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Inorganic Polyphosphate in the Yeast *Saccharomyces cerevisiae* with a Mutation Disturbing the Function of Vacuolar ATPase

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Abstract—A mutation in the *vma2* gene disturbing V-ATPase function in the yeast *Saccharomyces cerevisiae* results in a five- and threefold decrease in inorganic polyphosphate content in the stationary and active phases of growth on glucose, respectively. The average polyphosphate chain length in the mutant cells is decreased. The mutation does not prevent polyphosphate utilization during cultivation in a phosphate-deficient medium and recovery of its level on reinoculation in complete medium after phosphate deficiency. The content of short chain acid-soluble polyphosphates is recovered first. It is supposed that these polyphosphates are less dependent on the electrochemical gradient on the vacuolar membrane.

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The conception of tight relationships between energy metabolism and metabolism of inorganic polyphosphate (polyP) consisting of orthophosphate residues linked by energy-rich phosphoanhydride bonds is generally accepted [1, 2]. However, the details of these relationships need further study, especially in eukaryotes. One of the approaches to solution of this problem is investigation of the effect of mutations in the genes encoding energy-transforming enzymes. It would be particularly interesting to study the peculiar features of polyP metabolism in mutants with disturbed energization of the vacuolar membrane. Recently it has been shown that the protein Vtc4p, which plays an important role in vacuolar membrane fission and V-ATPase functioning [3], is able to synthesize polyphosphates [4]. Mutants in different V-ATPase subunits are extensively studied [5]; however, the effect of these mutations on polyP metabolism is still unknown.

The object of this study is a mutant in the *vma2* gene [5]. The *vma2* gene codes for protein B of subunit V1 of the V-ATPase domain, which is a proton pump providing the formation of membrane potential on the vacuolar membrane and acidification of the vacuolar lumen (http://www.yeastgenome.org/cgi-bin/locus.fpl?locus=VMA2). Deletions in the *vma2* gene decrease the ATP-hydrolyzing and proton-translocating activities of V-ATPase and result in a phenotype characterized by disturbed pH homeostasis in the cytoplasm and vacuoles, susceptibility to enhanced concentrations of extracellular calcium, copper, and iron ions, and decreased stress resistance [5]. It should be noted that resistance of microorganisms to heavy metal cations and oxidants is often associated with polyP metabolism [1, 2].

The goal of this work was to study the peculiar features of polyP metabolism in the yeast *Saccharomyces cerevisiae* under mutation in the *vma2* gene blocking the function of V-ATPase.

MATERIALS AND METHODS

The strains of *S. cerevisiae* BY 4741 (parent strain) and BY 4741 Vma2Δ (deletions in the *vma2* gene) were kindly provided by Dr. P. Kane (SUNY Upstate Medical University, USA) [5]. Yeast cultures were maintained on YPD agar medium. The strains were grown on a shaker (120 rpm) at 30°C on the YPD medium (1% yeast extract, 2% peptone, and 2% glucose). The P_i-deficient medium with 0.08 mM P_i was prepared according to [6], and media with 10 and 20 mM P_i were prepared by adding KH_2PO_4. In the P_i-deficient medium and in the medium with 10 mM P_i, the cells were grown to the stationary growth phase (24 h). Growth curves in the medium with 10 mM P_i are presented in the figure. The growth curves

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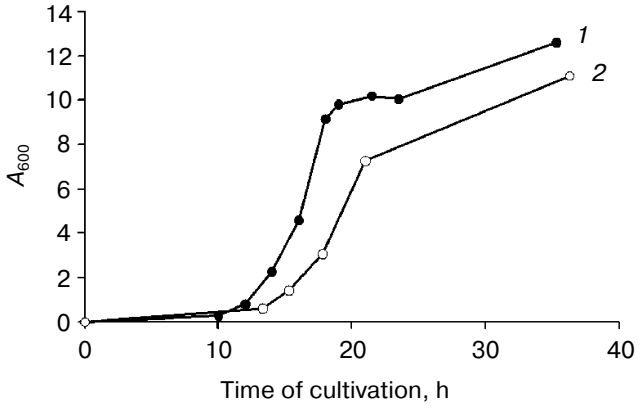

Growth curves of *S. cerevisiae* strains during cultivation on glucose: *1*) parent strain BY 4741; *2*) mutant strain BY 4741 Vma2Δ

in the P_i-deficient medium were the same. The polyP content under phosphate surplus was studied during short-term cultivation at high cell density [7]. Cells were grown in the P_i-deficient medium, harvested by centrifugation at 5000*g* for 10 min, washed with distilled water, put into the medium with 20 mM P_i (2 g of wet biomass per 200 ml flask), and cultivated for 1 h. Then the cells were harvested by centrifugation at 5000*g* for 10 min, washed with distilled water, and analyzed.

PolyP was extracted from the cells and assayed by the labile phosphorus content as described in [8]. The following polyP fractions were obtained: acid-soluble fraction polyP1 (extracted with 0.5 M $HClO_4$ at 0°C), salt-soluble fraction polyP2 (extracted with saturated $NaClO_4$ solution at 0°C), alkali-soluble fraction polyP3 (extracted with NaOH solution, pH 9-10, at 0°C), and alkali-soluble fraction polyP4 (extracted with 0.05 M NaOH at 0°C). The fraction polyP5 was a hot perchloric acid extract; its polyP content was determined by the amount of P_i released after the treatment of residual biomass with 0.5 M $HClO_4$ at 90°C for 40 min. The polyP content was assayed by the amount of labile phosphorus [8]; P_i was assayed by the methods described in [9].

PolyP chain length was determined by electrophoresis in 20% polyacrylamide gel in 200 mM Tris-borate buffer, pH 8.3, with 7 M urea [10]. Commercial polyphosphates with different average chain lengths (Sigma and Monsanto, USA) were used as markers. The average values of three experiments are presented.

RESULTS AND DISCUSSION

The P_i and polyP contents in cells of the parent strain of *S. cerevisiae* and the *vma2* mutant in the stationary growth phase are presented in Table 1. The parent strain contained 1.6-fold more P_i and 5-fold more polyP compared to the mutant strain. The polyP1 and polyP3 content in the mutant was 5-fold lower than in the parent strain; the polyP2 and polyP5 content was 10- and 1.5-fold lower, respectively. The content of polyP4 was actually the same. This fact is in agreement with the suggestion that this fraction is localized in the cell envelope and its metabolism is different compared to other fractions [8]. Thus, the polyP content in separate fractions decreased in the presence of the mutation under study to different extents.

Electrophoresis showed that the average chain length of polyP1 was ~15 phosphate residues both in the parent and mutant strains, this being similar to other yeast strains [11]. The average chain length of polyP2 was ~15 phosphate residues in both strains. The average chain length of polyP3 was 45-75 and 15-30 phosphate residues in the parent strain and in the mutant, respectively. On the whole, the average chain length in the mutant strain was shorter.

The accumulation of polyP in the active growth phase after phosphorus starvation was investigated. The mutation did not prevent polyP utilization during cultivation in the P_i-deficient medium (Table 2). After reinoculation in P_i-containing medium, polyP was accumulated in the cells of both strains. After 1 h of cultivation, the polyP level in the cells of the parent strain was restored to the initial value, while in the mutant cells it became higher than in the stationary growth phase. However, total polyP accumulation under these culture conditions in the mutant was three times less than in the parent strain. In the mutant cells under active growth, only the polyP1 fraction was accumulated, while the content of polyP2 decreased and the content of other fractions changed insignificantly.

So, the mutation in the *vma2* gene disturbing the function of V-ATPase decreases the ability of the yeast cell to accumulate polyP both in the active and station-

Table 1. PolyP content in *S. cerevisiae* cells in the stationary phase of growth in YPD medium with 10 mM P_i

Polyphosphate fraction	PolyP content, μmol P/g wet biomass	
	BY 4741, parent strain	BY 4741 Vma2Δ, the strain with disturbed functioning of vacuolar ATPase
P_i	16.3 ± 3	10.4 ± 1
polyP1	76 ± 10	14 ± 1.5
polyP2	19 ± 3	1.8 ± 0.1
polyP3	19 ± 3	3.6 ± 0.4
polyP4	2.6 ± 0.3	2.4 ± 0.3
polyP5	0.8 ± 0.1	0.51 ± 0.02
Total polyP	118 ± 10	23 ± 3

Table 2. PolyP content in *S. cerevisiae* cells during cultivation under P_i deficiency followed by reinoculation in medium with 20 mM P_i to the active growth phase

Polyphosphate fraction	PolyP content, μmol P/g wet biomass			
	BY 4741, parent strain		BY 4741 Vma2Δ, the strain with disturbed functioning of vacuolar ATPase	
	P_i deficit	transfer to P_i-containing medium	P_i deficit	transfer to P_i-containing medium
P_i	9.63	25.4	9.06	18.6
polyP1	1.27	36.4	0.19	18.4
polyP2	7.51	40.8	5.28	3.65
polyP3	2.89	23.7	2.25	5.52
polyP4	1.56	5.12	1.61	3.24
polyP5	0.894	0.3	0.515	0.544
Total polyP	14.1	106.3	9.84	31.3

ary growth phases. This indicates a relationship between the synthesis of these polymers and the electrochemical gradient on the vacuolar membrane. In contrast to strains with the defects in mitochondrial ATPase [12] with disturbance of polyP accumulation in the stationary growth phase, the mutant with defects in V-ATPase function has lower polyP content compared to the parent strain in the active and stationary growth phases. The polyP synthase of the vacuolar membrane [4] probably synthesizes not only the polyP of vacuoles but also the polyP transported to other cell compartments. In particular, polyP could arrive directly in the cytosol during the synthesis; such possibility is suggested by localization of the active center of this enzyme on the cytoplasmic side [4]. The data suggest that the polyP2 and polyP3 fractions are more associated with the vacuolar energetics. The cytosol probably has an independent polyP-synthesizing system responsible for the accumulation of short-chain polyP1.

Thus, the relationship between polyP metabolism and the state of vacuoles in *S. cerevisiae* cells and disturbance of polyP metabolism as a pleiotropic effect of *vma2* deletion have been demonstrated.

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