only the polyphosphatase of the nucleus has similar affinities to $polyP_{15}$ and $polyP_{208}$ (K_m value was 5 μM).

The antibodies against the purified cell envelope polyphosphatase were effective inhibitors of polyphosphatases from cell envelope and cytosol, but they did not affect the vacuolar, nuclear, or mitochondrial membrane-bound polyphosphatase activities. These antibodies inhibited the soluble mitochodrial polyphosphatase by ~30%, in contrast to the 80% inhibition of the cell envelope and cytosol polyphosphatases (4, 5, 62, 63).

Ethylenediaminetetraacetic acid (EDTA) had varied effects on polyphosphatase activities. Although EDTA decreased the polyphosphatase activities of vacuoles and nuclei by ~30% and had a poor effect on mitochondrial activities, it increased those of the purified cytosol and cell envelope enzymes 1.5-fold. Different effects of EDTA on polyphosphatase activities are probably caused by their different sensitivities to divalent metal cations (4).

The molecular mass, as determined by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was 40 kDa for the cell envelope

polyphosphatase (7) and the cytosol polyphosphatase (4) and 41 kDa for the soluble mitochondrial enzyme (LP Lichko, unpublished). These enzymes were monomers. The molecular mass of vacuolar polyphosphatase, as determined by gel filtration, was 240 kDa (5). For membrane-bound mitochondrial polyphosphatases molecular masses of 120 kDa and 76 kDa were reported (63).

Thus, the biochemical and immune properties of the cell envelope and cytosol polyphosphatases of the same yeast strain are similar. Polyphosphatases of nuclei, vacuoles, mitochondrial matrix, and membrane differ in terms of their kinetic and immune properties, substrate specificity, requirement for divalent cations, and in some effector actions both from cytosolic and cell-envelope polyphosphatases and from each other. This suggests differences in their functions.

Two polyphosphatases have been recently purified from homogenate of *S. cerevisiae* (67, 126). These enzymes have neutral pH optima and similar kinetic properties and substrate specificity, and they require divalent cations (preferably Mg^{2+} or Co^{2+}) for maximal activity. Their activity on tripolyphosphate is ~1.5-fold higher than that on long-chain polyP. These enzymes are monomeric proteins, one of 40 kDa (126) and the other of 28 kDa (67). The properties of these enzymes are similar to those of cytosolic and cell-envelope exopolyphosphatases. Therefore, all of these polyphosphatases seem to be products of the *PPXs* gene (127), with possible posttranscriptional modifications. The vacuolar polyphosphatase is most likely encoded by another gene, because it differs markedly from the product of the *PPXs* gene in structure and properties. The existence in the yeast genome of other genes encoding exopolyphosphatases was proposed by Wurst et al (127). The amino-terminal sequence of a polyphosphatase from the soluble preparation of mitochondria of *S. cerevisiae* shows that this enzyme is not encoded by *PPXs* (LP Lichko, unpublished data). The vacuolar enzyme has a certain similarity to bacterial exopolyphosphatases in the values of specific activity and substrate specificity. However, in contrast to bacterial enzymes, the vacuolar polyphosphatase does not require K^+. Comparison of the properties of bacterial polyphosphatases purified recently shows that they differ greatly from those of *S. cerevisiae*. There is no similarity between the properties of the soluble polyphosphatase from yeast mitochondria and those of the polyphosphatase from *E. coli* encoded by the *ppx* gene: the yeast mitochondrial enzyme is a monomer, whereas the enzyme of *E. coli* is a dimer; the mitochondrial enzyme hydrolyzes $polyP_3$, whereas the enzyme of *E. coli* fails; and the bacterial polyphosphatase is active only in the presence of K^+, which is in contrast to the mitochondrial enzyme of *S. cerevisiae*. It seems reasonable to say that bacterial polyphosphatase has not been conserved in yeast mitochondria in the evolutionary process or that the ancient mitochondrial precursor was quite different from *E. coli* in this respect. The polyphosphatases of other lower eukaryotes have been studied much less.

Exopolyphosphatase purified from *N. crassa* is close to the yeast cytosol exopolyphosphatase as far as molecular mass and divalent cations requirement. The fact that it actually did not hydrolyze tripolyphosphate may be because of its low affinity to this substrate, which was used in a ten-fold–lower concentration than

K_m for the yeast enzyme (54). Two other exopolyphosphatase activities were observed in the "slime" variant of *N. crassa*, which cannot synthesize cell walls (111, 112, 116). One of them was K^+ and Mg^{2+} dependent and hydrolyzed high-molecular polyP, and the other was K^+ and Mg^{2+} independent and hydrolyzed low-molecular polyP. The study of a number of exopolyphosphatases of the lower eukaryotes is important to clarify the polyP functions in each individual compartment of these microorganisms.

POLYPHOSPHATE FUNCTIONS

Phosphate Reserve

Prokaryotes The function of polyP as a phosphate reserve is well known (47, 52, 54). The content of polyP in the cells of microorganisms strongly depends on the phosphate content in the medium (52, 54). A large amount of polyP is characteristic of the bacteria from wastewaters with a high phosphate content. *A. johnsonii* (23), *Microlunatus phosphovorus* (74), and *Microthrix parvicella* (25), isolated from activated sludge, are examples of such bacteria. In *A. johnsonii*, these polymers make $\leq$30% of dry biomass (23). In *E. coli*, the level of polyP drops drastically under phosphate starvation, and the subsequent addition of orthophosphate to the medium restores the initial phosphate level (76).

Eukaryotes The accumulation of phosphate reserves as polyP and its use at phosphate starvation occur also in eukaryotic microorganisms (52, 54). Yeasts are characterized by the phenomenon of phosphate overplus. If the cells after phosphate starvation are transferred to a phosphate-containing medium, then they accumulate higher contents of polyP. If the function of phosphorus reservation in bacteria is performed by the cytosol polyP, phosphorus in eukaryotic microorganisms is also reserved as polyP in vacuoles and the cell envelope. Under yeast growth on the medium without phosphate, the polyP content drops more than an order of magnitude in the cytosol, vacuoles, and cell wall (51). polyP granules of cytosol quickly disappear after the yeast is placed in a phosphate-deficient medium. In a P_i-deficient medium, a sharp decrease in the level of polyP both in whole cells and in vacuoles was noted; after 7 h of starvation, the polyP level in vacuoles decreased by 85%, which indicates active utilization of the entire polyP pool for the needs of the cell under these growth conditions (51).

Vacuoles are an important phosphorus reserve in yeasts and fungi (21, 82, 105, 114, 122). Under phosphate overplus, the content of polyP in vacuoles of *S. carlsbergensis* grew dramatically (64, 82). Some mutants of *S. cerevisiae* that have no vacuoles are unable to grow on the medium without P_i (105).

Energy Source

Prokaryotes In bacteria, the functions of polyP are associated with the energy metabolism. Belozersky (12) suggested that polyP in very primitive organisms

could perform the functions of energy-rich compounds. These suggestions were supported experimentally in a number of works (15, 34, 54, 58, 83). In many prokaryotes, polyP is a direct phosphorus donor for biochemical reactions. Under certain conditions, polyP kinase performs an ATP-regenerating function (58, 73). Other enzymes carrying out the interaction of polyP metabolism and nucleoside phosphates have also been studied. polyP:AMP phosphotransferase was purified from *Acinetobacter* strain 210A (15). The polyP glucokinase activity revealed by Szymona & Ostrowsky (108) and the ATP glucokinase activity were shown to be catalyzed by a single enzyme (34, 83). This bifunctional enzyme was purified from *Propionibacterium shermanii* (83) and *Mycobacterium tuberculosis* (34). polyP and ATP have separate binding sites with different regulatory properties (34, 83). It was proposed that the ATP glucokinase activity of this enzyme was developed later in evolution than was the polyP glucokinase activity (34, 83).

Eukaryotes The function of polyP as an energy reserve is preserved also in eukaryotic microorganisms. Both the total content of polyP and its distribution by fractions in the yeast *S. cerevisiae* depend on the growth phase (117). Before glucose was consumed from the medium, the biomass and total cellular polyP content increased in parallel. After glucose depletion, the content of polyP in the cells fell sharply and then increased again. The significant decline of the content of intracellular polyP, while P_i was present in the growth medium at high concentrations, may imply that, in this growth phase, polyP is an energy rather than a phosphate source (117). The active synthesis of polyPs, accompanied by a dramatic decrease of their length in the logarithmic phase of *S. cerevisiae* growth in a carbon- and phosphorus-sufficient medium, also suggests that the energy derived from polyP hydrolysis is necessary to maintain the high rate of yeast growth (117). The induction of polyP synthesis in the yeast cell in parallel with the exit of K^+ ions from the cell also suggests the possibility of polyP participation in retention of energy of the transmembrane K^+ gradient (81). The experimental evidence of polyP participation in the mitochondrial energy metabolism has been obtained, and mitochondrial ATP generation directly from polyP has been proposed (11).

Cation Sequestration and Storage

Prokaryotes Another important function of polyP is involvement in detoxification of heavy metal cations. polyP sequesters nickel in *Staphylococcus aureus* (28). The cells of *Anacystis nidulans* with high intracellular polyP levels showed a greater tolerance to cadmium than those with small polyP reserves (45). The cadmium tolerance of *E. coli* also depends on the polyP metabolism (43). The following mechanism of polyP participation in the detoxification of heavy metals has been proposed: polyP sequesters heavy metals, and the entry of metal cations into cells stimulates exopolyphosphatase activity, which releases P_i from polyP. The metal-phosphate complexes are then transported out of the cells (41, 43).

Eukaryotes In eukaryotes, this function is observed in vacuoles. Vacuoles accumulate amino acids (123), K, Mg^{2+}, and Mn^{2+} (82). polyP is able to confine different cations in an osmotically inert form (21, 24). Arginine accumulated in vacuoles was shown to form a complex with polyP (21, 24). In the vacuoles of *N. crassa*, spermidine was found along with arginine, and almost half of the polyP in these organelles was considered to form complexes with these amines (21). The accumulation of K^+, Mn^{2+}, and Mg^{2+} ions in the vacuoles of *S. carlbergensis* correlates well with the increase in the polyP content (64). This reconfirms the proposal that these cations are able to form the appropriate complexes. This complex-forming function of polyP may be very important for yeast cells, because, under short-term phosphate starvation in the presence of metal cations in the medium, the vacuolar polyP content decreases, but only slightly (64, 82). A stable P_i content in the cytosol under the above conditions is maintained mainly because of a decrease in the vacuolar P_i pool but not in the vacuolar polyP pool (64). polyPs of the cell envelope could also be the first barrier that heavy metal cations encounter during penetration into a cell.

Cell Envelope Formation and Function

polyP participates in the cell envelope formation and function both in prokaryotic and eukaryotic microorganisms. *Neisseria* mutants lacking capsular polyP have a lower pathogenicity than does the wild-type strain (109).

polyP in the cell envelope is of great importance for the maintenance of the negative charge on the cell surface of fungi (37, 115). It can bind with the cationic dye 9-aminoacrydine (9AA) in the presence of thiamine, an inhibitor of the dye translocation across the plasma membrane (115). By measuring the 9AA absorption rate, one can determine variations in the polyP content in the cell envelope. Various P_i concentrations in the medium result in significant variations of the polyP content in the yeast, which has an effect on 9AA absorption by the cell envelope. The phosphate starvation of cells resulted in a significant decline of their ability to absorb 9AA, whereas their subsequent growth on a P_i-rich medium promoted higher absorption of the dye. The pretreatment of cells with the uranium dioxide anion decreased the 9AA sorption by ~80%. These results are evidence of the appreciable contribution of polyP to the total negative charge of the cell envelope (115). The extent of the cytoplasmic membrane damage by different ionic compounds depends on the polyP content in the cell envelope (37). The mechanism of polyP involvement in the cell wall biosynthesis has been described previously (100–102). The changes in the cellular content of polyP fractions located in the periphery of yeast cells were investigated, and their association with the formation of cell walls in *S. cerevisiae* was proposed (51, 54).

Gene Activity Control

Prokaryotes Direct evidence of polyP involvement in the regulation of gene expression in bacteria has now been obtained (46, 60, 89, 104). RNA polymerase isolated from the stationary-phase cells of *E. coli* was found to be closely associated

with polyP (60). Using two forms of the holoenzyme, one containing σ^{70} (the major sigma factor for transcription of the genes expressed during exponential cell growth) and the other containing σ^{38} (the sigma factor operating in the stationary phase), the inhibitory effects of polyP on transcription were examined. At low salt concentrations, polyP inhibited the transcription by both forms of RNA polymerase, with σ^{70} and with σ^{38}. At high salt concentrations, the σ^{38}-containing enzyme is activated, but the σ^{70}-containing enzyme is not able to function. These results show that polyP may play a certain role in the promoter selectivity control of RNA polymerase in *E. coli* growing under high osmolarity and during the stationary growth phase (60). The genetic data were also obtained, which confirmed that polyP is important for inducing the expression of the stationary-phase sigma factor in *E. coli* (104).

Eukaryotes The participation of polyP in the regulation of gene activity is one of the most important functions of these compounds in eukaryotic microorganisms. The mechanism of this involvement is still little studied; however, many facts favor this concept. Eukaryotes possess polyP in the nuclei (52, 54). It is well known that their biosynthesis occurs simultaneously with the total RNA synthesis (52). In the nuclei, polyP is related to the fraction of nonhistone proteins (52, 80). $polyP_{60}$ that is present in DNA preparations from filamentous fungal species of *Colletotrichum* inhibits restriction endonucleases and other enzymes (96). *N. crassa* nuclei were revealed to contain a polyP depolymerase (52, 54), and *S. cerevisiae* nuclei were revealed to contain a polyphosphatase (62). polyPs in fungi are probably involved in the on-and-off switching of large groups of genes during sporulation. Significant changes of nuclear polyP are observed during this process. Degradation of high-molecular polyP to low-molecular fragments in the nuclei during sporulation is observed in the fungus *Agaricus bisporus* (52, 54) and in the fungus *Physarvum polycephalum* (86).

Regulation of Enzyme Activities

Being a polyanion, polyP can exhibit an inhibitory activity for a number of enzymes. polyP inhibits restriction endonucleases (96) and RNA-degrading enzymes (13). polyP inhibited trehalase from vegetative yeast cells and, to a lesser extent, from spores (124). Polygalacturonase activity (which is important for pathogenicity) from the phytopathogenic fungus *Botrytis cinerea* was inhibited in vitro by extracellular polyP (71). In addition, polyP is most likely involved in the regulation of enzyme activities by participation in their phosphorylation. A protein kinase that uses not ATP but high-polymer polyP was found in the archaebacterium *Sulfolobus acidocaldarius* (106).

Stress Response and Stationary-Phase Adaptation

Prokaryotes The involvement of polyP in the regulation of both enzyme activities and expression of large groups of genes is the basis of survival of different bacteria, including pathogens, under stress conditions and adaptation to

the stationary-growth phase (46, 47, 49, 59, 88, 89, 104). Kornberg and coworkers (47, 49) have shown that polyP kinase and polyP synthesized by this enzyme play the key role in the transition of bacteria from active growth to the stationary phase, as well as in their survival in the stationary phase and under stress. The important role of polyP kinase in the survival of *E. coli* under stress and starvation was established by the study of mutants deficient in the *ppk* gene (22). Mutant cells show no phenotypic changes during the exponential phase of growth (22). During the stationary phase, mutants survive poorly and are less resistant to heat, oxidants, and osmotic challenge (22, 88). The *ppk* mutants of *N. gonorrhoeae* and *N. meningitidis* grew less vigorously than did wild-type cells and showed a striking increase in sensitivity to human serum (109). Various mechanisms providing the above processes have been proposed. First, polyP kinase may be involved in the regulation of the level of nucleoside triphosphates and deoxynucleoside triphosphates, whereas this enzyme can convert GDP and other nucleoside diphosphates to nucleotide triphosphates by using polyP (46, 58). Second, this enzyme may influence the mRNA stability (13). The polyP kinase in the degradosome appears to maintain the appropriate microenvironment by removing the inhibitory polyP and nucleoside diphosphates and permitting ATP regeneration (13). Third, polyP is directly involved in the regulation of RNA polymerase expression and activity (60, 104). Finally, bacteria possess pppGpp that yields guanosine tetraphosphate (ppGpp) upon hydrolysis. This is the so-called stringent factor, which is a known regulator of ribosome function and protein biogenesis in bacteria under the conditions of amino acid starvation. The pppGpp phosphohydrolase of *E. coli* exhibits an exopolyphosphatase activity, and polyP may be involved in the regulation of pppGpp hydrolysis (42, 46, 59). On the other hand, the major *E. coli* exopolyphosphatase is inhibited by pppGpp. This disturbs the dynamic balance between the polyP synthesis and hydrolysis (59). The interaction of polyP metabolism and both ppGpp and pppGpp metabolism in *E. coli* is being actively studied (42, 46, 59) and described in detail in reviews (49, 97). Here, we mention but a few important facts. Generation of high levels of ppGpp and pppGpp in response to amino acid starvation in *E. coli* results in a significant accumulation of polyP (59). This accumulation can be attributed to the inhibition by pppGpp and/or ppGpp of polyP hydrolysis by exopolyphosphatase (59). Accumulation of polyP requires the functional *PHOB* gene and higher levels of ppGpp or pppGpp (89). In *E. coli*, the genes *ppk* and *ppx* are in the same operon, which results in a coordinated regulation of their activities (89). It should be noted that different bacteria may have different predominant mechanisms of polyP participation for surviving under stress and stationary-growth phase, or they may have other mechanisms that have not been studied yet. For instance, in *H. pylori*, the pppGpp level does not rise as a result of amino acid starvation (99).

Eukaryotes For eukaryotic microorganisms, the involvement of polyP in biochemical regulation under stresses has also been observed. This function of polyP obviously remains one of the most important. The involvement of vacuolar polyP in survival under osmotic or alkaline stresses was shown in algae and fungi. In

the alga *D. salina*, alkalinization of the cytoplasm results in massive hydrolysis of polyP, which results in pH restoration. Pick and coworkers (84, 85) suggested that the hydrolysis of polyP provides the pH-stat mechanism to counterbalance the alkaline stress. It was suggested that the accumulation of amines within vacuoles (in response to amine-induced alkaline stress) activates a specific phosphatase that hydrolyzes long-chain polyP to tripolyphosphates (85). This phosphatase appears to be activated at the neutral or mildly alkaline pH and repressed at the physiological intravacuolar acidic pH. Currently, two enzymes that can catalyze this process have been purified. An exopolyphosphatase that hydrolyzes long-chain polyP but not tripolyphosphate was purified from yeast vacuoles (5). The optimal pH of this enzyme was neutral (5). An endopolyphosphatase could also be involved in this process. Such an enzyme has recently been purified from yeasts, and its vacuolar localization is assumed (57). It is important that yeasts showed the accumulation of tripolyphosphate after polyP hydrolysis was induced by amines and basic amino acids (29). The role of polyP as a buffer was demonstrated in *N. crassa* under osmotic stress (129). The hypoosmic shock produced a rapid hydrolysis of polyP with an increase in the concentration of cytoplasmic phosphate (129).

It has been reported that polyP participates in the repair of radiation damage in yeasts by acting as an alternative energy supply and phosphate source (33).

The metabolism of polyP in eukaryotic microorganisms might be directly related to the metabolism of such second messengers as diadenosinetetra-, penta-, and hexapolyphosphates and their derivatives. These second messengers are accumulated in yeasts under stress conditions (10). It was found that the exopolyphosphatase of the cytosol of *S. cerevisiae* is capable of hydrolyzing adenosine-5′-tetraphosphate and guanosine-5′-tetraphosphate about twofold more actively than $polyP_{15}$ (30, 51). The apparent K_m value is 80 μM for these substrates and 1 μM for $polyP_{208}$ (51). Thus, polyP may be an effective inhibitor of hydrolysis of the above compounds. It is possible that the metabolic pathways of inorganic polyP and diadenosine polyphosphates and their derivatives in yeasts are interrelated, just as the prokaryotes show an interrelation between the metabolic pathways of polyP and those of pppGpp and ppGpp.

Membrane Channels

One of the findings that greatly changed our views on the functions of polyP was the detection of polyP/hydroxybutyrate complexes in membranes of organisms of different evolutionary stages (91–95). Both prokaryotic and eukaryotic membranes possess complexes of polyP, Ca^{2+}, and poly-β-hydroxybutyrate. The ability of these complexes to form voltage-activated calcium channels was shown in the synthetic planar lipid bilayer and in membrane vesicles from *E. coli* (92, 93). An increase in the content of such complexes in the membranes is followed by the appearance of rigid domains and disruption of lipid bilayer conformation (19). The amount of the complexes increases in transformation-competent bacterial cells whose membranes are easily permeable for DNA (19, 35). These complexes were proposed to be involved in DNA transport into the cell (19, 35). Most intriguing

was the report that Ca^{2+}-ATPase purified from human erythrocytes contained such complexes and exhibited ATP-polyP transferase and polyP-ADP transferase activities (94). These findings suggest a novel supramolecular structure for the functional Ca^{2+}-ATPase (94). In the opinion of Reusch (93), polyP/polyhydroxybutyrate channels may be the most ancient form of ionic channels, which were conserved in the evolution from prokaryotes to eukaryotes.

Phosphate Pumps

The structure of polyP/polyhydroxybutyrate complexes suggests that they may function as a phosphate pump under the condition that the polyP chain is extended on one side of the membrane, whereas orthophosphate is split from its end on the other side. This hypothetical scheme includes Ca^{2+} and phosphate cotranslocation across the membrane (92, 93). The direction of transport in this case should depend on the location of polyP-synthesizing and polyP-hydrolyzing enzymes from different sides of the membrane. Probably, such a process occurs in lysosomes and vacuoles, their functional analogs in microorganisms. P_i transported into isolated lysosomes is quickly incorporated into high-molecular polyP (87). Yeast vacuoles are able to accumulate P_i independently of the electrochemical proton gradient at the vacuolar membrane, in contrast to some other transport systems at the vacuolar membrane (55). The process of P_i transport into yeast vacuoles is similar to that observed in lysosomes of animal cells; therefore, it is possible that polyP in these organelles participates in the P_i transport.

The mechanisms of polyP participation both in the process of transmembrane phosphate transfer and in its regulation in *A. johnsonii* were intensively studied (120). In particular, it was noted that the two systems, the polyP:AMP phosphotransferase/adenylate kinase system and the electrogenic excretion of $MeHPO_4$ in symport with a proton, are important for maintaining the proton motive force during anaerobiosis in *A. johnsonii* (120). The interrelation of polyP and phosphate transport seems to be evident from the fact that polyP is a phosphorus reserve and its accumulation depends on phosphate content in the medium. The genes encoding the enzymes of polyP metabolism were proposed to form a phosphate regulon together with a number of other genes, the products of which are involved in phosphate metabolism and transport (77). At present, the interrelation of polyP metabolism and the activities of PHO and PHOB regulons is supplemented with new details (49, 89). Possibly, polyP, a polyanionic molecule that could interact with many other biopolymers, was an ancient prototype of a membrane channel. Its role in the transport processes was probably preserved during the evolution from prokaryotes to eukaryotes.

POLYPHOSPHATES AND LIFE EVOLUTION

In the opinion of some authors, polyP could have an abiotic origin (121, 128), although other authors do not agree with this proposition (44). Model experiments showed that polyP probably played an important role in abiogenic synthesis

of nucleic acids and other macromolecules on the primordial Earth (52, 54). Quite a number of facts support the idea that these polymers are of very ancient evolutionary origin.

The study of some "fossil reactions" in primitive prokaryotes has shown an important role of polyP in their bioenergetics. Thus, glucokinase has a higher activity with polyP than with ATP in phylogenetically more ancient prokaryotic microorganisms, whereas the younger systematic groups show quite the opposite. In eukaryotes, this enzyme uses only ATP. A number of enzymes of the polyP metabolism are revealed in prokaryotes but not in eukaryotes. In the course of evolution from prokaryotes to eukaryotes, the energetic role of polyP decreased. However, other functions appeared, such as phosphate storage, cation chelation, regulation of enzyme activities, gene expression, and membrane transport. The significance of the regulatory functions of polyP increased in eukaryotes. These functions are predominant in animal cells, in which polyP participates mainly in the transport across the membranes and in the regulation of gene expression. The polyP amount in animals is quite low, but this compound occurs in various tissues and cell compartments.

Recent studies of the properties and functions of polyP-metabolizing enzymes from different cell compartments of eukaryotic microorganisms provide further support to the idea of the endosymbiotic origin of organelles (70, 75). According to the modern conception, archae played a key role in formation of the primary eukaryotic cell. Ancient archae were probably precursors of vacuoles and host cells (75). Therefore, future comparative investigations of polyP metabolism in archae and bacteria will provide a better understanding of the evolution of polyP functions.

CONCLUSION

In microbial cells, inorganic polyP plays a significant role in increasing cell resistance to unfavorable environmental conditions and in regulating different biochemical processes. polyP is a polyfunctional compound. Its most important functions are as follows: reservation of phosphate and energy, sequestration and storage of cations, formation of membrane channels, participation in phosphate transport, involvement in cell envelope formation and function, control of gene activity, and regulation of enzyme activities. As a result, polyP plays an important role in stress response and stationary-phase adaptation.

The functions of polyP changed greatly during the evolution of living organisms. In prokaryotes, the most important functions are energy source and phosphate reserve. In eukaryotic microorganisms, the regulatory functions predominate. Therefore, there is a great difference between prokaryotes and eukaryotes in their polyP-metabolizing enzymes. Eukaryotes do not possess some key prokaryotic enzymes, but they developed new polyP-metabolizing enzymes that are absent in prokaryotes. The synthesis and degradation of polyP in each specialized organelle and compartment of eukaryotic cells are mediated by different sets of

enzymes, which is consistent with the endosymbiotic hypothesis of eukaryotic cell origin.

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