RESEARCH ARTICLE

Adaptation of *Saccharomyces cerevisiae* to toxic manganese concentration triggers changes in inorganic polyphosphates

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Abstract

The ability of *Saccharomyces cerevisiae* to adapt to toxic Mn^{2+} concentration (4 mM) after an unusually long lag phase has been demonstrated for the first time. The mutants lacking exopolyphosphatase PPX1 did not change the adaptation time, whereas the mutants lacking exopolyphosphatase PPN1 reduced the lag period compared with the wild-type strains. The cell populations of WT and *ΔPPN1* in the stationary phase at cultivation with Mn^{2+} contained a substantial number of enlarged cells with a giant vacuole. The adaptation correlated with the triggering of polyphosphate metabolism: the drastic increase in the rate and chain length of acid-soluble polyphosphate. The share of this fraction, which is believed to be localized in the cytoplasm, increased to 76%. Its average chain length increased to 200 phosphate residues compared with 15 at the cultivation in the absence of manganese. DAPI-stained inclusions in the cytoplasm were accumulated in the lag phase during the cultivation with Mn^{2+}.

Introduction

Inorganic polyphosphates (PolyP), the linear polymers of orthophosphoric acid, form complexes with metal cations (Kulaev *et al.*, 2004). These complexes participate in many cellular functions (Reusch, 1992; Negoda *et al.*, 2009; Rao *et al.*, 2009; Docampo *et al.*, 2010; Pavlov *et al.*, 2010). The involvement of PolyP in heavy metal resistance was observed in many living organisms. PolyP sequestered Ni^{2+} in *Staphylococcus aureus* (Gonzales & Jensen, 1998). The Cd^{2+} tolerance of *Escherichia coli* depended on PolyP metabolism: the mutant strain carrying multiple copies of exopolyphosphatase and polyphosphate kinase encoding genes showed the enhanced resistance to Cd^{2+} (Keasling *et al.*, 2000). PolyP sequestered heavy metals, on the one hand, and the entry of metal cations into the cells stimulated the exopolyphosphatase activity that releases P_i from PolyP, on the other hand (Keasling *et al.*, 2000). The $MeHPO_4^-$ (metal phosphate) ions were then transported out of the cells (Keasling *et al.*, 2000). The archaea *Sulfolobus metallicus* with the high PolyP level was resistant to copper sulfate, while *Sulfolobus solfataricus* with the lower PolyP level could not grow in the presence of high concentrations of copper salts (Remonsellez *et al.*, 2006). The earthworm *Lumbricus rubellus* inhabiting soils polluted with high concentrations of Pb^{2+} and Zn^{2+} accumulated inorganic phosphates in its tissues (Andre *et al.*, 2009). In *Trichoderma harzianum*, the presence of cadmium induced a reduction in PolyP content (De Lima Freitas *et al.*, 2011). In protozoa and some algae, cation sequestration is one of the functions of acidocalcisome, an organelle that contains pyrophosphate and PolyP bound with Ca^{2+} and other cations (Docampo *et al.*, 2010). The addition of copper ions to the diet of the larva of *Anticarsia gemmatalis*, a lepidopteran pest of soybean, increased the PolyP content in cell lysate and spherites (specific vesicles similar to acidocalcisomes) (Gomes *et al.*, 2012). The relationship between the Mn^{2+} homeostasis in yeast cells and PolyP was observed in short-time (1 h) uptake experiments: the content of acid-insoluble PolyP increased during Mn^{2+} uptake by the yeast cells (Okorokov *et al.*, 1983).

As the content of heavy metals in the environment is continuously increasing as a result of natural and industrial contamination, living organisms have evolved the mechanisms for tolerating the presence of these compounds by efflux, complexation, or reduction in metal ions

(Spain, 2003). The study of living organisms resistant to heavy metals ion, including manganese, is of interest due to the growing presence of these contaminants in the environment. Manganese is an essential trace element in living cells, because it is a cofactor of many enzymes including oxidases, dehydrogenases, DNA and RNA polymerases, and sugar transferases (Crowley *et al.*, 1999). At high concentrations, manganese is also a toxic ion. Inhibition of the growth of *Saccharomyces cerevisiae* was observed at a concentration of 0.5 mM Mn^{2+} or higher (Blackwell *et al.*, 1998; Jensen *et al.*, 2003). Yeast cells possess many transport proteins for Mn^{2+} uptake, sequestration, and excretion; they are located in the plasma membrane, Golgi, vacuolar, and mitochondrial membranes (Culotta *et al.*, 2005; Reddi *et al.*, 2009). The phosphate transporter of the yeast plasma membrane, PHO84 (Reddi *et al.*, 2009), is responsible for Mn^{2+} uptake via manganese-phosphate complexes. Disruption of the *PHO84* gene results in a manganese-resistant phenotype (Jensen *et al.*, 2003). This resistance is associated with the inability of yeast cells to take up a large amount of Mn^{2+}. It is of interest to reveal the possibility of physiological adaptation of wild-type yeast cells to toxic Mn^{2+} concentrations. Such adaptation may be a useful model for understanding the tolerance of microorganisms to heavy metal cations.

The aim of this study was to reveal the changes in PolyP content and cellular localization during the adaptation of *S. cerevisiae* to the toxic concentration of Mn^{2+}.

Materials and methods

Strains and growth conditions

The wild-type strain of *S. cerevisiae* VKM Y-1173 was obtained from the All-Russian Collection of Microorganisms. The parent strain CRY (WT) and the strains with altered PolyP metabolism (Table 1) were kindly provided by N. Rao and A. Kornberg (Sethuraman *et al.*, 2001).

The yeast was cultivated in the YPD (2% glucose, 2% peptone, 1% yeast extract) phosphate-deficient medium to the stationary growth stage. The phosphate-deficient medium containing 0.02 mM of P_i was prepared according to Rubin (1973). The cells were harvested at 5000 g for 10 min, washed with sterile distilled water, and transferred into the YPD medium with 14 mM potassium phosphate. $MnSO_4$ was added to a final concentration of 4 mM. In the control YPD medium, Mn^{2+} concentration was estimated to be 0.0036 mM.

Polyphosphate extraction and assay

For PolyP extraction and assay, the cells were harvested by centrifugation at 5000 ***g*** for 10 min and washed twice with distilled water. Five PolyP fractions were extracted according to Vagabov *et al.*, 2000; : PolyP1 with 0.5 N $HClO_4$; PolyP2 with $NaClO_4$ (2 g) and 1 N $HClO_4$ (0.5 mL) per 1 g of wet biomass; PolyP3 with a weak NaOH solution, pH 9-10; and PolyP4 with 0.05 N NaOH, pH 12. All fractions were extracted at 0 °C. PolyP5 was assayed by the appearance of P_i during the hydrolysis of residual biomass in 0.5 N $HClO_4$ at 90 °C for 20 min. After each extraction procedure, the residual biomass was separated by centrifugation, and supernatants were analyzed. PolyP and P_i were assayed as described previously (Kulakovskaya *et al.*, 1999; Vagabov *et al.*, 2000). The total PolyP content was calculated as a sum of all fractions. For electrophoresis, PolyP5 fraction was extracted with cold water according to Vagabov *et al.*, 2008.

PolyP was precipitated from the extracts with saturated $Ba(NO_3)_2$ solution, dissolved (Vagabov *et al.*, 2000), and subjected to electrophoresis in 30% polyacrylamide gel with 7 M urea (Kumble & Kornberg, 1995). The gels were stained with 0.05% toluidine blue, a specific dye to PolyP, in a water solution containing 25% methanol and 1% glycerol, and then washed with distilled water (Kumble & Kornberg, 1995). Commercial PolyP with average chain lengths of 15, 25, and 75 phosphate residues from Sigma and the one with an average chain length of 208 phosphate residues from Monsanto were used as markers. The average chain lengths of PolyP were taken as the values indicated by Sigma and Monsanto.

The manganese content was assayed by atomic absorption spectroscopy after samples burning at 180 °C in 32% $HClO_4$ (Lichko & Okorokov, 1976).

The average data of three independent experiments with the standard deviation are presented.

Fluorescence microscopy

Staining with fluorochrome 4′,6′-diamino-2-phenylindole 2HCl (DAPI) is one of the effective methods of PolyP

Table 1. The mutant strains of *Saccharomyces cerevisiae* (Sethuraman *et al.*, 2001) used in the study

Strain	Name	Genotype	Alterations in PolyP metabolism
CRY	WT	*MATa ade2 his3 leu2 trp1 ura3*	Parent strain
CRX	*ΔPPX1*	*MATa ade2 his3 trp1 ura3 ppx1Δ::LEU2*	No activity of exopolyphosphatase PPX1
CRN	*ΔPPN1*	*MATa ade2 his3 ura3 ppn1Δ:: CgTPR1*	No activity of exopolyphosphatase PPN1
CNX	*ΔPPX1ΔPPN1*	*MATa ade2 his3 ura3 ppn1Δ:: CgTPR1 ppx1Δ::LEU2*	No activities of exopolyphosphatase PPX1 and PPN1

detection (Serafim *et al.*, 2002; Kulakova *et al.*, 2011). DAPI is used for DNA detection at 0.1 to 1 μg mL^{-1} and for PolyP detection at 3–50 μg mL^{-1} (Serafim *et al.*, 2002). The emission maximum of DAPI and DAPI-DNA complexes is 456 nm. The emission maximum of DAPI-PolyP complexes is 525 nm. The fluorescence of pure DAPI-DNA complexes was blue and white, while that of pure DAPI-PolyP complexes was yellow. Living cells may have mixed fluorescence spectra. DAPI fluoresces green when PolyP is colocalized with DNA or when DAPI and DAPI-PolyP have a mixed spectrum.

For examination under fluorescent and phase-contrast microscopes (AXIO Imager A1, ZEISS, Germany), the cells were incubated in PBS, pH 7.4, with 10 μg mL^{-1} DAPI (Sigma) at 30° for 15 min, centrifuged, and resuspended in PBS. Filter set 49 (ZEISS) was used with the excitation maximum at 359 nm and the emission maximum at 460 nm.

Results

Saccharomyces cerevisiae adapts to manganese after an unusually long lag phase

We have observed for the first time the ability of wild-type strain *S. cerevisiae* VKM Y-1173 (see Fig. S1, Supporting Information) and some mutant strains (Fig. 1a) to grow in the presence of toxic Mn^{2+} concentration (4 mM). This growth was characterized by an unusually long lag phase. All mutants under study had a 2-h lag phase in the medium without Mn^{2+}. The lag phases of the strains WT (Fig. 1a) and *ΔPPX1* (data not shown) lasted for about 96 h in the presence of Mn^{2+}. The lag phases of the strains *ΔPPN1* (Fig. 1a) and *ΔPPX1ΔPPN1* (data not shown) lasted for about 65 h in the presence of Mn^{2+}. If re-inoculated into a fresh medium with Mn^{2+}, the adapted cells showed only a slight growth delay (Fig. 1a). The strain *ΔPPX1* lacks exopolyphosphatase PPX1 (Wurst *et al.*, 1995) hydrolyzing mainly short-chained PolyP, while the strain *ΔPPN1* lacks exopolyphosphatase PPN1 (Sethuraman *et al.*, 2001) hydrolyzing mainly long-chained PolyP (Andreeva *et al.*, 2006). The strain *ΔPPX1ΔPPN1* is a double mutant in both enzymes (Sethuraman *et al.*, 2001). Thus, the absence of exopolyphosphatase PPX1 did not change the adaptation time, whereas the absence of exopolyphosphatase PPN1 did reduce the lag period.

Manganese uptake

The absorption of Mn^{2+} by the cells of the strains WT and *ΔPPN1* was estimated by the decrease in its concentration in the cultivation medium (Fig. 1b). Both strains

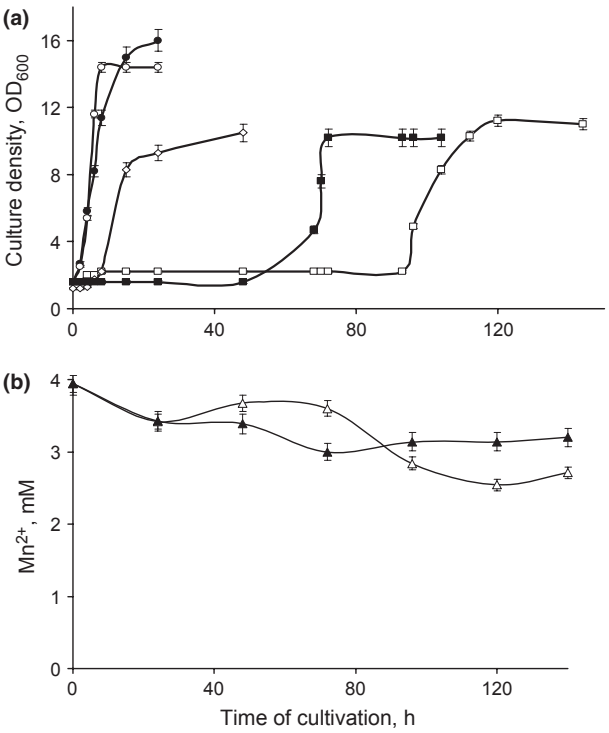

Fig. 1. (a) Growth curves of the strains of *Saccharomyces cerevisiae* in YPD medium: ○ –WT strain, without Mn^{2+}; ● – *ΔPPN1* strain, without Mn^{2+}; □ – WT strain, with 4 mM Mn^{2+}; ■ – *ΔPPN1* strain, with 4 mM Mn^{2+}; ◇ – WT strain, the cells adapted to Mn^{2+} were placed into the fresh medium with 4 mM Mn^{2+}. (b) Manganese concentration in the medium during cultivation of strains of *S. cerevisiae*: △ –WT strain; ▲ – *ΔPPN1* strain.

absorbed Mn^{2+} from the medium. Absorption begins as early as in the lag phase. The difference in the kinetics of decrease in Mn^{2+} concentration in the medium corresponded to the difference between the growth curves of strains under study. The Mn^{2+} content in the cells of both strains was ~1.4 mmol g^{-1} dry biomass in 24 h of cultivation. It was ~0.56 mmol g^{-1} dry biomass for both strains in 120 h of cultivation. It should be noted that the yield of biomass in 120 h was 2.3 and 1.8 g dry biomass L^{-1} for WT and *ΔPPN1*, respectively. So, there were no significant differences in manganese content in the biomass of the two strains.

Changes in PolyP during adaptation to manganese

As the *ΔPPN1* mutant lacking exopolyphosphatase PPN1 adapted to manganese before WT, we compared manganese-induced changes in PolyP in these two strains. It is known that PolyP of separate fractions in *S. cerevisiae* differs in the average chain length (Vagabov *et al.*, 2008). For example, the average chain length of acid-soluble

PolyP1 is usually ~15 phosphate residues, while that of acid-insoluble PolyP5 is more than 200 phosphate residues (Vagabov *et al.*, 2008). The amounts of PolyP fractions are shown in Fig. 2. In the stationary phase in the presence of Mn^{2+}, the content of PolyP increased in both strains compared with the cultivation without Mn^{2+} (Fig. 2a and b). This enhancement was due to the increase in acid-soluble PolyP1: The contribution of PolyP1 to total PolyP was unusually high (~76% in both strains). The PolyP1 content in the WT cells was enhanced during the cultivation with Mn^{2+} already in the lag phase. This increase was not observed in the *ΔPPN1* strain.

It is notable that the content of the highest molecular weight PolyP5 fraction is considerably enhanced in the cells of both strains during the cultivation with Mn^{2+} in the lag phase (Fig. 2a and b). The cells of the WT strain at this stage also show the enhanced content of PolyP3 fraction.

The average PolyP chain lengths in different fractions were assessed by electrophoresis in polyacrylamide gel. The average chain length of PolyP1 was ~15 phosphate residues during the cultivation of both strains without Mn^{2+} (Fig. 3). There was a drastic increase in the chain length of PolyP1 in the presence of Mn^{2+}: The average chain length of PolyP1 was estimated as ~45-65 phosphate residues in the lag phase and ~200 phosphate residues in the stationary phase in both strains (Fig. 3).

The average chain lengths of PolyP2, PolyP3, and PolyP4 fractions were ~ 65, 75, and 75-200 phosphate residues, respectively; the average chain length of PolyP5 fraction was more than 200 phosphate residues in the stationary phase of the parent strain and the *ΔPPN1* strain without Mn^{2+} (data not shown). These values are similar to those obtained earlier for the yeast *S. cerevisiae* (Vagabov *et al.*, 2008; Breus *et al.*, 2012). In the presence of Mn^{2+}, the average chain lengths of PolyP2, PolyP3, and PolyP4 increased but insignificantly, while that of PolyP5 remained the same in both strains (data not shown).

Excess Mn^{2+} similarly alters the PolyP1 content and chain length in the stationary phase in the WT and *ΔPPN1* strains. When the adapted cells were placed into a fresh medium with Mn^{2+}, the PolyP content, the proportion, and the average chain length of PolyP1 remained at the same high level (data not shown).

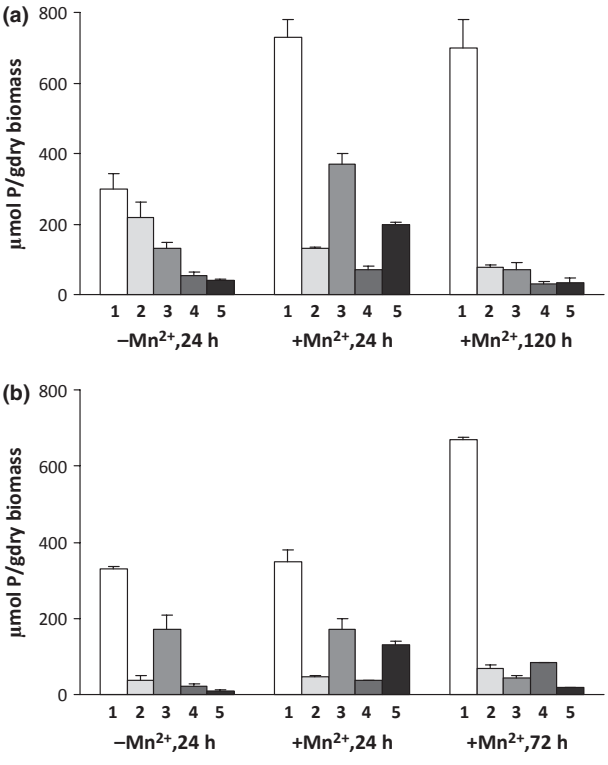

Fig. 2. The content of PolyP of separate fractions in the cells of WT strain (a) and *ΔPPN1* strain (b) of *Saccharomyces cerevisiae* at cultivation without manganese for 24 h (stationary stage) and with 4 mM $MnSO_4$ for 24 h (lag stage) and 72 and 120 h (stationary stage). 1– PolyP1, 2 – PolyP2, 3 – PolyP3, 4 – PolyP4, 5 – PolyP5.

Changes in cell morphology and DAPI staining with excess Mn^{2+}

Light microscopy revealed the changes in cell morphology in the presence of excess Mn^{2+}. The damaged and

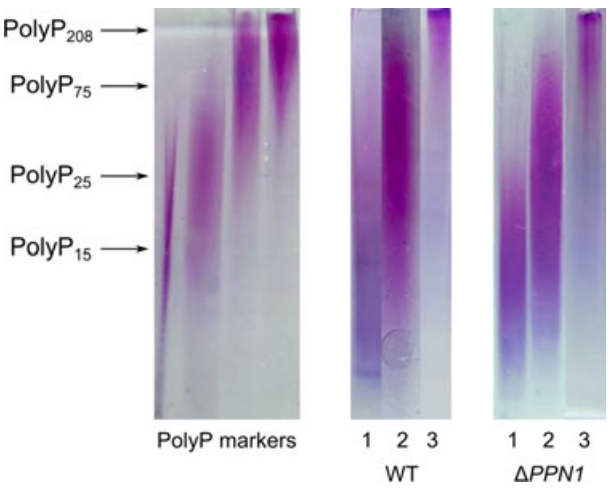

Fig. 3. Polyacrylamide gel electrophoresis of PolyP1 fraction from the cells of WT strain and *ΔPPN1* strain: 1 – cultivation without Mn^{2+}, stationary phase; 2 – cultivation with 4 mM Mn^{2+}, lag phase; 3 – cultivation with 4 mM Mn^{2+}, stationary stage. Commercial PolyP with average chain lengths of 15, 25, and 75 phosphate residues from Sigma and the one with an average chain length of 208 phosphate residues from Monsanto was used as markers.

deformed cells were predominant in the population of WT strains after 24 h of cultivation with Mn^{2+} (Fig. 4c). The cell population of the *ΔPPN1* mutant was more heterogeneous after 24-h cultivation in the presence of Mn^{2+}: Large round cells and budding cells appeared together with the damaged cells (Fig. 5c). This observation is consistent with the earlier adaptation of the *ΔPPN1* mutant to Mn^{2+}. The cell populations of both strains in the stationary phase during the cultivation with Mn^{2+} contained a significant number of cells twice as large (Figs 4e and 5e) as the cells grown in the medium without Mn^{2+} (Figs 4a and 5a). The enlarged cells had a giant vacuole. It was probably due to Mn^{2+} accumulation in these organelles. It is known that the vacuoles of *S. cerevisiae* accumulate manganese (Lichko *et al.*, 1980; Culotta *et al.*, 2005). The cells re-inoculated in the fresh medium with Mn^{2+} retained this morphological feature (see Fig. S2, Supporting Information).

Intense yellow-orange fluorescence of DAPI-stained cells was observed after 24 h of cultivation in the presence of Mn^{2+} (Figs 4d and 5d). This fluorescence is typical for DAPI-PolyP complexes (Serafim *et al.*, 2002;

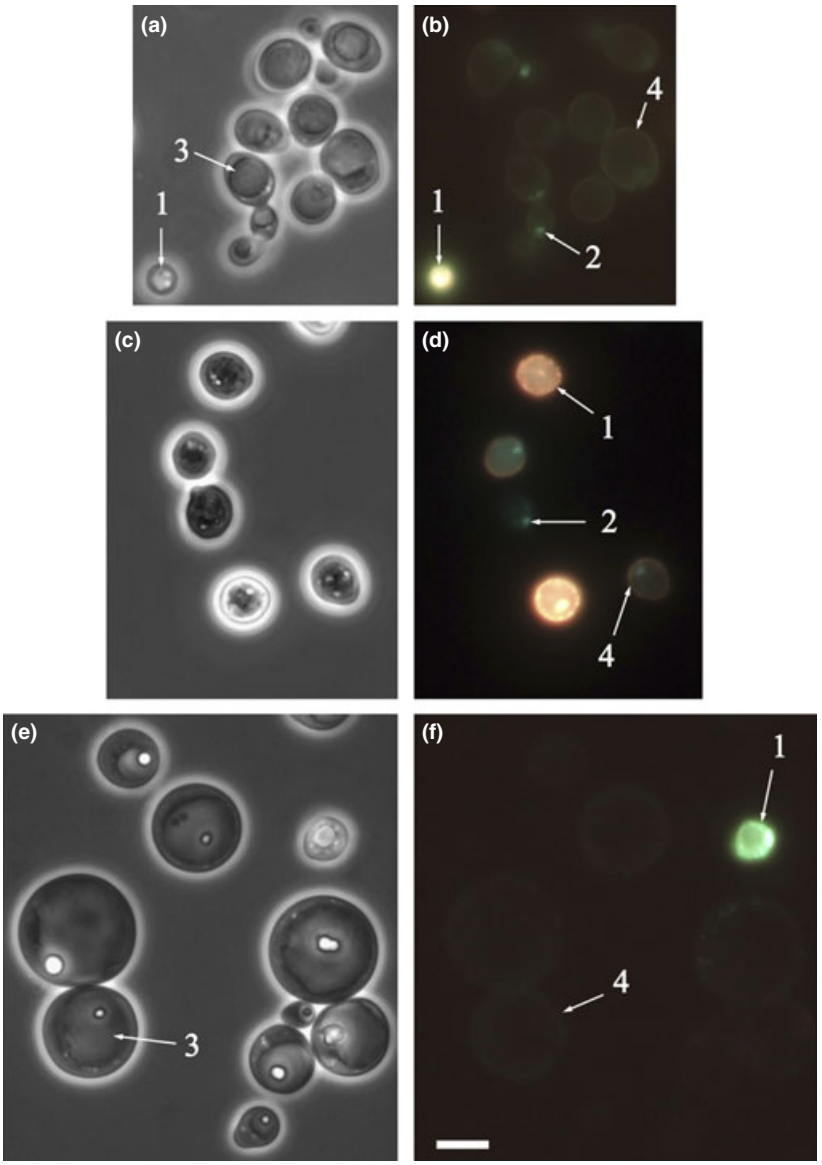

Fig. 4. Micrographs of the cells of WT strain of *Saccharomyces cerevisiae*. (a, c, e) phase-contrast microscopy; (b, d, f) fluorescence microscopy of the DAPI-stained cells. (a, b) cultivation without Mn^{2+}, 24 h; (c, d) cultivation in the presence of Mn^{2+}; 24 h; (e, f) cultivation in the presence of Mn^{2+}, 120 h. The bar line is 5 μm. 1 – mineralized cell with high PolyP content, 2 – nucleus, 3 – vacuole, 4 – cell wall.

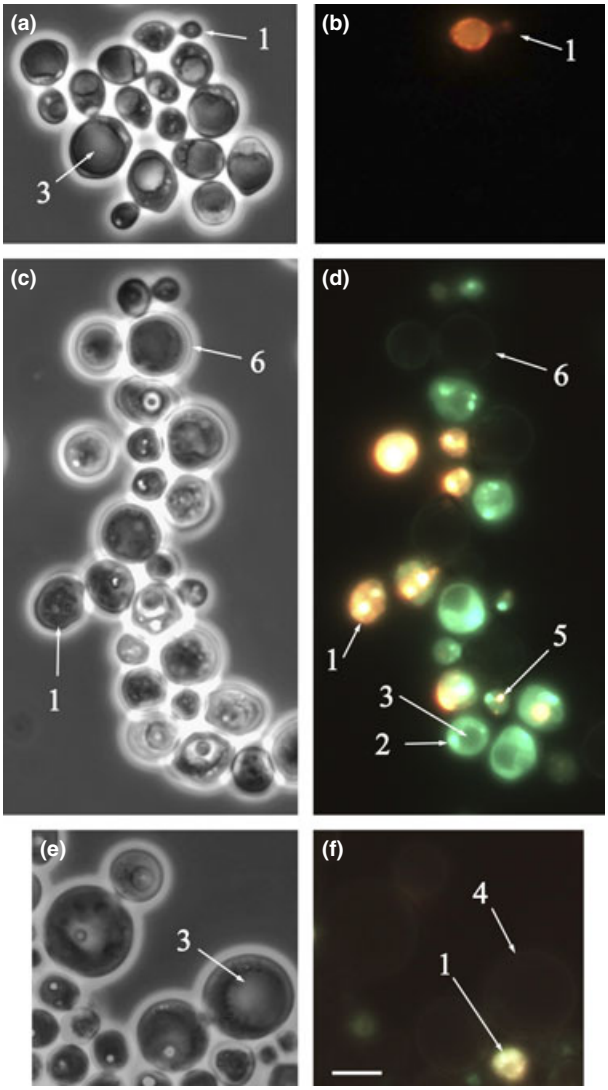

Fig. 5. Micrographs of the cells of *ΔPPN1* strain of *Saccharomyces cerevisiae*. (a, c, e) phase-contrast microscopy; (b, d, f) fluorescence microscopy of the DAPI-stained cells. (a, b) cultivation without Mn^{2+}, 24 h; (c, d) cultivation in the presence of Mn^{2+}; 24 h; (e, f) cultivation in the presence of Mn^{2+}, 72 h. The bar line is 5 μm. 1 – mineralized cell with high PolyP content, 2 – nucleus, 3 – vacuole, 4 – cell wall, 5 – PolyP containing inclusion, 6 – round low-fluorescent cell

Kulakova *et al.*, 2011). Some cells were wholly fluorescent, while fluorescent granules could be seen in the cytoplasm of other cells. The fluorescence of the cell envelope was observed as well. It confirms the multiple localization of PolyP in yeast cells (Kulaev *et al.*, 2004). However, weakly fluorescent cells were already observed in the population of the *ΔPPN1* mutant (Fig. 5d).

The cells of both strains of *S. cerevisiae* were weakly stained by DAPI in the stationary phase independent of the presence or absence of Mn^{2+} (Figs 4 and 5b and f). It is noteworthy that the cell of *S. cerevisiae* does not always show intense fluorescence with DAPI even at high concentrations of PolyP (Ryazanova *et al.*, 2011). The absence of DAPI-fluorescence is not always indicative of the low PolyP level in the yeast, and therefore, chemical extraction of this polymer is still necessary for its quantification. We suppose that intensive fluorescence with DAPI in the lag phase of growth with Mn^{2+} correlates with the accumulation of alkali-soluble PolyP3 and acid-insoluble PolyP5. Similar coincidence was observed earlier in *S. cerevisiae* during PolyP accumulation in a nitrogen-limited medium (Breus *et al.*, 2012).

Discussion

In this study, we have demonstrated for the first time the ability of *S. cerevisiae* to adapt to toxic Mn^{2+} after an unusually long lag phase. The adaptation correlates with the triggering of PolyP metabolism: the drastic increase in the rate and chain length of acid-soluble PolyP1. This fraction is supposed to be localized in the cytoplasm (Kulaev *et al.*, 2004).

We believe that excess manganese affects PolyP accumulation in several ways. First, Mn^{2+} may stimulate P_i uptake via the transport protein, PHO84, taking up manganese-phosphate complexes (Jensen *et al.*, 2003). Second, Mn^{2+} stimulates the PolyP-synthase activity of Vtc4, the protein of the vacuolar membrane of *S. cerevisiae* (Hothorn *et al.*, 2009), whereas Mn^{2+} inhibits the activity of some yeast exopolyphosphatases (Andreeva *et al.*, 1993). The accumulated PolyP may contribute to Mn^{2+} detoxification by forming complexes with these metal cations.

There is still no obvious answer to the question why the mutation in the *PPN1* gene results in acceleration of adaptation to Mn^{2+}. As PolyP biosynthesis is an energy-consuming process, it is probable that the *ΔPPN1* mutant with decreased PolyP hydrolysis saves energy and can use it to start growth earlier. This is another pleiotropic effect of the *PPN1* gene in addition to its previously known effects. The Δ*PPN1* mutants are unable to grow on ethanol and lactate (Pestov *et al.*, 2005) and have the lower expression of exopolyphosphatase PPX1 (Lichko *et al.*, 2004). The cause of the lower level of PolyP in the *ΔPPN1* mutant cells in the lag phase in the presence of Mn^{2+} is still unknown. Indirect effects of the *PPN1* gene products on PolyP synthesis are not improbable.

The functional significance of exopolyphosphatase PPN1 for yeasts cannot be considered as completely understood. The mutants in this gene do not show any growth variations during cultivation on the media with phosphate limitation or excess (Lichko *et al.*, 2008). The products of this gene are processed by proteinases (Sethuraman *et al.*, 2001) and have multiple localizations in the cell, including vacuolar and mitochondrial

membranes (Lichko *et al.*, 2006), and cytoplasm (Andreeva *et al.*, 2006); the protein sequence contains a transmembrane domain (http://www.uniprot.org/uniprot/Q04119). Further experiments on transformation of the mutants by the *PPN1* gene may clear up the question about the functions of this presumably polyfunctional protein.

Acknowledgements

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References

Andre J, Charnock J, Stürzenbaum SR, Kille P, Morgan AJ & Hodson ME (2009) Accumulated metal speciation in earthworm populations with multigenerational exposure to metalliferous soils: cell fractionation and high-energy synchrotron analyses. *Environ Sci Technol* **43**: 6822–6829.

Andreeva NA, Lichko LP, Kulakovskaya TV & Okorokov LA (1993) Characteristics of polyphosphatase activity of vacuoles in *Saccharomyces cerevisiae* cells. *Biokhimiia (Moscow)* **58**: 737–744.

Andreeva NA, Kulakovskaya TV & Kulaev IS (2006) High molecular mass exopolyphosphatase from the cytosol of the yeast *Saccharomyces cerevisiae* is encoded by the *PPN1* gene. *Biochemistry (Mosc)* **71**: 975–977.

Blackwell KJ, Tobin JM & Avery SV (1998) Manganese toxicity towards *Saccharomyces cerevisiae*: dependence on intracellular and extracellular magnesium concentrations. *Appl Microbiol Biotechnol* **49**: 751–757.

Breus NA, Ryazanova LP, Dmitriev VV, Kulakovskaya TV & Kulaev IS (2012) Accumulation of phosphate and polyphosphate by *Cryptococcus humicola* and *Saccharomyces cerevisiae* in the absence of nitrogen. *FEMS Yeast Res* **12**: 617–624.

Crowley JA, Traynor DA & Weatherburn DC (1999) Enzymes and protein containing manganese: an overview. *Manganese and Its Role in Biological Processes. Metal Ions in Biological Systems, Vol. 37* (Siegel A, Siegel H, eds), pp. 209–257. Marcel Dekker, New York.

Culotta VC, Yang M & Hall MD (2005) Manganese transport and trafficking: lesson learned from *Saccharomyces cerevisiae*. *Eukaryot Cell* **4**: 1159–1165.

De Lima Freitas A, Ferreira de Moura G, Barbosa de Lima MA, Mendes de Souza P, Alves da Silva CA, de Campos Takaki GM & do Nascimento AE (2011) Role of the morphology and polyphosphate in *Trichoderma harzianum* related to cadmium removal. *Molecules* **16**: 2486–2500.

Docampo R, Ulrich P & Moreno SN (2010) Evolution of acidocalcisomes and their role in polyphosphate storage and osmoregulation in eukaryotic microbes. *Philos Trans Royal Soc B Biol Sci* **365**: 775–784.

Gomes FM, Carvalho DB, Peron AC, Saito K, Miranda K & Machado EA (2012) Inorganic polyphosphates are stored in spherites within the midgut of *Anticarsia gemmatalis* and play a role in copper detoxification. *J Insect Physiol* **58**: 211–219.

Gonzales H & Jensen TE (1998) Nickel sequestering by polyphosphate bodies in *Staphylococcus aureus*. *Microbios* **93**: 179–185.

Hothorn M, Neumann H, Lenherr ED *et al.* (2009) Catalytic core of a membrane-associated eucaryotic polyphosphate polymerase. *Science* **324**: 513–516.

Jensen LT, Ajua-Alemandji M & Culotta VC (2003) The *Saccharomyces cerevisiae* high affinity phosphate transporter encoded by PHO84 also functions in manganese homeostasis. *J Biol Chem* **278**: 42036–42040.

Keasling JD, Van Dien SJ, Trelstad P, Renninger N & McMahon K (2000) Application of polyphosphate metabolism to environmental and biotechnological problems. *Biochemistry (Mosc)* **65**: 324–331.

Kulaev IS, Vagabov VM & Kulakovskaya TV (2004) *The Biochemistry of Inorganic Polyphosphates*. Wiley, Chichester.

Kulakova AN, Hobbs D, Smithen M, Pavlov E, Gilbert JA, Quinn JP & McGrath JW (2011) Direct quantification of inorganic polyphosphate in microbial cells using 4′-6-diamidino-2-phenylindole (DAPI). *Environ Sci Technol* **45**: 7799–77803.

Kulakovskaya TV, Andreeva NA, Karpov A, Sidorov I & Kulaev IS (1999) Hydrolysis of tripolyphosphate by purified exopolyphosphatase of *Saccharomyces cerevisiae* cytosol: kinetic model. *Biochemistry (Mosc)* **64**: 990–993.

Kumble KD & Kornberg A (1995) Inorganic polyphosphate in mammalian cells and tissues. *J Biol Chem* **270**: 5818–5822.

Lichko LP & Okorokov LA (1976) The compartmentalization of magnesium and phosphate ions in *Saccharomyces carlsbergensis* cells. *Dokl Akad Nauk SSSR (In Russian)* **227**: 756–758.

Lichko LP, Okorokov LA & Kulaev IS (1980) Role of vacuolar ion pool in *Saccharomyces carlsbergensis*: potassium efflux from vacuoles is coupled with manganese or magnesium influx. *J Bacteriol* **144**: 666–671.

Lichko L, Kulakovskaya T & Kulaev I (2004) Inactivation of endopolyphosphatase gene *PPN1* results in inhibition of expression of exopolyphosphatase PPX1 and high-molecular-mass exopolyphosphatase not encoded by *PPX1* in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1674**: 98–102.

Lichko L, Kulakovskaya T, Pestov N & Kulaev I (2006) Inorganic polyphosphates and exopolyphosphatases in cell compartments of the yeast *Saccharomyces cerevisiae* under inactivation of *PPX1* and *PPN1* genes. *Biosci Rep* **26**: 45–54.

Lichko LP, Kulakovskaya TV, Kulakovskaya EV & Kulaev IS (2008) Inactivation of *PPX1* and *PPN1* genes encoding exopolyphosphatases of *Saccharomyces cerevisiae* does not prevent utilization of polyphosphates as phosphate reserve. *Biochemistry (Mosc)* **73**: 985–989.

Negoda A, Negoda E, Mo Xian, Rosetta N & Reusch RN (2009) Role of polyphosphate in regulation of the *Streptomyces lividans* KcsA channel. *Biochim Biophys Acta* **1788**: 608–614.

Okorokov LA, Lichko LP & Andreeva NA (1983) Changes of ATP, polyphosphate and K^+ contents in *Saccharomyces carlsbergensis* during uptake of Mn^{2+} and glucose. *Biochem Int* **6**: 481–488.

Pavlov E, Aschar-Sobbi R, Campanella M, Turner RJ, Gómez-García MR & Abramov AY (2010) Inorganic polyphosphate and energy metabolism in mammalian cells. *J Biol Chem* **285**: 9420–9428.

Pestov NA, Kulakovskaya TV & Kulaev IS (2005) Effects of inactivation of the *PPN1* gene on exopolyphosphatases, inorganic polyphosphates and function of mitochondria in the yeast *Saccharomyces cerevisiae*. *FEMS Yeast Res* **5**: 823–828.

Rao NN, Gómez-García MR & Kornberg A (2009) Inorganic polyphosphate: essential for growth and survival. *Ann Rev Biochem* **78**: 605–647.

Reddi AR, Jensen LT & Culotta VC (2009) Manganese homeostasis in *Saccharomyces cerevisiae*. *Chem Rev* **109**: 4722–4732.

Remonsellez F, Orell A & Jerez CA (2006) Copper tolerance of the thermoacidophilic archaeon *Sulfolobus metallicus*: possible role of polyphosphate metabolism. *Microbiology* **152**: 59–66.

Reusch RN (1992) Biological complexes of poly-β-hydroxybutyrate. *FEMS Rev* **103**: 119–130.

Rubin GM (1973) The nucleotide sequence of *Saccharomyces cerevisiae* 5.8 S ribosomal ribonucleic acid. *J Biol Chem* **11**: 3860–3875.

Ryazanova L, Andreeva N, Kulakovskaya T, Valiakhmetov A, Yashin V, Vagabov V & Kulaev I (2011) The early stage of polyphosphate accumulation in *Saccharomyces cerevisiae*: comparative study by extraction and DAPI staining. *Adv Biosci Biotechnol* **2**: 293–297.

Serafim LS, Lemos OC, Levantesi C, Tandoi V, Santos H & Reis MA (2002) Methods for detection and visualization of intracellular polymers stored by polyphosphate-accumulating microorganisms. *J Microbiol Meth* **51**: 1–18.

Sethuraman A, Rao NN & Kornberg A (2001) The endopolyphosphatase gene: essential in *Saccharomyces cerevisiae*. *P Natl Acad Sci USA* **98**: 8542–8547.

Spain A (2003) Implication of microbial heavy metal tolerance in the environment. *Rev Undergrad Res* **2**: 1–6.

Vagabov VM, Trilisenko LV & Kulaev IS (2000) Dependence of inorganic polyphosphate chain length on the orthophosphate content in the culture medium of the yeast *Saccharomyces cerevisiae*. *Biochemistry (Mosc)* **65**: 349–355.

Vagabov VM, Trilisenko LV, Kulakovskaya TV & Kulaev IS (2008) Effect of carbon source on polyphosphate accumulation in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **8**: 877–882.

Wurst H, Shiba T & Kornberg A (1995) The gene for a major exopolyphosphatase of *Saccharomyces cerevisiae*. *J Bacteriol* **177**: 898–906.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Growth curves of *Saccharomyces cerevisiae* wild strain VKM Y-1173.

Fig. S2. Micrographs of the cells of parent strain CRY of *Saccharomyces cerevisiae*.