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EXPERIMENTAL ARTICLES

The Effect of Inactivation of the Exo- and Endopolyphosphatase Genes *PPX1* and *PPN1* on the Level of Different Polyphosphates in the Yeast *Saccharomyces cerevisiae*

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Abstract—The inactivation of the *PPX1* and *PPN1* genes, which encode the major enzymes of polyphosphate degradation (exopolyphosphatase and endopolyphosphatase, respectively), was found to exert different effects on the content of different polyphosphates in the yeast *Saccharomyces cerevisiae.* The content of relatively low-molecular-weight acid-soluble polyphosphates in mutant yeast strains is inversely proportional to the exopolyphosphatase activity of the cytosol. At the same time, the mutation of these genes exerts no effect on salt-soluble polyphosphates. The content of high-molecular-weight alkali-soluble polyphosphates increases twofold in a mutant with inactivated genes of both exopolyphosphatase and endopolyphosphatase. The data obtained confirm the earlier suggestion that the metabolic pathways of particular polyphosphates in yeasts are different.

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Key words: inorganic polyphosphates, exopolyphosphatase, endopolyphosphatase, gene inactivation, *Saccharomyces cerevisiae.*

Inorganic polyphosphates (polyP), which are linear polymers of *ortho*phosphoric acid, and the enzymes involved in their metabolism play an important part in microbial cells, being responsible for phosphate and energy conservation, storage of cations in a bound state, regulation of gene expression, adaptation to unfavorable environmental conditions, and other functions [1, 2]. Yeast cells contain polyphosphates in various organelles and compartments. The successive treatment of yeast cells with cold diluted perchloric acid, $NaClO_4$, diluted solutions of alkalies, and hot perchloric acid makes it possible to obtain five fractions of polyphosphates, which differ in molecular mass, metabolism, and cellular location [3, 4].

One approach to studying polyphosphates is analysis of the effect of directed mutations induced in the enzymes of polyphosphate metabolism. The laboratory headed by Kornberg succeeded in deriving *Saccharomyces cerevisiae* mutants that contain the inactivated *PPX1* gene of exopolyphosphatase (EC 3.6.1.11), the enzyme that cleaves a terminal *ortho*phosphate from the polyphosphate chain [5–8], and the inactivated *PPN1* gene of endopolyphosphatase (EC 3.6.1.10), the enzyme that cleaves long-chain polyphosphate molecules into shorter fragments [9, 10]. These mutants are very useful in studying the functions of polyphosphates and the role of exo- and endopolyphosphatases in the metabolism of particular polyphosphates.

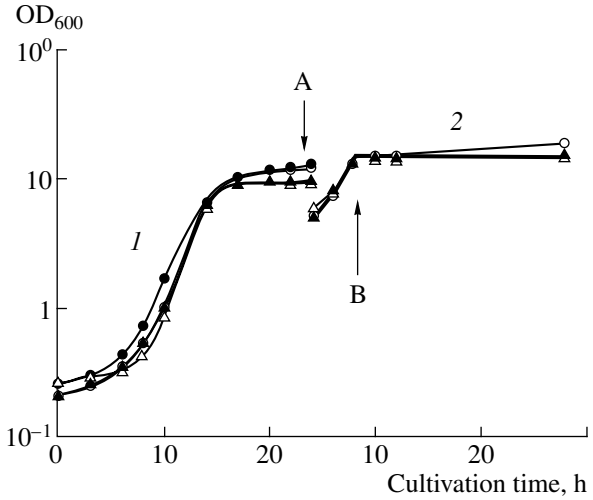

Fig. 1. Growth of the *S. cerevisiae* strains CRY (●), CRX (○), CRN (▲), and CNX (△) in the glucose–peptone medium: (*1*) the medium was inoculated with cells washed off the agar slants; (*2*) the fresh medium was inoculated with the stationary-phase cells taken from the previous cultivation at point A. The arrows at points A and B show the moments when yeast cells were taken for assay of exopolyphosphatase activity and the content of various polyphosphates.

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Table 1. Percentage of budding *S. cerevisiae* cells in the stationary (point A) and retardation (point B) growth phases

Strain	Budding cells, %	
	A (24 h of growth)	B (5 h of growth)
CRY	33	92
CRX	34	91
CRN	19	90
CNX	19	90

This work was undertaken to study the effect of the *PPX1* and *PPN1* mutations on the content of certain polyphosphates in two different physiological stages of yeast cells, namely, in the stationary and the retardation growth phases.

MATERIALS AND METHODS

The mutants *Saccharomyces cerevisiae* CRY (parent strain), CRX (mutant strain with the inactivated *PPX1* gene), CRN (mutant strain with the inactivated *PPN1* gene), and CNX (strain with mutations of both *PPX1* and *PPN1* genes) were kindly provided by Kornberg and Rao from Stanford University (United States) [7, 10]. The strains were cultivated at 30°C on a shaker in flasks with 250 ml of a medium containing 1% yeast extract, 2% peptone, and 2% glucose. The medium was inoculated with cells washed off agar slants of the same nutrient composition. Cells to be analyzed were taken in the stationary growth phase (24 h of cultivation, Fig. 1, point A) and in the retardation growth phase (5 h of cultivation after cell transfer to the fresh medium, Fig. 1, point B). To obtain retardation-phase cells, stationary-phase cells taken at point A were washed with distilled water and inoculated, at a high concentration, into a fresh medium. The number of budding cells was determined using a Goryaev chamber.

Exopolyphosphatase activity in the cytosol was determined in 50 mM Tris–HCl buffer (pH 7.2) containing 0.1 mM $CoSO_4$ and 0.01 mM $polyP_{208}$. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of *ortho*phosphate in 1 minute.

Polyphosphates were extracted and analyzed for the content of labile phosphorus as described earlier [3, 4]. Acid-soluble polyphosphates (polyP 1) were extracted with 0.5 M $HClO_4$ at 0°C. Salt-soluble polyphosphates (polyP 2) were extracted with a saturated solution of $NaClO_4$ at 0°C. Two fractions of alkali-soluble polyphosphates (polyP 3 and polyP 4) were extracted at 0°C with diluted solutions of NaOH (pH 9–10) and 0.05 M NaOH, respectively. Fraction polyP 5 was obtained by extracting the residual biomass with 0.5 M $HClO_4$ at 90°C for 40 min.

Table 2. Content of polyphosphates in the stationary-phase cells of the parent and mutant *S. cerevisiae* strains

Strain	Polyphosphates											
	Σ PolyP		PolyP 1		PolyP 2		PolyP 3		PolyP 4		PolyP 5	
	1	2	1	2	1	2	1	2	1	2	1	2
CRY	283	100	130	46	66	23	61	21	13	5	13	5
CRX	359	100	183	51	85	23	61	17	13	4	17	5
CRN	392	100	272	69	44	11	61	16	7	2	8	2
CNX	507	100	292	58	62	12	142	28	7	1.3	4	0.7

Note: Columns 1 and 2 show the polyphosphate content in μmol P/g dry cell wt. and % of the total, respectively.

Table 3. Content of polyphosphates in the retardation-phase cells of the parent and mutant *S. cerevisiae* strains

Strain	Polyphosphates											
	Σ PolyP		PolyP 1		PolyP 2		PolyP 3		PolyP 4		PolyP 5	
	1	2	1	2	1	2	1	2	1	2	1	2
CRY	393	100	254	65	66	17	49	12	17	4	7	2
CRX	452	100	290	64	82	18	48	11	26	5.7	6	1.3
CRN	340	100	163	48	73	21.5	89	26	10	3	5	1.5
CNX	545	100	265	49	77	14	192	35	6	1	5	1

Note: Columns 1 and 2 show the polyphosphate content in μmol P/g dry cell wt. and % of the total, respectively.

RESULTS AND DISCUSSION

It is known that mutant yeast strains with the inactivated *PPN1* gene of endopolyphosphatase enter the stationary phase earlier and survive it more poorly than the parent strains [10]. Our experiments showed that, at point A, when glucose is almost completely exhausted in the medium (data not shown), the fraction of budding cells was lower in the case of the CRN and CNX strains containing the inactivated *PPN1* gene than in the case of the parent CRY strain and the mutant CRX strain containing the inactivated *PPX1* gene (Table 1). However, mutations induced in the *PPX1* and *PPN1* genes did not affect the ability of the cells transferred to the fresh medium to grow and to bud. This is evident from the nearly the same number of budding cells at point B in the case of the parent and all the mutant strains (Table 1).

A comparison of the content of total and particular polyphosphates in the parent and mutant cells at points A (Table 2) and B (Table 3) showed that cells of the parent CRY and mutant CRX strains, but not of the mutant strains CRN and CNX that contain the inactivated *PPN1* gene, had 30% more total polyphosphates at point B than at point A. The greatest difference at these points was observed for the low-molecular-weight fraction polyP 1. This circumstance is in agreement with the earlier finding that the mutation of the *PPN1* gene prevents an increase in the content of acid-soluble polyphosphates in *S. cerevisiae* cells actively growing on glucose [3].

Acid-soluble polyphosphates comprise up to 70% of all polyphosphates in the *S. cerevisiae* strains under study. The content of salt- and alkali-soluble polyphosphates is lower (11–28%), and the content of the polyP 4 and polyP 5 fractions does not exceed 5%. It should be noted that this fractional composition of polyphosphates considerably differs from that of the strain *S. cerevisiae* VKM Y-1173 studied earlier [3, 4]. This circumstance can be related to strain differences, as well as to the use of different cultivation media (glucose–peptone medium in this work and Reader medium in earlier works [3, 4]).

The inactivation of the *PPN1* gene considerably diminished the exopolyphosphatase activity of the cytosol, whereas the mutation of both genes (*PPN1* and *PPX1*) completely abolished it (Fig. 2). The content of polyphosphates and the level of exopolyphosphatase activity were found to be inversely related, especially in the stationary growth phase.

Figure 3 shows the effect of mutations on the content of particular polyphosphates in *S. cerevisiae* cells. As is evident from this figure, the content of the acid-soluble polyP 1 fraction increased as the exopolyphosphatase activity decreased. In contrast, the content of the salt-soluble polyP 2 fraction virtually did not depend on the mutations. The content of the alkali-soluble polyP 3 fraction increased twofold in the double mutant CNX. These data are in agreement with the earlier suggestion that the metabolic pathways of particular polyphosphates in yeasts are different.

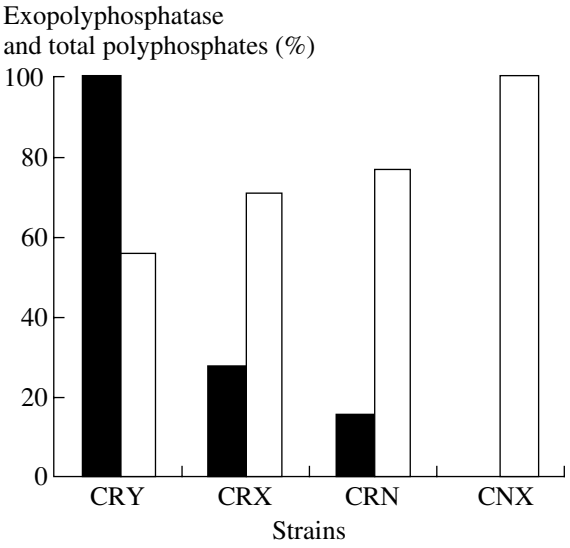

Fig. 2. Exopolyphosphatase activity (dark bars) and the total content of polyphosphates (open bars) in the stationary-phase cells (taken at point A) of the parent and mutant *S. cerevisiae* strains.

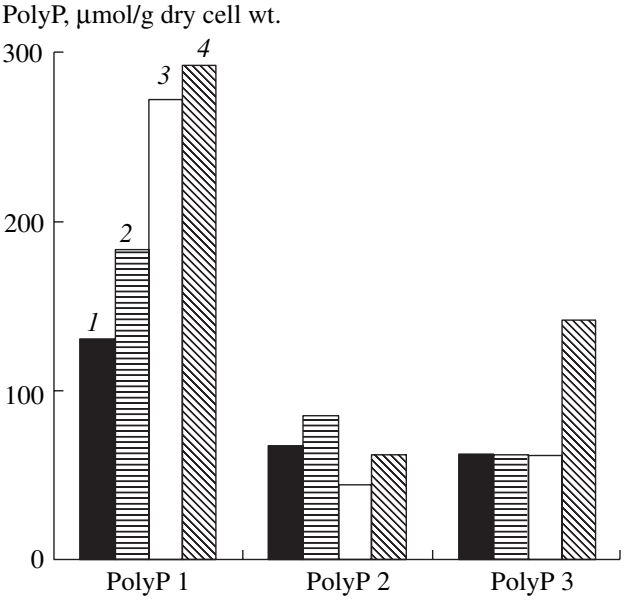

Fig. 3. The content of particular polyphosphates in the stationary-phase cells (taken at point A) of the parent CRY (*1*) and the mutant strains CRX (*2*), CRN (*3*), and CNX (*4*). CRX contains the inactivated gene *PPX1* of exopolyphosphatase, CRN contains the inactivated gene *PPN1* of endopolyphosphatase, and CNX contains the inactivated genes of both exopolyphosphatase and endopolyphosphatase.

The inhibitory effect of the *PPN1* gene inactivation on the accumulation of polyphosphates in the actively

growing cells suggests a possible regulatory role of this gene in the biosynthesis of particular polyphosphates, especially acid-soluble polyphosphates, and their important role in energy conservation. The specific role of the *PPN1* gene, which is also known as the *PMN5* gene, was earlier emphasized by Ogawa *et al.* [11].

It should be noted that the presence of polyphosphates in the mutants lacking exopolyphosphatase and endopolyphosphatase (the latter enzyme may also possess exopolyphosphatase activity) indicates that these two enzymes are not the only enzymes of polyphosphate metabolism in yeasts. It is noteworthy in this context that little is known about the enzymes involved in polyphosphate synthesis.

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