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REVIEW

Inorganic Polyphosphates and Exopolyphosphatases in Different Cell Compartments of *Saccharomyces cerevisiae*

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Abstract—The cytosol, nuclei, vacuoles, and mitochondria of the yeast *Saccharomyces cerevisiae* possess inorganic polyphosphates (polyPs). PolyP levels, spectra of polyP chain lengths, and their dependence on the growth phase are distinguished in the mentioned compartments. Inactivation of the *PPX1* gene has no effect on the polyP metabolism under cultivation of the yeast in medium with glucose and 5-7 mM P_i. Inactivation of the *PPN1* gene results in elimination of the high-molecular-mass exopolyphosphatases (~120 to 830 kD) of the cytosol, nuclei, vacuoles, and mitochondria of *S. cerevisiae* suggesting that it is just *PPN1* that encodes these enzymes. Expression of the low-molecular-mass exopolyphosphatase of ~45 kD encoded by the *PPX1* gene decreases under *PPN1* inactivation as well. While *PPN1* inactivation has negligible effect on polyP levels, it results in increase in the long-chain polyPs in all the compartments under study.

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Inorganic polyphosphates (polyPs) are linear polymers of orthophosphoric acid found in every organism studied so far. PolyPs are involved in many functions including phosphate and energy storage, metal chelating, regulation of gene expression, and formation of transport channels [1-3]. This suggests their localization in different cell compartments of eukaryotic cells. However, it is widely believed that nearly all polyPs are located in the yeast vacuoles [4-6]. At the same time, there is evidence for the presence of polyPs in other compartments of these microorganisms: cell envelope [7-9], cytosol [8, 10], mitochondria [11, 12], plasma membranes [2], and nuclei [1]. There is another argument in favor of multiple polyP localization, which involves the presence of exopolyphosphatases, specific enzymes of polyP metabolism, in all yeast compartments [13].

At present, two genes encoding polyP-metabolizing enzymes are identified in the yeast. The first is *PPX1* encoding ~45-kD exopolyphosphatase [14] localized in the cytosol, cell envelopes [15], and soluble mitochondrial fraction [16]. Exopolyphosphatases of nuclei, vacuoles, and mitochondrial membranes are not encoded by this gene [13]. The second gene, *PPN1*, was first regarded as encoding an endopolyphosphatase, an enzyme splitting long polyP chains to shorter ones [17, 18]. But later it turned out that this enzyme is able to split out orthophosphate from the polyP chain [19], and therefore it can be considered as an exopolyphosphatase. Moreover, the *PPN1* gene is identical to the *PHM5* gene found in the yeast genome. This gene encodes the vacuolar exopolyphosphatase [5] and possesses a signal sequence characteristic of vacuolar proteins [20, 21].

In this review, we will consider our new data on polyP localization and the effects of *PPX1* and *PPN1* inactivation on exopolyphosphatase activities, polyP levels, and chain lengths in the cytosol, nuclei, vacuoles, and mitochondria of *Saccharomyces cerevisiae*.

EVIDENCE FOR DIFFERENT POLYPHOSPHATE LOCALIZATION IN THE YEAST CELL

Until recently, the study of polyP localization in the yeast cell was performed using cytochemistry and NMR spectroscopy [22, 23]. In a recent publication, polyP localization in yeast vacuoles has been proved with the cytochemical method based on polyP binding to exopolyphosphatase of *Escherichia coli* [6]. However, one cannot exclude the possibility that not all the polyP fractions of the yeast are able to interact with this enzyme.

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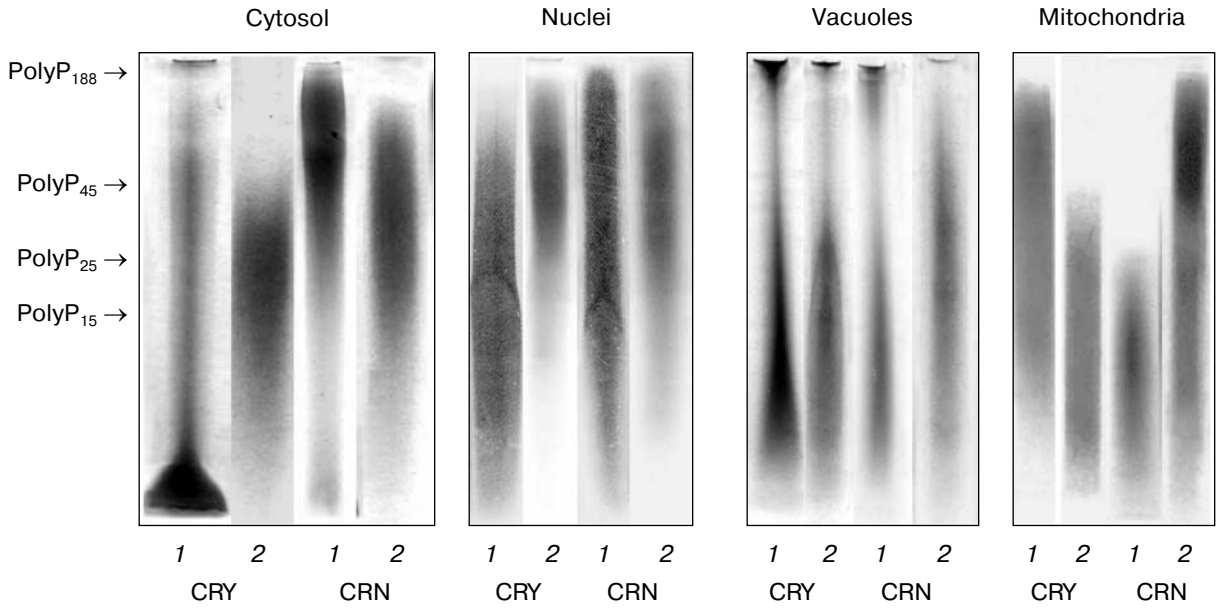

Electrophoresis of polyPs in 20% polyacrylamide gel: *1*) exponential growth phase; *2*) stationary growth phase. Chain lengths of polyP size markers are indicated on the left

There are indications that the yeast possesses polyPs beyond the reach of exopolyphosphatases [18]. NMR spectroscopy has a number of restrictions as well.

An analysis of pure subcellular fractions revealed that the cytosol, nuclei, vacuoles, and mitochondria possessed polyPs characterized by individual spectra of polyP chain lengths (see the figure). The most part (~90%) of the polyPs was found in the cytosol [24]. These data were obtained under yeast cultivation with glucose, peptone, and sufficient P_i supply in the medium (5-7 mM). The principal localization of polyPs in vacuoles was revealed under cultivation of the yeast with arginine as the only nitrogen source [4] or under phosphate starvation [25].

PolyP metabolism in the compartments under study strongly depended on the growth phase of the yeast *S. cerevisiae*. In these compartments, polyP levels and their chain lengths changed differently under transition from exponential to stationary growth phase as demonstrated for the parent strain CRY (Table 1 and figure). In the cytosol and vacuoles, polyP levels increased ~3- and ~5-fold, respectively, in the stationary growth phase as compared with the exponential phase. In these growth conditions, polyP levels decreased 1.5- and 2.5-fold in the nuclei and mitochondria, respectively (Table 1).

In the stationary growth phase, the shortest polyP chains (<15 phosphate residues), characteristic of the cytosol and nuclei in the exponential growth phase, disappeared from these compartments (figure). No effect on polyP chains was observed in the vacuoles under transition of the yeast to stationary growth phase (figure). In

Table 1. PolyP levels (μmol P_i/mg protein) in cell compartments of *S. cerevisiae*

Compartment	Parent strain CRY		Strain CRN with inactivated *PPN1* gene	
	exponential growth phase	stationary growth phase	exponential growth phase	stationary growth phase
Cytosol	1.6	4.6	1.3	3.4
Nuclei	5.0	3.4	4.7	3.4
Vacuoles	2.1	10.6	5.1	26.6
Mitochondria	1.0	0.4	0.3	0.8

Note: Heparin (4 mg/ml) and 20 mM EDTA were added to all the solutions under isolation procedures of all the subcellular fractions to prevent polyP hydrolysis by exopolyphosphatases.

the same conditions, the average chain length of mitochondrial polyPs decreased from >45 to ~15 phosphate residues (figure).

Thus, the data confirm different localization, heterogeneity, and multiplicity of the metabolic pathways of polyPs in the yeast cell.

EFFECT OF *PPX1* INACTIVATION ON POLYPHOSPHATES AND EXOPOLYPHOSPHATASE ACTIVITIES

Activity of the enzyme encoded by the *PPX1* gene was 60% of the total exopolyphosphatase activity of the yeast cell [13, 26]. Exopolyphosphatases of vacuoles, nuclei, and mitochondrial membranes were not encoded by this gene [13]. Therefore, an absence of substantial changes in the polyP levels and their chain lengths in these compartments was not a surprise under *PPX1* inactivation.

PPX1 inactivation was followed by elimination of ~45-kD enzyme encoded by this gene and localized in the cytosol and mitochondrial matrix and had no effect on the polyP levels and polyP chain lengths in the cytosol in both growth phases. It seems likely that this enzyme was not involved in the polyP metabolism in these conditions.

EFFECT OF *PPN1* INACTIVATION ON EXOPOLYPHOSPHATASE ACTIVITIES

Inactivation of the *PPN1* gene (CRN strain) resulted in decrease in the cytosol exopolyphosphatase activities as compared with the parent strain CRY: ~3- and 4.5-fold in the exponential and stationary growth phases, respectively (Table 2). The cytosol of the parent strain possessed two exopolyphosphatases, an exopolyphosphatase of ~45 kD encoded by the *PPX1* gene and a high-molecular-mass exopolyphosphatase of ~830 kD not encoded by this gene [15, 27]. The latter was observed only under exponential growth phase [27]. Its activity increased under elimination of *PPX1* [15] and phosphate surplus [28].

The *PPN1* inactivation resulted in disappearing of ~830-kD exopolyphosphatase of the cytosol and only ~45-kD enzyme was revealed in this compartment [27]. This inactivation unexpectedly inhibited the expression of low-molecular-mass enzyme in both studied growth phases [27]. Activity of this enzyme was considerably less as compared with the parent strain CRY: ~25% and ~12% of activity in the exponential and stationary growth phases, respectively [27].

Exopolyphosphatase of ~200 kD differing in physicochemical properties from exopolyphosphatases found in other compartments was revealed in the nuclei of *S. cerevisiae* [13, 29]. Nuclear exopolyphosphatase in the CRY strain little depended on the growth phase (Table 2). Under inactivation of the *PPN1* gene (CRN strain), elimination of the nuclear exopolyphosphatase was observed in both growth phases (Table 2).

Exopolyphosphatases of ~440 and >1000 kD were revealed in the vacuoles of the parent strain CRY of *S. cerevisiae* [30]. They differed from each other by their requirements for bivalent cations and sensitivity to heparin, the known inhibitor of exopolyphosphatases [13]. Under *PPN1* inactivation, elimination of ~440-kD exopolyphosphatase was observed. This inactivation had no effect on the other, previously unknown exopolyphosphatase with apparent molecular mass of >1000 kD [30]. This vacuolar enzyme of >1000 kD was not encoded by *PPX1* either. The main part of exopolyphosphatase activity (~70%) was due to the functioning of ~440-kD enzyme in both growth phases. This is why inactivation of *PPN1* resulted in decrease in specific activities of the vacuolar enzyme as compared with CRY and it was most expressed in the stationary growth phase (Table 2).

Table 2. Exopolyphosphatase activities (mU/mg protein) in the cell compartments of *S. cerevisiae*

Compartment	Parent strain CRY		Strain CRN with inactivated *PPN1* gene	
	exponential growth phase	stationary growth phase	exponential growth phase	stationary growth phase
Cytosol	180	130	70	30
Nuclei	70	100	2	3
Vacuoles	250	375	125	20
Mitochondrial soluble fraction	130	135	—	15
Mitochondrial membranes	80	100	0	0

Two exopolyphosphatases were found in the mitochondria. The soluble exopolyphosphatase of ~45 kD was localized in the mitochondrial matrix and encoded by *PPX1* gene [16]. *PPN1* inactivation resulted in decrease in ~45-kD enzyme activity (Table 2) as observed in case of the cytosol [27].

The membrane-bound exopolyphosphatase of ~120 kD tightly bound to the membranes and inhibited by divalent cations [13] was not encoded by the *PPX1* gene [13] and totally disappeared under *PPN1* inactivation (Table 2).

Inactivation of the *PPN1* gene resulted in elimination of the high-molecular-mass exopolyphosphatases of the cytosol (~830 kD), nuclei (~200 kD), vacuoles (~440 kD), and mitochondria (~120 kD). This suggests that it is just *PPN1* that encodes all these enzymes. Mass spectrometric analysis of the purified high-molecular-mass exopolyphosphatase isolated from the cytosol of wild strain *S. cerevisiae* revealed this enzyme was encoded by the *PPN1* gene (N. A. Andreeva, unpublished). However, to determine if this gene encodes other mentioned exopolyphosphatases it is necessary to elucidate their amino acid sequences.

All enumerated exopolyphosphatases were similar in substrate specificity and sensitivity to certain reagents including antibodies against *PPX1*, heparin, EDTA, and fluoride [13]. The only distinction was found for the membrane-bound exopolyphosphatase of mitochondria, which was inhibited by divalent metal cations as compared with other exopolyphosphatases stimulated by these cations [13]. The distinctions in molecular masses and some other properties might be due to posttranslational modification of the enzymes and their interaction with other cell components. Many proteins have been reported to form complexes with polyPs [31]. One example is polyphosphate glucokinase, an essential enzyme of polyP metabolism in bacteria, which has numerous forms differing from each other in molecular mass of the native enzyme and isoelectric point [32]. Another example is the high-molecular-mass exopolyphosphatase from the cytosol of *S. cerevisiae*, which forms a stable complex of ~500 kD in the presence of polyP [33].

Inactivation of the *PPN1* gene resulted also in decrease in expression of ~45-kD exopolyphosphatases encoded by the *PPX1* gene and located in the cytosol and mitochondria [15, 27]. This effect remains unclear and requires further investigation.

EFFECT OF *PPN1* INACTIVATION ON POLYPHOSPHATES IN SEPARATE YEAST COMPARTMENTS

The effect of *PPN1* inactivation on the polyP levels in the cytosol and nuclei was insignificant, while in the vacuoles it increased ~2.5-fold in both growth phases (Table 1). The tendency for the polyP level in vacuoles to increase in the stationary phase, observed for the parent strain, still persisted under *PPN1* inactivation.

PPN1 inactivation was followed by ~3-fold decrease in the polyP level of mitochondria under exponential growth phase and its 2-fold increase under stationary phase (Table 1). In mitochondria, the polyP level decreased ~2.5-fold and increased ~2.5-fold under transition to stationary growth phase of CRY and CRN strains, respectively (Table 1).

In the cytosol, *PPN1* inactivation resulted in displacement of the spectrum of polyP chain lengths to higher values under both exponential and stationary growth phases (figure). PolyP chain lengths in the nuclei of the *PPN1*-deficient strain CRN were just the same as in CRY except for the appearance of some amount of higher polymeric polyPs in both growth phases (figure). In the vacuoles, inactivation of the *PPN1* gene resulted in increase in the content of longer polyP chains only in the stationary growth phase (figure). *PPN1* inactivation was followed by elongation of polyP chains in mitochondria from ~15 to ~150 phosphate residues in the stationary growth phase and their shortening from ~130 to ~15 phosphate residues in the exponential phase (figure). It should be mentioned that the yeast cells in the exponential phase of growth on glucose possessed not functionally active mitochondria but promitochondria [34].

As shown earlier, the total polyP level in cells does not increase under *PPN1* inactivation [5, 18, 35]. This correlates well with the idea that the most part of polyP is localized in the cytosol in conditions under study, since polyP level in this compartment changes little under this mutation (Table 1). *PPN1* inactivation was followed by displacement of the spectra of polyP chain lengths to higher values in all the compartments under study, but it was the most expressed only in the cytosol (figure).

The double *PPX1* and *PPN1* mutant was similar to the single *PPN1* mutant in polyP levels and chain lengths [24, 34].

The data on polyP location and peculiarities of their structure discussed above indicate either different pathways of biosynthesis and degradation of these biopolymers or a sophisticated system of their distribution throughout the compartments. These different polyP pools might be involved in a variety of functions including phosphate and energy storage (cytosol, vacuoles, mitochondria), metal chelating (vacuoles), regulation of gene expression (nuclei), and formation of transport channels (mitochondria) [23].

The products of the two known genes, *PPX1* and *PPN1*, play different roles in polyP metabolism in the yeast. Under cultivation of the yeast on a glucose-containing medium with sufficient P_i content, *PPX1* inactivation has no effect on the polyP metabolism in all the compartments under study. Therefore, the enzyme encoded

by *PPX1* gene is not involved in polyP metabolism in the conditions under study.

PPN1 inactivation results in elimination of exopolyphosphatases having preference for splitting long-chain polyPs in the yeast cells [24]. This appears to explain the increase in amount of long-chain polyPs in all the compartments under *PPN1* inactivation. It is likely that there is an interrelation between increase in the content of long-chain polyPs and decrease in *PPX1* expression. Decrease in *PPX1* expression in parallel with increase in the long-chain polyPs is also observed under polyP surplus [28, 36].

PPN1 and *PPX1* are not the only genes encoding the enzymes of polyP metabolism in the yeast. Apart from the above-mentioned vacuolar exopolyphosphatase, the enzymes of polyP synthesis are required as well, since polyP levels and their chain lengths change during growth in separate yeast compartments even in the double mutant [24, 30].

No genes homologous to *ppk* encoding a polyphosphate kinase, which catalyzes polyP synthesis in bacteria, have been found in the genomes of eukaryotes [37]. At the same time, an effective synthesis of polyPs using ATP was revealed in *Candida humicola* [38]. It is possible that the enzymes carrying out such synthesis and having no homology with bacterial polyphosphate kinases are available in *S. cerevisiae*. There are a number of indirect evidences that high-energy phosphoanhydride bonds in polyPs are directly synthesized from P_i using the proton motive force as a source of energy [5, 23, 39]. It is possible that yet unknown enzymes, depolymerases or/and phosphotransferases, take part in the polyP metabolism.

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