Potassium uptake system Trk2 is crucial for yeast cell viability during anhydrobiosis

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Abstract

Yeasts grow at very different potassium concentrations, adapting their intracellular cation levels to changes in the external environment. Potassium homeostasis is maintained with the help of several transporters mediating the uptake and efflux of potassium with various affinities and mechanisms. In the model yeast Saccharomyces cerevisiae, two uptake systems, Trk1 and Trk2, are responsible for the accumulation of a relatively high intracellular potassium content (200–300 mM) and the efflux of surplus potassium is mediated by the Tok1 channel and active exporters Ena ATPase and Nha1 cation/proton antiporter. Using a series of deletion mutants, we studied the role of individual potassium transporters in yeast cell resistance to dehydration. The Trk2 transporter (whose role in S. cerevisiae physiology was not clear) is important for cell viability in the stationary phase of growth and, moreover, it plays a crucial role in the yeast survival of dehydration/rehydration treatments. Mutants lacking the TRK2 gene accumulated significantly lower amounts of potassium ions in the stationary culture growth phase, and these lower amounts correlated with decreased resistance to dehydration/rehydration stress. Our results showed Trk2 to be the major potassium uptake system in stationary cells, and potassium content to be a crucial parameter for desiccation survival.

Introduction

In a natural environment, most microorganisms, including yeasts, may be periodically subjected to quite intense dehydration, resulting in the state of anhydrobiosis. This unique state of live organisms is linked with a temporary reversible suspension of metabolism for the periods of unfavorable environmental conditions. Upon rehydration, the cell functions can be restored and the cells start to grow and divide. This ability is widely utilized, mainly in food-related biotechnology processes producing or employing so-called ‘dry yeast’.

Detailed studies of anhydrobiosis in yeasts revealed structural and functional changes in the main cellular organelles as well as a number of protective intracellular reactions which take place in the cells upon their dehydration and subsequent rehydration/reactivation (Beker & Rapoport, 1987). One of the most important factors to determine the maintenance of cell viability under these conditions is linked with the maximal preservation of the molecular organization of cell membranes, including the plasma membrane (Crowe et al., 1989; Rapoport et al., 1997). The transfer of yeast cells into the state of anhydrobiosis results in a very significant decrease in cell volume (up to 60%). Such a huge decrease in cell volume is accompanied by the formation of large invaginations of the plasma membrane inside the cytosol (Beker & Rapoport, 1987). Cell volume and the normal shape of the plasma membrane is restored during a rather long process of cell reactivation that follows the rehydration process (Beker & Rapoport, 1987; Gervais & Beney, 2001). Besides the importance of trehalose and polyols for membrane protection under conditions of dehydration-rehydration (Panek et al., 1987; Krallish et al., 1997; Rapoport et al., 2009), several studies pointing to the importance of the plasma-membrane lipid composition and structural organization have been published recently (e.g. Dupont et al., 2010, 2011). On the other hand, almost nothing is known about the role of plasma membrane transporters in the yeast survival of desiccation. A
recent whole-genome study identified more than 100 genes whose absence increased the cell sensitivity to desiccation (Rodriguez-Porrata et al., 2012).

Potassium (K⁺) homeostasis inside the yeast cell is a complex process which is important for the survival of all organisms. Yeast cells usually spend a lot of energy to accumulate and maintain the high intracellular concentration of potassium that is required for many physiological processes [regulation of cell volume and intracellular pH, protein synthesis, enzyme activation, a constant level of membrane potential, response to osmotic shock and maintenance of low cytosolic concentrations of toxic cations such as sodium or lithium (Rodriguez-Navarro, 2000; Arino et al., 2010; Navarette et al., 2010; Zahradka & Sychrova, 2012)]. As potassium ions efficiently bind many molecules of water, potassium accumulated inside the cells contributes significantly to the cell size and turgor necessary for cell growth and division (Rodriguez-Navarro, 2000).

The plasma membrane of Saccharomyces cerevisiae possesses at least seven transport systems with different substrate specificities and diverse mechanisms to maintain optimal cytosolic K⁺ concentration (c. 200–300 mM). Five main potassium transporters have been extensively studied in S. cerevisiae cells (for a review see Arino et al., 2010), and recently two new low-affinity potassium uptake systems, Kch1 and Kch2, have been partly characterized (Stefan et al., 2013).

K⁺ uptake is mainly mediated by the plasma membrane Trk1 and Trk2 uniporters. K⁺ accumulation in the cytosol via these systems is driven by the electrochemical H⁺ gradient across the plasma membrane generated by H⁺-ATPase Pma1 (Serrano et al., 1986). Trk1 is the primary high-affinity K⁺ transport system (Kₘ c. 25 μM) (Rodriguez-Navarro & Ramos, 1984; Gaber et al., 1988). The activity of Trk1 has been described to be important for K⁺ and pH homeostasis (Madrid et al., 1998; Yenush et al., 2002), turgor (Merchan et al., 2004) and plasma membrane potential (Δψ) (Madrid et al., 1998; Mulet et al., 1999). Although the potassium uptake via Trk2 is much lower than via Trk1 in exponentially growing cells (Ramos et al., 1994), a recent study showed that Trk2 activity contributes significantly to the maintenance of membrane potential in growing cells (Petrezselyova et al., 2011).

To export surplus potassium, S. cerevisiae cells use three types of exporters. The potassium-specific channel Tok1 (Gustin et al., 1986) opens upon plasma-membrane depolarization (Bertl et al., 2003) and serves to fine tune plasma membrane potential (Bertl et al., 2003; Maresova et al., 2006) The other two K⁺ transporters have an active mechanism of potassium extrusion and have a broader substrate specificity, as they serve to export surplus potassium and eliminate toxic sodium and lithium cations from the cytosol. The Na⁺/K⁺ antiporter is a constitutively expressed housekeeping protein that uses the inward gradient of H⁺ (created by the Pma1 H⁺-ATPase) as a driving force to export alkali metal cations and whose activity plays a role in the maintenance of plasma-membrane potential and regulation of cell volume and internal pH (Sychrova et al., 1999; Kinclova-Zimmermannova et al., 2006; Arino et al., 2010). The third system exporting alkali metal cations, Na⁺(K⁺)-ATPase (Haro et al., 1991), is the main sodium and lithium detoxifying system in S. cerevisiae, but it also contributes significantly to high potassium tolerance (Banuelos et al., 1998).

To study the role of the five main S. cerevisiae potassium transporters in anhydrobiosis, we used a set of isogenic strains lacking one or more genes encoding the plasma-membrane K⁺ transporters in the BY4741 genetic background and studied the ability of mutant cells to survive desiccation and the subsequent rehydration processes. Our results revealed that whereas the functionality of potassium exporting systems is not important for surviving desiccation, it is the activity of potassium uptake systems, and mainly that of Trk2, which is crucial to successfully survive anhydrobiosis.

Materials and methods

Strains and growth conditions

The S. cerevisiae BY4741 strain (MATa his3Δ1 leu2Δ met15Δ ura3Δ; EUROSCARF) and its derivatives were used. Mutants lacking genes for potassium transporters were prepared by homologous recombination using the Cre-loxP system (Guldener et al., 1996) and their genotypes are listed in Table 1. To verify the phenotypes of single trk1Δ or trk2Δ mutants, two or three independently prepared mutants were used.

<table>
<thead>
<tr>
<th>Table 1. Saccharomyces cerevisiae strains used in this study</th>
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<td>Strain</td>
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</tr>
<tr>
<td>BY4741</td>
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<tr>
<td>BYT345</td>
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<td>BYT45</td>
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<tr>
<td>BYT12</td>
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<td>BYT11</td>
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<td>BYT2</td>
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Yeast strains were routinely grown in standard liquid YPD medium (1% extract, 2% peptone, 2% glucose) supplemented with 50 mM or 100 mM KCl in an orbital shaker at 160 r.p.m. min⁻¹ at 30 °C. Solid YPD media were supplemented with 2% agar. To follow the growth resumption of stationary cells, the growth rate of 100-μL cultures in a 96-well plate was followed in an absorbance microplate reader (BioTek Instruments, Winooski, VT); eight parallel cultures for one strain were run in each experiment, and the experiment was repeated three times.

**Dehydration and rehydration procedures**

Yeast cells were grown to the stationary phase (40–42 h) in YPD with 50 mM KCl, harvested, washed and dehydrated by convective drying at 30 °C for 15–16 h. Dehydrated biomass was rehydrated in distilled water or in 50 mM KCl for 10 min at room temperature. Cell survival was estimated using either the fluorochrome primulin and fluorescence microscopy (Rapoport & Meyssel, 1985) or after appropriate dilution of the rehydrated biomass, plating on solid YPD with 50 mM KCl and counting the colonies (CFU) after 2 days of growth at 30 °C. The experiments were repeated three times, all strains processed in parallels of three in each experiment. In Figs 1 and 2 and in Table 2, the viability of cells determined as CFU is shown.

**Estimation of internal K⁺ content**

The internal K⁺ content in cells from the stationary growth phase was estimated as described earlier (Kinclova, et al., 2001). Briefly, cells (three aliquots per strain) were collected on Millipore membrane filters (0.8 μm pore diameter) and quickly washed with 20 mM MgCl₂. The cells were then extracted with HCl and analyzed with a flame atomic absorption spectrophotometer. The experiments were repeated three times.

**Results and discussion**

To characterize the role of plasma membrane potassium transporters upon cell dehydration and subsequent rehydration, we first estimated the desiccation survival of cells lacking either the two main potassium uptake systems (BYT12, trk1Δ trk2Δ), the two active potassium efflux systems (BYT45, nha1A ena1-5Δ) or all three K⁺ exporters (BYT345, tok1A nha1A ena1-5Δ). The experimental conditions (cf. Materials and methods) were set to achieve c. 70% survival of the parental BY4741 strain, so that a better or worse survival rate of the mutants could be easily observed. All strains were grown in YPD supplemented with 50 mM KCl [to achieve a comparable growth of strains lacking the Trk transporters; (Navarette et al., 2010)] to the stationary phase of growth, as it has been repeatedly shown that exponentially growing cells
are, compared with stationary cells, much more sensitive to various types of stress, including anhydrobiotic stress (Beker & Rapoport, 1987).

Figure 1a shows that the absence of potassium exporting systems (BYT45 and BYT345 cells) did not significantly change the ability of cells to survive dehydration/rehydration treatment. About 65–70% of cells lacking potassium exporters were able to survive the desiccation and revitalization processes. On the other hand, the absence of potassium uptake systems (BYT12, trk1A trk2A) brought about a dramatic decrease in the survival rate. Only about 8% of cells were able to form colonies after dehydration/rehydration treatment. This result suggested the importance of potassium uptake for anhydrobiosis. To distinguish which of the two Trk transporters’ absence causes the observed phenotype, the same experiment was repeated with single mutants lacking either the Trk1 (BYT1) or Trk2 (BYT2) transporter. It was the absence of Trk2 that diminished the ability of cells to survive desiccation stress (Fig. 1b). Since the deletion of the TRK2 gene has almost no phenotype in exponential cells harboring an intact copy of TRK1 (Petrezselyova et al., 2011), we were aware of a risk of a non-specific mutation that could occur during the construction of the BYT2 mutant, e.g. upon electroporation. To be sure that the observed phenotype is related to the absence of the TRK2 gene and not to an additional non-specific mutation, we tested the survival of two independently prepared BYT1 (trk1A) and three BYT2 (trk2A) mutants (Fig. 2). Whereas about 55–65% of cells lacking TRK1 were able to survive in our experiment, all three trk2A mutants exhibited very low viability after the treatment, with only about 10% survival. This result showed unambiguously that the role of Trk2 in the cell survival of desiccation stress is much more important than that of the Trk1 transporter.

One of the reasons for the decreased viability could be the need for the active uptake of potassium during the rehydration process. As mentioned above, desiccation is accompanied by a substantial decrease in cell volume. Such a decrease in cell volume may be not only related to a loss of water but may be accompanied by a loss of ions to preserve sustainable intracellular osmotic conditions. After obtaining our initial results, we hypothesized that a substantial amount of intracellular potassium content may be lost during desiccation, and it is the Trk2 (and not Trk1) transporter that mediates the reuptake of required potassium during the rehydration procedure. To confirm this hypothesis, we followed the survival of cells that were first desiccated in the standard way described in Materials and methods, and then rehydrated in either water or 50 mM KCl. If the regeneration of internal potassium content during rehydration were crucial, the increased availability of potassium in the rehydration solution would enhance the survival of cells. As shown in Table 2, the presence of KCl during the rehydration of cells had no significant effect. The survival of wild-type BY4741 cells was almost the same under both sets of conditions; the survival of cells lacking potassium exporters (BYT345 and BYT45) was slightly decreased in the presence of KCl, probably due to the impaired ability of potassium flux and membrane potential regulation (Zahradka & Sychrova, 2012). The survival of BYT1 cells (trk1A) was not changed upon the addition of potassium, and the same was found for cells lacking either Trk2 alone (BYT2) or in combination with the trk1 mutation (BYT12, trk1A trk2A). These results showed clearly that the uptake or efflux of potassium by cells during the rehydration process is not crucial for their desiccation survival.

Another important role of Trk2 might be supplying potassium to stationary cells. Stationary cells need to have a basal level of continuous potassium influx and efflux to maintain their membrane potential. This role of Trk2 in stationary cells has not been studied in detail so far; the only hint may be the low level of expression of TRK1 in stationary cells (Gasch et al., 2000). To verify the possibility of the effect of the absence of TRK2 on stationary cells, we measured the potassium content in cells from the stationary phase of growth harvested for desiccation. As shown in Table 3, cells lacking the potassium exporter contained a significantly lower amount of potassium, which confirmed the presumption that Trk1 was not very active in the stationary cells. Measurements of $K^+$ content in cells lacking the active exporters Nha1 and Ena1 (BYT45) revealed a slightly higher potassium content compared with the parental BY4741 strain, and this higher content diminished upon the deletion of TOK1 (BYT345), which depolarizes the plasma membrane potential and thus slightly diminishes potassium uptake (Zahradka & Sychrova, 2012). In summary, the measurements of potassium content revealed a lower level of potassium in BYT2 (trk2A) and BYT12 (trk1A trk2A) stationary cells and confirmed the importance of Trk2 activity for the potassium homeostasis and desiccation survival of stationary cells.

### Table 3. Potassium content in cells grown in YPD supplemented with 50 mM KCl to the stationary phase

<table>
<thead>
<tr>
<th>Strain</th>
<th>$K^+_{\text{in}}$ (mg g$^{-1}$ dry weight)</th>
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<tbody>
<tr>
<td>BY4741</td>
<td>18.6 ± 1.0</td>
</tr>
<tr>
<td>BYT45</td>
<td>21.4 ± 1.8</td>
</tr>
<tr>
<td>BYT345</td>
<td>19.3 ± 1.7</td>
</tr>
<tr>
<td>BYT1</td>
<td>17.8 ± 4.2</td>
</tr>
<tr>
<td>BYT2</td>
<td>12.7 ± 3.1</td>
</tr>
<tr>
<td>BYT12</td>
<td>14.6 ± 1.45</td>
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</tbody>
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Another way of verifying the importance of Trk2 for stationary cells was by testing the growth resumption of stationary cells. Cells grown in YPD supplemented with 50 mM KCl for 40 h, were harvested, washed, resuspended in fresh YPD with KCl, and the growth of cultures followed in a microplate reader. In parallel, the CFU was estimated to know the amount of viable cells in the inoculum. The growth of parental strains BY4741 and the BYT1 strain lacking the Trk1 system was almost the same, but the strain lacking the Trk2 transporter had a significantly longer lag phase (about 3 h longer) than the other two strains (not shown) despite the number of viable cells in the inoculum being almost the same (c. 5% difference, not shown). This result suggests that a relatively quick growth resumption depends on the presence and activity of Trk2, and the prolonged lag phase of BYT2 (trk2Δ) cells might be due to the need to first synthesize/reactivate Trk1.

When we compared our results with those obtained from a whole-genome study (Rodriguez-Porrata et al., 2012) we found some differences. First, the study employing the EUROSCARF single null mutant collection in the BY4742 background, found, among other things, the nha1Δ mutant to be sensitive to desiccation. In our experiments, we did not see a significant difference between the parental strain BY4741 and the two strains lacking Nha1 and other potassium efflux systems (BYT45 and BYT345). This could be due to the different experimental conditions. The experimental conditions used for the whole-genome study were much more severe than our conditions (20% vs. 70% survival of the parental strains, respectively). The fact that the study with the mutant collection did not reveal the TRK2 gene to be important for desiccation survival might be due to the use of minimal YNB medium and no addition of extra KCl. When we used YNB media supplemented with KCl, we observed a poorer survival of YNB-grown cells in our conditions of dehydration/rehydration. Nevertheless, significant differences in desiccation survival, although lower than for YPD-grown cells, were observed between the strains; c. 18% of BY4741 cells and 6.5% of BYT2 (trk2Δ) cells survived.

In summary, our results show that the activity of the Trk2 potassium uptake system is important in stationary phase cells because it influences their potassium homeostasis, and high intracellular potassium content is a crucial factor in the dehydration/rehydration survival of stationary S. cerevisiae cells.

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K+ homeostasis influences yeast survival of desiccation


