ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 1, pp. 30–33. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © A.A. Tomashevsky, L.P. Ryazanova, T.V. Kulakovskaya, I.S. Kulaev, 2010, published in Mikrobiologiya, 2010, Vol. 79, No. 1, pp. 35–38.

EXPERIMENTAL ARTICLES

Inorganic Polyphosphates of Different Fractions in the Mutant Yeast *Saccharomyces cerevisiae* with Impaired Mitochondrial ATP Synthesis

A. A. Tomashevsky, L. P. Ryazanova, T. V. Kulakovskaya[1], and I. S. Kulaev

Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received April 9, 2009

Abstract—Impaired synthetase function of the mitochondrial ATPase induced by mutation in the *ATP22* gene results in decreased accumulation of inorganic polyphosphates in the stationary growth phase of the yeast *Saccharomyces cerevisiae* grown on glucose. The content of polyphosphates in the mutant strain in this phase is 2.5 times lower than in the parent strain. This difference is most pronounced for the acid-soluble polyP1 fraction and the alkali-soluble polyP3 fraction. Polyphosphate chain length in mutant cells is less than in the parent cells in both the acid-soluble polyP1 and in the salt-soluble polyP2 fractions. The mutation had no effect on polyphosphates content in the mitochondria.

Key words: inorganic polyphosphates, *Saccharomyces cerevisiae*, mutant, *ATP22* gene, ATPase, mitochondria, glycolysis.

DOI: 10.1134/S0026261710010042

The concept of close connection between the metabolism of inorganic polyphosphates (polyP), consisting of phosphoric acid residues linked by high-energy phosphorus–anhydride bonds, and energy metabolism is prevalent in the literature [1, 2].

At the same time, the following question requires further investigation: in what way do different energetic states of yeast cells influence the metabolism of these polymers? One of the approaches to the solution of this problem is to study the effect of mutations in the genes encoding the enzymes involved in energy conversion on the content and chain length of polyP. Such mutants have been obtained at different laboratories and are presently available for application, including the mutants with defective synthetic function of the mitochondrial ATPase. The *ATP22* gene encodes the protein involved in posttranslational assembly of the hydrophobic part of F_o of the mitochondrial ATPase [3]. In the mutants by this gene, the mitochondrial ATPase retains only hydrolase activity; ATP synthesis in mitochondria is inhibited, and the cells cannot utilize oxidized substrates [3].

The goal of this work was to reveal the peculiarities of polyP metabolism in different growth phases of the yeast *S. cerevisiae* with mutations in the *ATP22* gene blocking ATP synthesis in the mitochondria.

[1] Corresponding author; e-mail: alla@ibpm.pushchino.ru

MATERIALS AND METHODS

The strains of *Saccharomyces cerevisiae* D273 (parent strain), N417, and E232 (different deletions in the *ATP22* gene) were kindly provided by A. Tzagoloff (Columbia University, United States) [3]. The genotypes of the strains were presented in [3]. Yeast cultures were maintained on YPD agar medium. All strains were grown on a shaker (120 rpm) at 30°C on YPD medium (1% yeast extract, 2% peptone, and 2% glucose). KH_2PO_4 was added to the medium to a concentration of 10 mM. The growth was controlled by optical density at 600 nm.

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 as labile phosphorus content as described [4]. The following polyP fractions were obtained: acid-soluble fraction polyP1 (extracted with 0.5 N $HClO_4$ 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 N 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 N $HClO_4$ at 90°C for 40 min. The polyP5 fraction for electrophoresis was extracted with hot distilled water.

PolyP chain length was determined by electrophoresis in 20% polyacrylamide gel prepared in

200 mM Tris–borate buffer, pH 8.3, with 7 M urea [5]. Commercial polyphosphates with different average chain lengths (Sigma and Monsanto, United States) were used as markers.

Mitochondria were obtained by the method of differential centrifugation [6].

Exopolyphosphatase activity was determined by the rate of P_i release at 30°C during 30 min in 1 ml of the reaction mixture containing the following: Tris–HCl, 50 mM, pH 7.2; $MgSO_4$, 2.5 mM; and $polyP_{188}$, 1 mM (by labile phosphorous). The activity unit (U) was determined as the amount of the enzyme producing 1 μmol P_i per 1 min. ATPase activity was determined by the rate of P_i release at 30°C for 30 min in 1 ml of the reaction mixture containing the following: Tris–HCl, 50 mM, pH 8.5; $MgSO_4$, 1 mM; Triton X-100, 0.1%; and ATP, 1 mM. P_i was assayed as described [7].

Protein concentration was assayed by the modified Lowry method [8]. Bovine serum albumin was used as a standard.

The average values of three experiments are presented.

RESULTS AND DISCUSSION

The growth curves of *S. cerevisiae* strains are presented in Fig. 1. The strains with mutations in the *ATP22* gene grew more slowly and yielded less biomass than the parent strain, which is typical of many so-called petite mutants with impaired ATP synthesis in mitochondria [9]. The growth of strains N417 and E232 was similar (data not shown). The phenotypes of both mutant strains used in the work are characterized by a smaller cell size compared to the parent strain. The parent and mutant strains contain 1.8×10^{10} and 3.2×10^{10} cells per 1 g of wet biomass, respectively.

The cells of the logarithmic growth phase (7 h for the parent strain (Fig. 1, curve *1*, point A) and 12 h for the mutant strains (Fig. 1, curve *2*, point A)) and of the stationary growth phase (14 h for the parent strain (Fig. 1, curve *1*, point B) and 17 h for the mutant strains (Fig. 1, curve *2*, point B)) were used for polyP extraction.

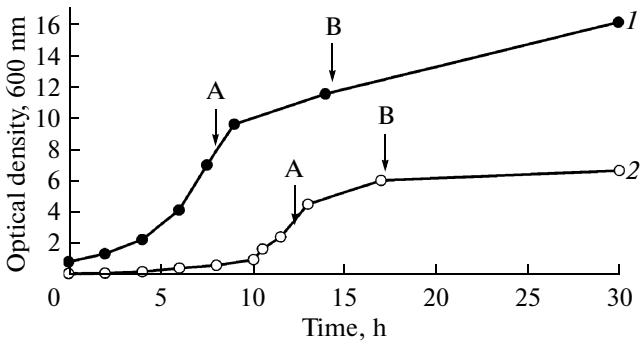

Fig. 1. Growth curves of different *S. cerevisiae* strains grown on glucose. A, B: the points of biomass sampling for determination of polyphosphate content. Strain D273 (*1*) and strain E232 (*2*).

The data on polyP content in the cells of the parent and mutant strains are presented in Table 1. In the cells from the logarithmic growth phase, the content of different polyP fractions was nearly the same. This result had been anticipated, because glycolysis is the main energy source for yeast cells in this growth phase, while oxidative phosphorylation at the level of the respiratory chain is suppressed by the mechanism of catabolic repression [10]. The polyP content increased in both strains during the stationary growth phase, which is typical also of other *S. cerevisiae* strains [11]. However, the total polyP content increased almost fourfold in the parent strain but only 1.8-fold in the mutant. As a result, the parent strain in the stationary growth phase contained 2.5 times more polyP than the mutant one. This difference is the greatest in the fractions polyP1 and polyP3, which are the most dynamic ones and are accumulated in larger quantities than other fractions [4, 11]. Thus, a significant dependence of the content

Table 1. PolyP content in *S. cerevisiae* cells in the logarithmic and stationary growth phases (μmol P/g of dry biomass)

Polyphosphate fraction	Yeast strain			
	D273, parent strain		E232, the strain with impaired mitochondrial ATP synthesis	
	Logarithmic	Stationary	Logarithmic	Stationary
P_i	170 ± 10.6	95.4 ± 12.2	144 ± 16.9	159 ± 19.9
PP1	36.6 ± 3.7	170 ± 26.5	31.8 ± 2.98	64.7 ± 8.9
PP2	6.36 ± 0.53	37.1 ± 5.6	4.975 ± 0.99	12.9 ± 4.9
PP3	13.8 ± 3.18	63.6 ± 7.42	11.4 ± 0.99	22.4 ± 2.4
PP4	1.43 ± 0.21	4.98 ± 1.59	1.12 ± 0.149	2.69 ± 0.3
PP5	3.66 ± 0.26	4.93 ± 1.06	2.09 ± 0.34	3.93 ± 0.4
Total polyP	61.8 ± 6.8	281 ± 26.5	51.4 ± 4.48	107 ± 12

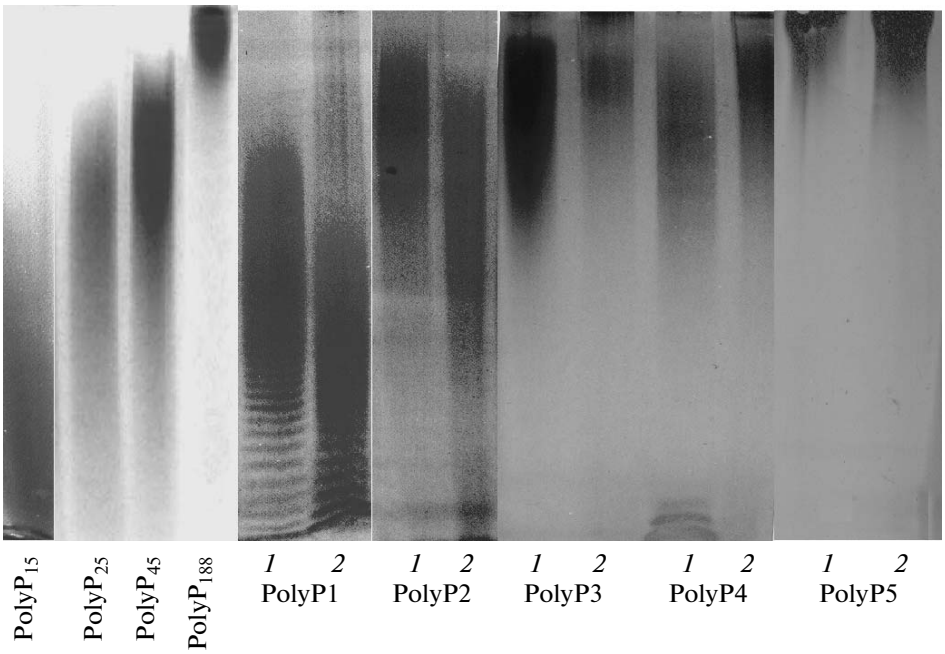

Fig. 2. Electrophoresis of polyphosphates of different fractions obtained from *S. cerevisiae* cells in the stationary growth phase. Parent strain D273 (*1*); strain E232, mutant by the *ATP22* gene (*2*). $PolyP_{15}$, $PolyP_{25}$, $PolyP_{45}$, and $PolyP_{188}$ are polyphosphate markers with the chain lengths of 15, 25, 45, and 188 phosphate residues, respectively.

of these two fractions on the energetic state of yeast cell was confirmed [12, 13].

Electrophoresis of the polyP obtained from the cells at the stationary growth phase revealed that the average polyP chain length in the fractions polyP1 and polyP2 was less in the mutant than in the parent strain. For other fractions, the differences in the chain length between the parent and mutant strains were not revealed (Fig. 2).

Mutant strains N417 and E232 showed no essential differences in the contents and chain length of polyphosphates; however, the mitochondrial fraction could be more effectively obtained from strain N417. We compared the polyP content, as well as ATPase and exopolyphosphatase activities, in the fractions of isolated mitochondria of the parent strain D273 and of the mutant strain N417.

Hydrolase activity of mitochondrial ATPase and its sensitivity to sodium azide were preserved in the mutant (Table 2). Sodium azide inhibited it by 90%, demonstrating that the preparation was free from other ATPase-containing organelles and membranes. Exopolyphosphatase activities in the isolated mitochondria of strains D273 and N417 were similar (Table 2). The mutant mitochondria exhibited a certain decrease of polyP content, the significance of which in the cellular polyP metabolism requires further investigation.

Table 2. PolyP quantity and activity of the enzymes of phosphorus metabolism in isolated mitochondria of yeast strains D273 and N417

Strain	ATPase activity, U/mg of protein	Exopolyphosphatase activity, U/ mg of protein	PolyP, μmol P/mg of protein)
D273, parent	0.47	0.16	0.096
N417, mutant	0.38	0.14	0.088

Thus, the mutation in the *ATP22* gene, which blocks mitochondrial ATP synthesis and results in the petite phenotype, reduces the ability of yeast cells to accumulate polyP in the stationary growth phase, primarily at the expense of the acid-soluble fraction polyP1 and the alkali-soluble fraction polyP3. This finding indicates close interrelation between polyP accumulation and the capacity of yeast cells for oxidative phosphorylation at the level of the respiration chain. The interrelation of polyphosphate synthesis and ATP formation in yeast cells is probably not direct. However, some data suggest the possibility of synthesis of these polymers due to the electrochemical gradient on the membranes [1] that is generated on the cytoplasmic and vacuolar membranes by the ATP energy. An ATP-dependent polyphosphate synthetase was recently found in the vacuolar membrane [14]. It is also not improbable that additional regulatory mechanisms exist providing the dependence of polyphosphate synthesis on ATP concentration, because this synthesis requires a lot of energy.

ACKNOWLEDGMENTS

The authors are grateful to Prof. A. Tzagoloff for kindly providing the mutant strains.

The work was supported by the Russian Foundation for Basic Research (project no. 08-04-00472) and the Program of Support of Leading Scientific Schools of the Russian Federation (project NSh-1004.2008.4).

REFERENCES

1. Kulaev, I.S., Vagabov, V.M., and Kulakovskaya, T.V. *Vysokomolekulyarnye neorganicheskie poliP: biokhimiya, kletochnaya biologiya, biotekhnologiya* (High-Molecular Inorganic PolyP: Biochemistry, Cell Biology, and Biotechnoilogy), Moscow: Nauchnyi Mir, 2005.
2. Kornberg, A., Rao, N.N., and Ault-Riche, D., Inorganic Polyphosphate: A Molecule with Many Functions, *Ann. Rev. Biochem.*, 1999, vol. 68, pp. 89–125.
3. Helfenbein, K., Ellis, T., Dieckmann, C., and Tzagoloff, A., ATP22, a Nuclear Gene Required for Expression of the F_0 Sector of Mitochondrial ATPase in *Saccharomyces cerevisiae*, *J. Biol. Chem.,* 2003, vol. 278, pp. 19751–19756.
4. Vagabov, V.M., Trilisenko, L.V., and Kulaev, I.S., Dependence of Inorganic Polyphosphate Chain Length on the Orthophosphate Content in the Culture Medium of the Yeast *Saccharomyces cerevisiae*, *Biokhimiya*, 2000, vol. 65, pp. 414–420 [*Biochemistry* (Moscow) (Engl. Transl.), vol. 65, no. 3, pp. 349–355].
5. Lichko, L.P. Kulakovskaya, T.V., and Kulaev, I.S., Inorganic Polyphosphates and Exopolyphosphatases in Different Cell Compartments of *Saccharomyces cerevisiae, Biokhimiya*, 2006, vol. 71, pp. 1445–1450 [*Biochemistry* (Moscow) (Engl. Transl.), vol. 71, no. 11, pp. 1171–1175].
6. Lichko, L., Kulakovskaya, T., Pestov, N., and Kulaev, I., Inorganic Polyphosphates and Exopolyphosphatases in Cell Compartments of the Yeast *Saccharomyces cerevisiae* under Inactivation of PPX1 and PPN1 genes, *Biosci. Rep.,* 2006, vol. 26, pp. 45–54.
7. Kulakovskaya, T.V., Andreeva, N.A., Karpov, A.V., Sidorov, I.A., and Kulaev, I.S., Hydrolysis of Tripolyphosphate by Purified Exopolyphosphatase from *Saccharomyces cerevisiae* Cytosol: Kinetic Model, *Biokhimiya,* 1999, vol. 64, pp. 1180–1184 [*Biochemistry* (Moscow) (Engl. Transl.), vol. 64, no. 9, pp. 1180–1184].
8. Bensandoun, A. and Weinstein, D. Assay of Proteins in the Presence of Interfering Materials, *Anal. Biochem.*, 1976, vol. 70, pp. 241–250.
9. Schatz, G. J. Impaired Binding of Mitochondrial Adenosine Triphosphatase in the Cytoplasmic Petite Mutants of *Saccharomyces cerevisiae, J. Biol. Chem.,* 1967, vol. 243, pp. 2192–2199.
10. Meijer, M.M.C., Boonstra, J., Verkleij, A., and Verrips C.T., Glucose Repression in *Saccharomyces cerevisiae* Is Related to the Glucose Concentration rather than the Glucose Flux, *J. Biol. Chem.*, 1998, vol. 273, pp. 24102–24107.
11. Vagabov, V.M., Trilisenko, L.V., Shchipanova, I.N., Sibel'dina, L.A., and Kulaev, I.S., Changes in Inorganic Polyphosphate Length during the Growth of *Saccharomyces cerevisiae, Mikrobiologiya*, 1998, vol. 67, pp. 193–198 [*Microbiology* (Engl. Transl.), vol. 67, no. 2, pp. 153–157].
12. Vagabov, V.M., Trilisenko, L.V., Kulakovskaya, E.V., and Kulaev, I.S., Study of the Content of Inorganic Polyphosphates in *Saccharomyces cerevisiae* Grown on Different Carbon Sources with Different O_2 Concentrations in the Medium, *Mikrobiologiya*, 2008, vol. 77, pp. 611–616 [*Microbiology* (Engl. Transl.), vol. 67, no. 5, pp. 541–546].
13. Vagabov, V.M., Trilisenko, L.V., Kulakovskaya, T. V., and Kulaev I.S. Effect of Carbon Source on Polyphosphate Accumulation in *Saccharomyces cerevisiae*, *FEMS Yeast Res.*, 2008, vol. 8, pp. 877–882.
14. Hothorn, M., Neumann, H., Lenherr, E.D., Wehner, M., Rybin, V., Hassa P.O., Uttrnweiler, A., Reinhardt, M., Schmidt, A., Seiler, J., Ladurner, A.G., Hermann, C., Scheffzek, K., and Mayer, A. Catalytic Core of a Membrane-Associated Eukaryotic Polyphosphate Polymerase, *Science*, 2009, vol. 324, pp. 513–516.