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Role of *Saccharomyces cerevisiae* Trk1 in stabilization of intracellular potassium content upon changes in external potassium levels

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ABSTRACT

Saccharomyces cerevisiae cells are able to grow at very different potassium concentrations adapting its intracellular cation levels to changes in the external milieu. Potassium homeostasis in wild type cells resuspended in media with low potassium is an example of non-perfect adaptation since the same intracellular concentration is not approached irrespective of the extracellular levels of the cation. By using yeasts lacking the Trk1,2 system or expressing different versions of the mutated main plasma membrane potassium transporter (Trk1), we show that Trk1 is not essential for adaptation to potassium changes but the dynamics of potassium loss is very different in the wild type and in *trk1,2* mutant or in yeasts expressing Trk1 versions with highly impaired transport characteristics. We also show that the pattern here described can be also fulfilled by heterologous expression of *NcHAK1*, a potassium transporter not belonging to the TRK family. Hyperpolarization and cationic drugs sensitivity in mutants with defective transport capacity provide additional support to the hypothesis of connections between the activity of the Trk system and the plasma membrane H^+ ATPase (Pma1) in the adaptive process.

1. Introduction

Potassium is required by all living cells and it is essential for the proper function of many cellular processes that are critical for physiological parameters such as membrane potential, intracellular pH or cell volume. The model yeast *Saccharomyces cerevisiae* is able to grow in the presence of a broad range of external K^+-concentrations and the adaptation of intracellular cation levels to changes in the external milieu requires the coordinated regulation of at least 10 different alkali metal cation-specific transporters [1]. However, the exact mechanism of this homeostatic regulation of transport activity is still not sufficiently understood.

In particular, the activity of ScTrk1, the main plasma membrane potassium transporter, is tightly regulated. Both the Vmax and the affinity of the transporter increase strongly with decreasing potassium concentrations. In addition, mutants lacking the system show increased potassium requirements and impaired transport characteristics [2–4]. The existence of another Trk protein (Trk2) was later found out in *S. cerevisiae*, but it seems to play a minor role in potassium homeostasis [5,6]. Probably as a consequence of the defective potassium transport observed in the mutants, a low content of K^+ in *trk1,2* strains grown at low K^+ has also been reported [7]. In turn, differences between wild type and *trk1,2* strains in membrane potential and antibiotic resistance have been early documented [8,9].

TRK type transporters are present in fungi and plants and their general structure is based on four M1PM2 motifs being $M2_D$ helix unique with abundant positively charged amino acid residues, most probably forming part of the pore segment of the transporter and interacting with P segments [10–13].

The existence of elements sensing nutrients or regulating cell wall integrity and triggering signaling pathways has been proposed [14] although nothing has been reported about specific sensors of cations such as potassium or sodium and little is known about processes involved in the adaptation to changes in external potassium. By following a multidisciplinary approach, we have recently concluded that potassium homeostasis in wild type cells is an example of non-perfect adaptation, since the same intracellular concentration (output) is not approached irrespective of the extracellular levels (input). In addition we have shown that activation of Pma1 and bicarbonate systems is involved in the responses to potassium starvation [15]. However, the detailed sensing and signaling mechanism regulating potassium fluxes and intracellular stable potassium levels remains to be elucidated.

Haro and Rodríguez-Navarro reported in 2003 a mutational analysis of the $M2_D$ helix of the ScTrk1 transporter. They showed that single mutations affected the capacity of K^+ (Rb^+) transport in very different degrees, but the introduction of only one positively charged residue practically abolished the function of the transporter [13]. In this paper we have used *trk* mutants to study the adaptation to a decrease of the

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external potassium in yeast cells lacking the Trk system or expressing different versions of the mutated Trk1 protein. We conclude that, although Trk1 is not essential for adaptation to potassium changes, the dynamics of potassium loss is very different in the wild type and in *trk1,2* mutant or in yeasts expressing Trk1 versions with highly impaired transport characteristics.

2. Materials and methods

2.1. Strains and growth conditions

The *S. cerevisiae* wild type W303.1A (*Mata*, *ade2*, *ura3*, *leu2*, *his3*, *trp1*; EUROSCARF; Germany) and the isogenic double mutant WΔ6 (*trk1*Δ::*LEU2 trk2*Δ::*HIS3*), have been used in this work. Both strains were transformed with the empty plasmid" pFL38. Moreover, WΔ6 was transformed with pFL38-derivated plasmids carrying different constructions of *TRK1*: ScTRK1 (original Trk1 transporter), K1147N and M1153R [13]. In an additional experiment, WΔ6 was transformed with *NcHAK1* cloned into the pYPGE15 yeast expression vector [16]. Yeast cultures were routinely grown at 28 °C in YNB-Translucent K^+ -free medium with appropriate auxotrophic supplements (FORMEDIUM™), and the indicated amount of KCl (pH 5.8). Solid media were prepared by adding 2% (w/v) agar [17]. Potassium requirements and cationic drugs sensitivity were studied by analyzing growth on the same YNB-Translucent medium or YPD (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose) plates supplemented with several potassium concentrations or with the indicated amount of the drug. Cell $O.D._{600}$ was adjusted to 0.1 and plates were inoculated with serial 10-fold dilutions of these cultures and incubated at 28 °C during 48 h.

2.2. Determination of stationary internal concentrations and dynamics of potassium loss

To determine the stationary internal concentration of potassium, cells were grown in Translucent K^+ -free medium supplemented with the indicated amount of KCl during 18–36 h. When $O.D._{600}$ reached values of 0.2-0.4, cell samples were collected on Millipore filters, which were rapidly washed with 20 mM $MgCl_2$. The cells were then extracted with acid and potassium content analyzed by atomic emission spectrophotometry [18]. The experiments were repeated at least three times and the SDs calculated.

The time course of potassium loss was studied by resuspending 100 mM KCl grown cells in the same Translucent medium containing the indicated KCl concentrations [15]. Cell samples were collected at different times and potassium content was measured as described above. Experiments were repeated at least three times and the SDs calculated.

2.3. Cell volume determination

Cell volume was analyzed in a Cell Counter Z2 (Beckman-Coulter) and expressed in femtoliters (fL) [17]. The experiment was repeated at least three times, each time $6x10^4$ cells were analyzed for each strain and each condition.

2.4. Fluorescence measurement of membrane potential (diS-C_3(3) assay)

The membrane potential of yeast cells was estimated by fluorescence assay based on the redistribution of the fluorescence probe diS-C_3(3) (3,3′-dipropylthiacarbocyanine iodide; 0.1 mM stock solution in ethanol) [19,20]. Cells were inoculated in YNB-F medium containing 100 mM KCl, harvested during exponential phase of growth, washed twice with distilled water and resuspended in assay buffer (10 mM MES buffer, pH 6.0 adjusted by triethanolamine) to $O.D._{600}$ of 0.1 and the probe was added to a final concentration of 0.2 μM. Fluorescence emission spectra of the cell suspensions were measured every 4–6 min on ISS PC1 spectrofluorimeter equipped with a xenon lamp. Excitation wavelength was 531 nm, emission range 560–590 nm, duration of one spectral scan 20 s. Scattered light was eliminated by an emission filter with a cutoff wavelength at 540 nm. Samples were kept at room temperature and occasionally gently stirred. The staining

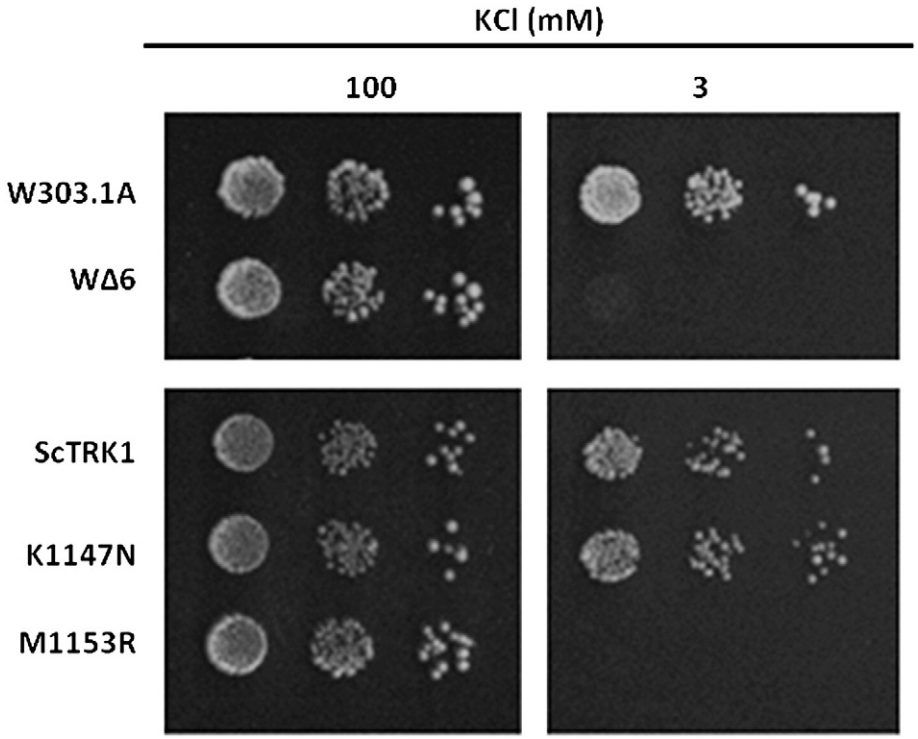

Fig. 1. Growth in low potassium depends on an efficient Trk1 version. Potassium requirements of wild type (W303.1A), double *trk1,2* mutant (WΔ6), and yeasts with different versions of Trk1 (see text) were pre-grown in YNB-F synthetic medium (pH 5.8) containing 100 mM KCl. Serial 10-fold dilutions were spotted on the same YNB-F medium plus KCl at the indicated concentrations. Cells were incubated at 28 °C for 2 days.

curves recorded the dependence of fluorescence emission maximum wavelength λ_{max} on the time of staining [21].

3. Results

To characterize the behavior of the different yeast, strains we performed growth tests in YNB-Translucent medium supplemented with several K^+ concentrations (Fig. 1). All strains grew similarly well in solid media and at non limiting K^+ (100 mM KCl). However, at low K^+ (3 mM KCl), impaired growth of the *trk1,2* mutant was observed. Expression of a wild type *TRK1* in the double *trk1,2* mutant improved the performance at limiting K^+ (ScTRK1). Yeasts transformed with the K1147N version of *TRK1* carrying a substitution of a lysine by an asparagine in the $M2_D$ helix had still a Vmax approximately half of the wild type's Vmax and were able to grow at low potassium. On the contrary, yeasts carrying version M1153R of the transporter (substitution of a methionine by an arginine) with almost no detectable high affinity transport behaved very similar to the double *trk* mutant and did not grow on plates with 3 mM KCl.

3.1. Role of the main plasma membrane potassium transporter in adaptation to changes in external potassium concentrations

We have recently published that intracellular potassium adjustment is an example of non-perfect adaptation: Wild type yeasts maintain a certain amount of internal potassium even at relatively low external concentrations. In addition, the internal potassium concentration of wild type cells depends at least partially on the external concentration. Cells resuspended from 50 mM potassium into media of lower external potassium had, after a transient period of potassium loss, a constant intracellular potassium concentration identical (within experimental error) to the concentration of cells grown overnight in these external concentration. These experiments showed, that after some relaxation time the intracellular potassium concentration attains a stationary value that is independent of the history of the cell and no hysteresis effects are observable. However, the adaptation to external potassium is not perfect in the sense that this steady state is influenced by the external concentration.

We wondered whether the lack of the main plasma membrane potassium transporters would affect that adaptive process. Therefore we grew our wild type and *trk1,2* mutant strains at non limiting KCl concentrations and resuspended them in media with several external potassium concentrations. Results in Fig. 2A and B show that wild type and double mutant were able to adapt to external changes, but, at low potassium, stationary internal concentrations were different in the two strains. Interestingly, the initial rate of change of internal K^+ (between 0 and 60 min) appears to be, in fact, higher for wild type than for *trk1,2* strains at 0,3 and 0,5 mM. This pattern seems to be consistent with the claim that enhanced hyperpolarization helps the cells to retain K^+. Supplementary Fig. 1 shows the time course of changes in OD_{600} (growth) after resuspension of wild type (A) and *trk1,2* (B) in media with lower potassium amounts. As expected, mutant cells resulted much more affected than wild type cells. We tested in our genetic background whether these stationary intracellular concentrations depend on the initial conditions or are determined solely by the external concentrations, we grew cells overnight in media with different external potassium concentrations (Fig. 2C). In both wild type and mutant strains the intracellular potassium concentration is limited to approx. 500 nmol/mg (497 ± 21 and 510 ± 28, respectively) when the external potassium concentration is high enough (100 mM). For low external potassium, the internal concentration is proportional to the external and, in the wild type, agrees with the stationary states of Fig. 2A. The decline of internal K^+-concentration was greater for *trk1,2* than for wild type cells long-term exposed to a low external K + -concentration (0.3-1 mM) (compare the time course in Fig. 2A and B for external concentrations between 0.3 and 1 mM KCl). In contrast to the wild type,

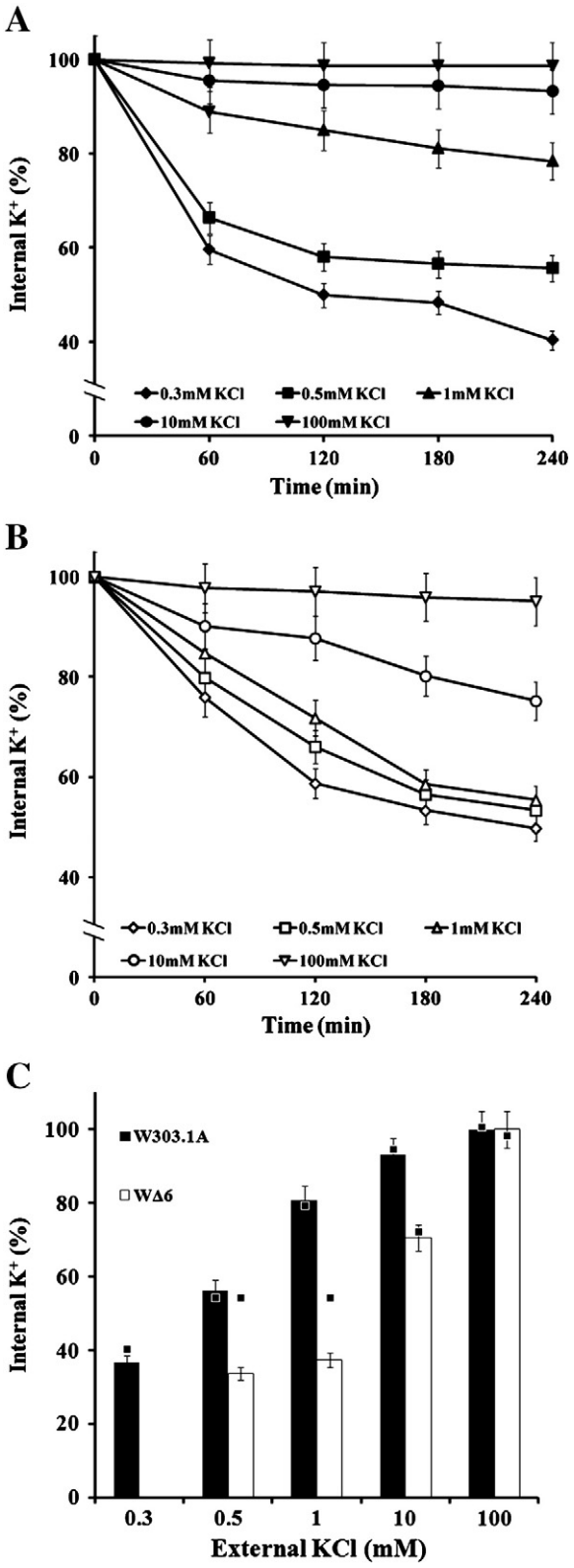

Fig. 2. Relationship of external and internal potassium in wild type and double *trk1,2* mutant. W303.1A (A) and WΔ6 (B) strains grown in 100 mM KCl were resuspended in several KCl concentrations and the time course of internal potassium was monitored. (C) Internal potassium concentration in wild type and double mutant strains grown overnight at different external potassium concentrations. The steady state concentrations from (A) and (B) are indicated as squares. At 0.3 mM KCl the *trk1,2* strain did not grow to allow internal potassium measurements. Experiments were repeated at least three times and the SDs calculated.

trk1,2 cells grown overnight at these limiting external concentrations (0.5-1 mM), contained a slight but significant lower amount of potassium suggesting impaired adaptation to reach the respective stable stationary states when grown at non limiting potassium and resuspended in low concentrations of the cation. As an example, wild type cells incubated 4 h or grown overnight in 1 mM KCl contained around 80% of the potassium in cells grown under non limiting conditions while *trk1,2* mutant cells contained around 55% when incubated 4 h in 1 mM KCl and only 37% when grown overnight under these conditions. At 0.3 mM KCl the double mutant did not grow at all and we did not get enough biomass for the experiment (Fig. 2C).

These results suggest an important role of the Trk1,2 system in the adaptation to low external potassium. Cells lacking the Trk1,2 system are able to adapt to low external potassium but the stationary intracellular concentrations depend on the initial conditions. This effect might be related to defective transport and/or lack of Trk1,2 dependent signaling activity. To gain insight into the possible relationship between the function of the Trk system in potassium transport and in potassium adjustment at different external potassium levels, we repeated the same kind of experiments using our double mutant transformed with plasmids carrying different versions of *TRK1* (Fig. 3). As expected from the results above, the different strains grown under non-limiting potassium contained similar amounts of the cation (519 ± 21, 496 ± 29 and 511 ± 20 nmol/mg in yeasts expressing wild type, K1147N or M1153R versions of Trk1 respectively). When resuspended in media with lower amounts of KCl, all yeasts were able to adapt their intracellular potassium levels to the external concentrations. However, the transport capacity and not the sole presence of Trk1 determines the stationary internal concentrations. The strains expressing wild type or K1147N version of Trk1 behaved similarly to the wild type and yeasts carrying version M1153R of the transporter behaved similarly to the double *trk* mutant (compare Figs. 2 and 3). Similarly to what was shown in Supplementary Fig. 1, Supplementary Fig. 2 shows the time course of changes in OD_{600} after resuspension of yeasts expressing ScTrk1 (A), K1147N (B) or M1153R (C) in media with lower potassium concentrations. Likewise to what was mentioned above for the wild type and the double mutant, cells expressing ScTrk1 or the K1147N version contained around 80% of the maximum potassium when incubated 4 h (Fig. 3A and B) or grown overnight in 1 mM KCl (Fig. 3D) and, on the other hand, cells expressing the M1153R version of Trk1 contained

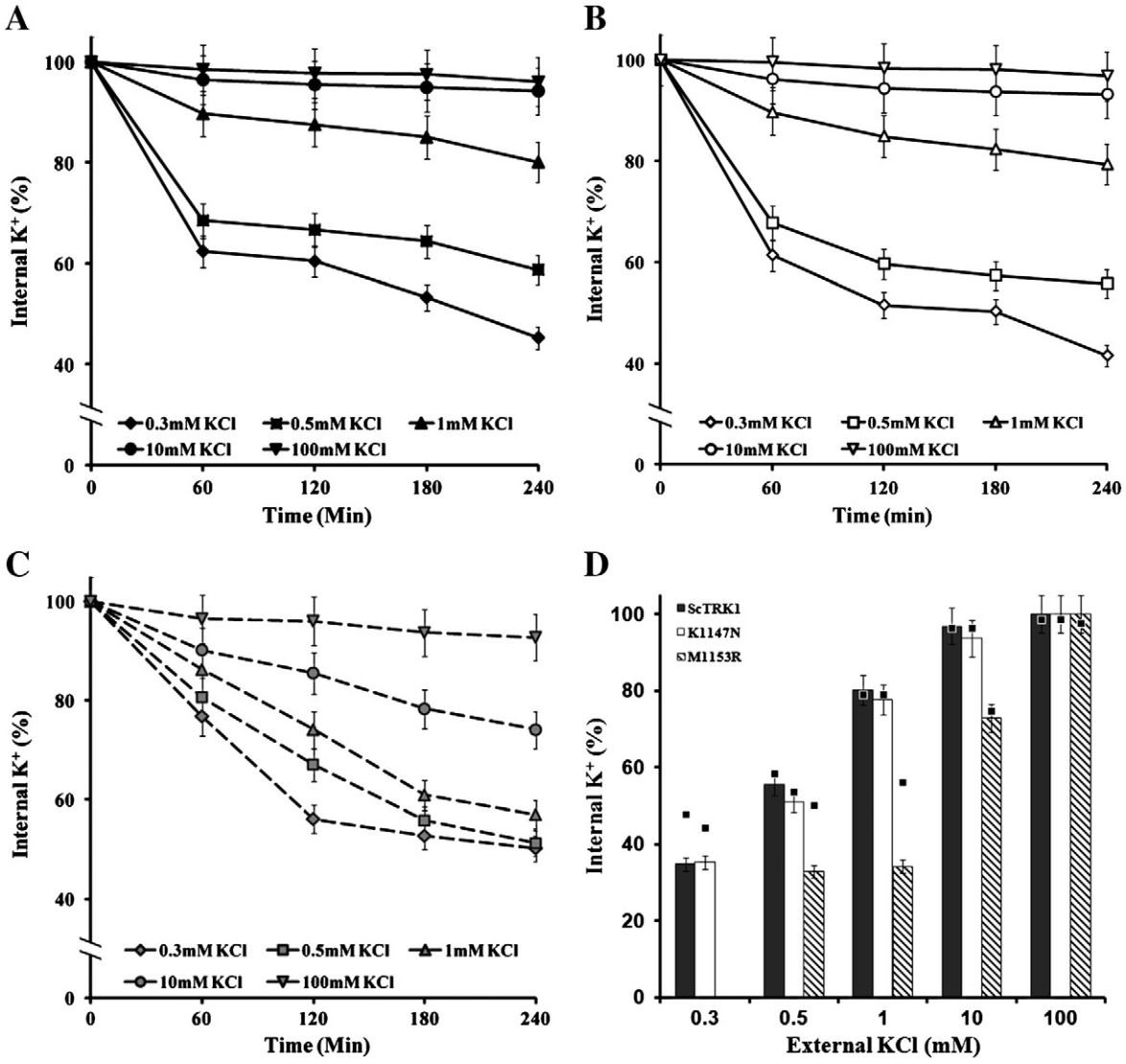

Fig. 3. Relationship of external and internal potassium in cells carrying different versions of Trk1. WΔ6 transformed with ScTRK1 (A), K1147N (B) or M1153R (C) was grown in 100 mM KCl, resuspended in several KCl concentrations and the time course of internal potassium was monitored. (D) Internal potassium concentration in yeasts carrying ScTRK1, K1147N or M1153R grown overnight at different external potassium concentrations. The steady state concentrations from (A), (B) and (C) are indicated as squares. At 0.3 mM KCl the cells carrying M1153R did not grow to allow internal potassium measurements. Experiments were repeated at least three times and the SDs calculated.

around 57% when incubated 4 h in 1 mM KCl (Fig. 3C) and 34% when grown under these conditions (Fig. 3D).

In order to elucidate if the pattern here reported is specific to Trk1 or could be fulfilled by any yeast or fungi K^+ transporter, the *trk1,2* strain expressing the "High Affinity K^+ transporter from *Neurospora crassa* (NcHak1) [22,23] was studied. Results are presented in Fig. 4. *trk1,2* strain expressing *NcHAK1* (Fig. 4A) or transformed with the "empty plasmid" (Fig. 4B). Cells were grown without potassium limitation and then resuspended in media containing lower KCl. The strain carrying the *Neurospora* transporter was able to adapt to decreasing external potassium levels in a very efficient mode which fits with the high affinity transport capacity provided by the heterologous system. However, also in this case, for low external potassium, the internal concentration was proportional to the external one (Fig. 4C) and, in that strain, agrees with the stationary states of Fig. 4A. In summary, these results provide additional account on the possibility that *TRK1* does not have specific sensor/signaling functions.

3.2. Membrane potential, cell volume and sensitivity to cationic drugs

We have previously published that *trk* mutants are hyperpolarized and that, probably as a consequence, an additional phenotype of these mutants is their sensitivity to some cationic drugs. Now, we determined the relative membrane potential, cell volume and the effect of Hygromycin B (Hyg B) and Tetramethylammonium (TMA) on growth in the *trk1,2* mutant carrying different versions of the Trk1 protein (Fig. 5). Relative membrane potential measurements confirmed that, in our genetic background, cells of the double *trk1,2* mutant grown at high potassium (100 mM KCl) show a hyperpolarized state when compared to the wild type. Moreover we found a direct correlation between the capacity to transport potassium and membrane potential. Strains WΔ6 and carrying M1153R were the most hyperpolarized while values measured in strains carrying ScTrk1 or K1147N were lower (Fig. 5A). In all strains, incubation in 0.3 mM KCl triggered a slight hyperpolarization when compared to 100 mM KCl (Fig. 5A). In an additional experiment, cells were grown in 100 mM KCl, washed, transferred to the assay buffer (that does not contain K^+), probe was added, relative membrane potential measured and, after 18 min, 100 or 0.3 mM KCl was added. Addition of 0.3 mM KCl did not change significantly the membrane potential, but 100 mM KCl caused depolarization in all cells, the biggest one in WΔ6 with empty vector (Fig. 5B). Under these growth (100 mM KCl) conditions all these strains had comparable cell volume (Fig. 5C). Drop tests in plates containing Hyg B or TMA showed a clear relationship between the capacity to transport potassium and tolerance to both drugs since the WΔ6 strain carrying a wild type Trk1 or the K1147N version of the transporter was similarly tolerant while the strain with the empty plasmid or expressing a Trk1 version very defective in transport capacity (M1153R), was clearly more sensitive to both drugs (Fig. 5D).

4. Discussion

In an attempt to understand the coordinated functioning of ion regulation in *S. cerevisiae*, an integrative mathematical model has been recently published [24]. The authors predicted the functioning of several transporters and pathways after stress perturbations but they found inconsistency between experimental data and the model prediction in the case of Trk because the model predicts that, for example, in the presence of Na^+, increases in Trk activity would increase intracellular Na^+/K^+ ratio which contradicts the known Trk role of discriminating against Na^+ uptake. This fact is explained by the authors on the basis of the over-simplified representation of the Trk system and can illustrate the complexity of potassium homeostasis. In addition, by using a novel inference method we recently showed that the main regulators under conditions of potassium starvation are proton fluxes responding to changes of potassium concentrations and that regulation of the main

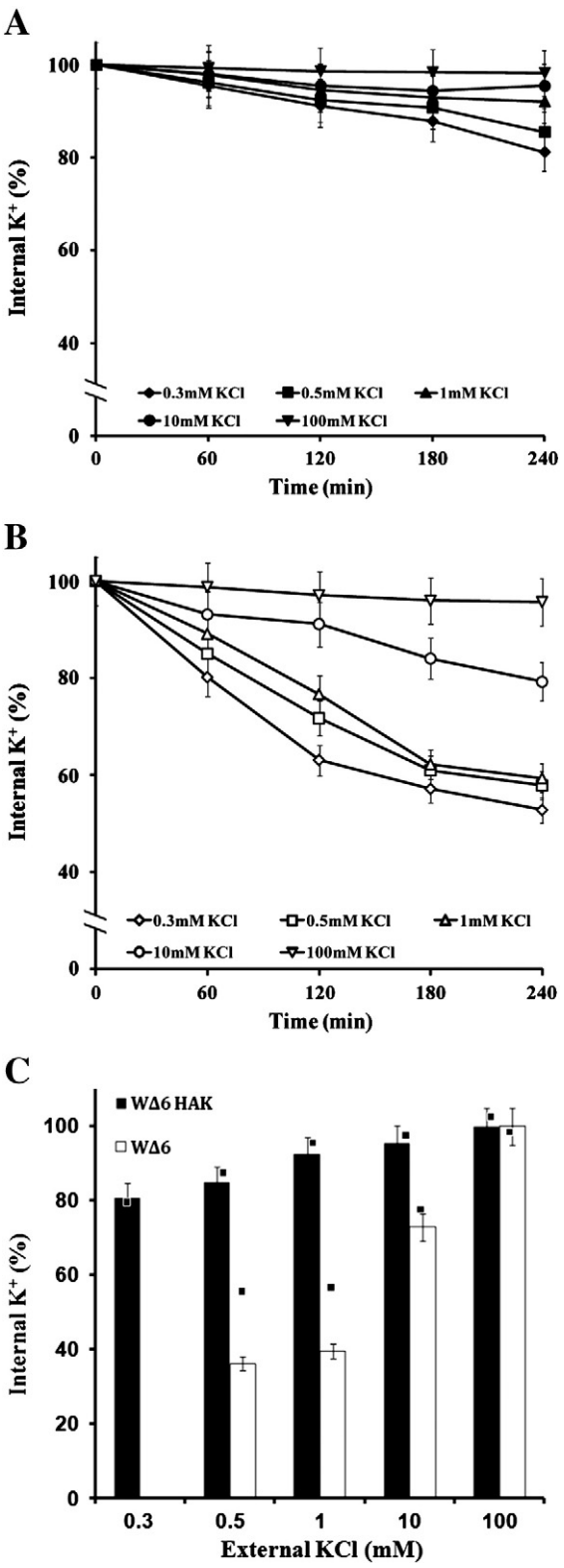

Fig. 4. Relationship of external and internal potassium in *trk1,2* cells expressing *NcHAK1*. WΔ6 transformed with pYPGE15 carrying *NcHAK1* (A) or with the "empty plasmid" (B) were grown in 100 mM KCl, resuspended in several KCl concentrations and the time course of internal potassium was monitored. (C) Internal potassium concentration in both strains grown overnight at different external potassium concentrations. The steady state concentrations from (A) and (B) are indicated as squares. At 0.3 mM KCl the cells carrying the "empty plasmid" did not grow to allow internal potassium measurements. Experiments were repeated at least three times and the SDs calculated.

potassium transport systems (Trk1,2 and Nha1) in the plasma membrane is not sufficient to achieve homeostasis [15]. We also reported that in wild type cells resuspended in media with different external potassium concentrations, the potassium efflux and the stationary internal concentrations are different for the different external KCl levels [15], therefore indicating that this process is not regulated by integral control which would mean a perfect adaptation, where the steady state input is independent of the steady state output [25].

Now we have extended our study to mutants in the main plasma membrane transport system. We first confirmed the mentioned results working with a different genetic background and found that both wild type and *trk1,2* mutants, lost internal potassium when they were grown in 100 mM KCl and then transferred to lower external concentrations. However, the dynamics of the potassium loss process seems to be clearly different in the two strains. In the wild type, the steady-state level reached was proportional to the external KCl amount while in the mutant the potassium loss process was not affected by low external potassium in a wide range of concentrations probably due to the impaired transport capacity (compare the process at concentrations between 0.3 and 1 mM) (Fig. 2A,B). It is relevant that when external potassium was high enough, the mutants were also able to adapt to the internal content to the external one. No adaptation to extracellular potassium changes would have meant that the mutant cells could not cope with a decrease in external potassium and eventually would stop growing or die. We conclude now that, similarly to the wild type, the *trk1,2* strain shows non-perfect adaptation, that is, the intracellular potassium concentration depends on the external one, but it is still larger than the minimum value required for cell survival.

Results from experiments using strains obtained by Haro and Rodríguez-Navarro [13] did not indicate the existence of specific and well differentiated functions (potassium transport and signaling/steady-state regulation) in different regions of the unique $M2_D$ helix of the Trk1 protein. As expected, when a double mutant was transformed with a plasmid carrying *TRK1*, it behaved as a wild type. Additionally, a mutation in a specific AA residue that very significantly affected transport capacity induced the dynamics of intracellular stable potassium very similar to the one in the double *trk1,2* mutant. On the other hand, when a mutation in the same motif of the protein slightly affected transport, the dynamics of potassium loss and stable intracellular potassium was again comparable to the one in the wild type (Fig. 3A,

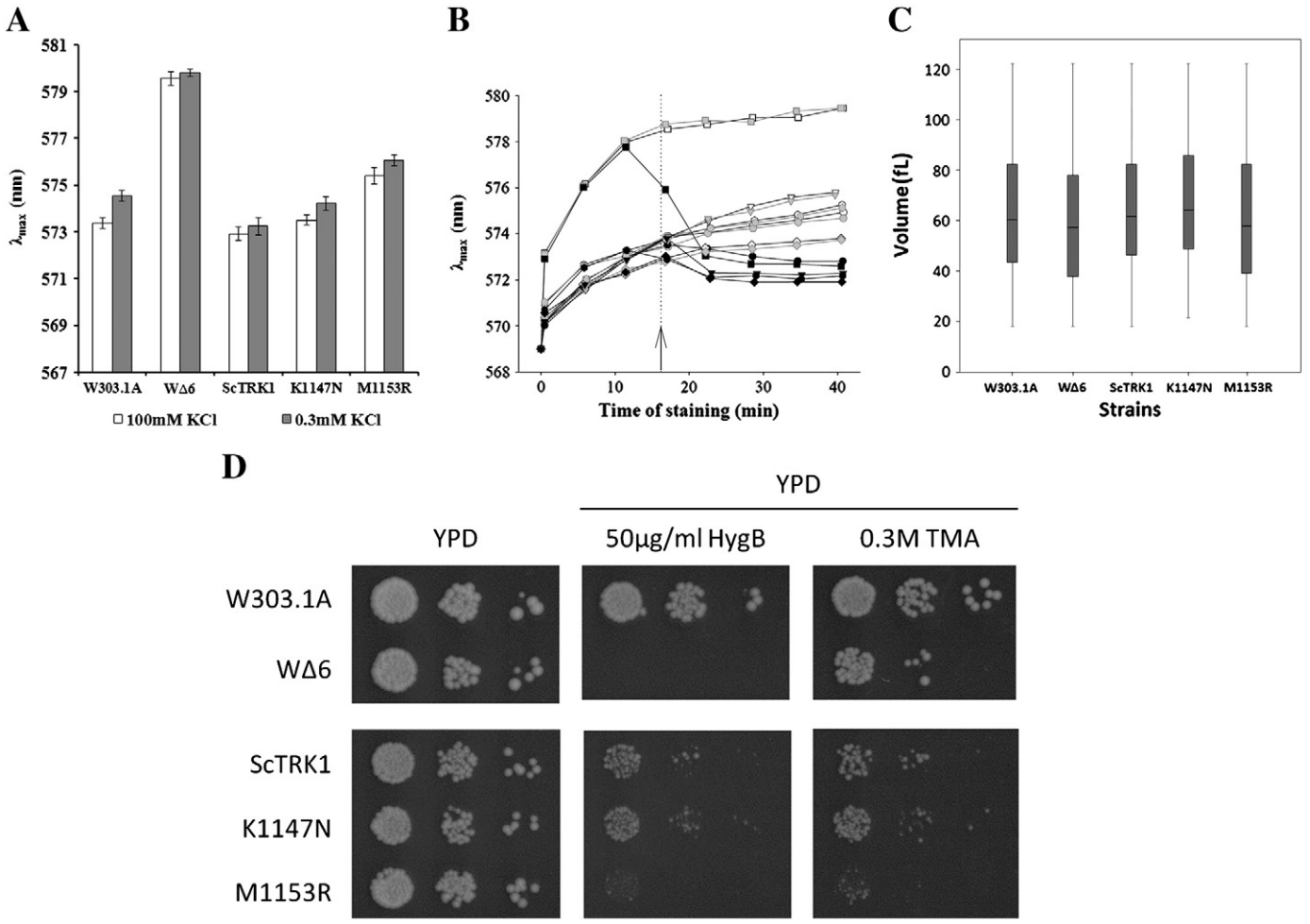

Fig. 5. Physiological characterization of wild type (W303.1A), double *trk1,2* mutant (WΔ6), and strains with different versions of Trk1. Comparison of relative plasma membrane potential (A and B), cell volume (C) and effect of cationic drugs on growth (D). Yeast cells were grown in YNB-F medium containing 100 mM KCl, washed, resuspended in fresh YNB-F medium supplemented with 100 mM KCl (white columns) or 0.3 mM KCl (gray columns) and incubated 10 min prior to the transfer to the assay buffer. Staining curves were fitted and the values of λ_{max} at 30 min of staining were plotted. Determinations were repeated at least three times and the SDs calculated (A). Response of the relative plasma membrane potential to addition of KCl. Cells were grown in YNB-F medium containing 100 mM KCl, washed twice with distilled water, resuspended in the assay buffer and 100 mM KCl (black symbols), 0.3 mM KCl (gray symbols) or no KCl (white symbols) was added to cell suspensions after 17 min of staining (indicated with arrow and dotted line). Staining curves of exponential cells W303.1A[pFL38] (circles), WΔ6[pFL38] (squares), WΔ6[ScTRK1] (diamonds), WΔ6[K1147N] (hexagons) and WΔ6[M1153R] (triangles). A representative assay of three independent experiments is shown (B). Yeast cells were grown in YNB-F synthetic medium (pH 5.8) containing 100 mM KCl to determine cell volume as described in text (C). The effect of Hygromycin B (Hyg B) and Tetramethylammonium (TMA) on growth in the *trk1,2* mutant carrying different versions of Trk1 protein was studied in YPD solid medium containing the indicated amounts of the drugs. Serial 10-fold dilutions were spotted on the plates and cells were incubated at 28 °C for 2 days (D). Cell volume experiments were repeated at least three times, each time $6x10^4$ cells were analyzed for each strain and each condition. Relative membrane potential determinations were repeated at least three times and the SDs calculated.

B,C and D). These results suggest, that there are no specific and well differentiated transport and signaling/steady-state functions in different regions of the Trk1 protein and that alterations in the signaling/steady-state function are only an indirect consequence of impaired transport capacity. In consequence, although very relevant for internal K^+ adjustment, Trk1 does not have additional specific sensor/signaling functions. This idea was reinforced by the fact that NcHak1 a potassium transporter not belonging to the TRK family also reproduced the pattern here reported although with different quantitative characteristics that are in agreement with the high affinity transport constants reported for this transporter [22,23]. However, we cannot rule out that the regulation of the activity of Hak1 during the adaptation to a new external K^+-concentration may occur at different levels and may play an additional role [23].

Present results on relative membrane potential measurements and cationic drugs sensitivity, together with results in Kahm et al.'s study [15] fit with the idea that the activity of the Trk system is connected to Pma1. A logical picture to explain the situation would be the following: when grown in high potassium and resuspended in low levels (0.3–0.5 mM), potassium flux is first outward and then, after some time, there is a reuptake. As mentioned above, the initial rate of change of internal K^+ (between 0 and 60 min) was higher for wild type than for strains lacking the Trk system at 0.3 and 0.5 mM (Fig. 2A,B). This behavior seems to be consistent with the claim that enhanced hyperpolarization helps the cells to retain K^+. Once K^+ reuptake becomes an important component, the rate of decline of internal K^+ becomes lower for wild type than for the mutant because this reuptake is not so effective in *trk1,2* mutants. In Kahm et al.'s study [15], it was shown for the wild type and for the *trk1,2* double mutant, that the Pma1 ATPase increases its activity after resuspension. For the wild type, it was also shown, that the gene expression of carbonic anhydrase increases. It is likely that these increased proton fluxes will hyperpolarize the membrane. As an immediate consequence, the increased Vm limits the loss of potassium and moreover the increased Vm generates the thermodynamic force for a reuptake of potassium via Trk1,2. If this is absent or nonfunctional, the Pma1-effect is still there, but the free energy stored in the membrane potential cannot be used for the reuptake, resulting in a hyperpolarized membrane state and higher sensitivity to cationic drugs.

Currently, the exact mechanism for the increased proton fluxes underlying hyperpolarization is unknown, but it has been shown that Pma1 can be regulated by kinase-mediated phosphorylation [26] and the gene *NCE103* coding for carbonic anhydrase is transcriptionally regulated [27].

In short, we propose that phenotypes associated to mutants lacking the Trk potassium transport system such as hyperpolarization, sensitivity to cationic drugs or alterations in the signaling/steady-state function are directly linked to the transport capacity of the cell and can be explained as just a consequence of the defective potassium transport process.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2013.08.022.

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References

[1] J. Ariño, J. Ramos, H. Sychrová, Alkali metal cation transport and homeostasis in yeasts, Microbiol. Mol. Biol. Rev. 74 (2010) 95–120.
[2] A. Rodríguez-Navarro, J. Ramos, Dual system for potassium transport in *Saccharomyces cerevisiae*, J. Bacteriol. 159 (1984) 940–945.
[3] J. Ramos, P. Contreras, A. Rodríguez-Navarro, A potassium transport mutant of *Saccharomyces cerevisiae*, Arch. Microbiol. 143 (1985) 88–93.
[4] R.F. Gaber, C.A. Styles, G.R. Fink, TRK1 encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 8 (1988) 2848–2859.
[5] C.H. Ko, R.F. Gaber, TRK1 and TRK2 encode structurally related K+ transporters in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 11 (1991) 4266–4273.
[6] J. Ramos, R. Alijo, R. Haro, A. Rodríguez-Navarro, TRK2 is not a low-affinity potassium transporter in *Saccharomyces cerevisiae*, J. Bacteriol. 176 (1994) 249–252.
[7] D.B. Lauff, G.E. Santa-María, Potassium deprivation is sufficient to induce a cell death in *Saccharomyces cerevisiae*, FEMS Yeast Res. 19 (2010) 497–507.
[8] R. Madrid, M.J. Gómez, J. Ramos, A. Rodríguez-Navarro, Ectopic potassium uptake in trk1 trk2 mutants of *Saccharomyces cerevisiae* correlates with a highly hyperpolarized membrane potential, J. Biol. Chem. 273 (1998) 14838–14844.
[9] J.M. Mulet, M.P. Leube, S.J. Kron, G. Rios, G.R. Fink, R. Serrano, A novel mechanism of ion homeostasis and salt tolerance in yeast: the Hal4 and Hal5 protein kinases modulate Trk1-Trk2 potassium transporter, Mol. Cell. Biol. 19 (1999) 3328–3337.
[10] S.R. Durell, Y. Hao, T. Nakamura, E.P. Bakker, H.R. Gay, Evolutionary relationship between K(+) channels and symporters, Biophys. J. 77 (1999) 775–788.
[11] S.R. Durell, H.R. Guy, Structural models of the KtrB, and Trk1,2 symporters based of the structure of the KcsA K(+) channel, Biophys. J. 77 (1999) 789–807.
[12] R. Haro, A. Rodríguez-Navarro, Molecular analysis of the mechanism of potassium uptake through the TRK1 transporter of *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1564 (2002) 114–122.
[13] R. Haro, A. Rodríguez-Navarro, Functional analysis of the MD(2) helix of the TRK1 potassium transporter of *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1613 (2003) 1–6.
[14] J.M. Thevelein, K. Voordeckers, Functioning and evolutionary significance of nutrients transceptors, Mol. Biol. Evol. 26 (2009) 2407–2414.
[15] M. Kahm, C. Navarrete, V. Llopis-Torregrosa, R. Herrera, L. Barreto, L. Yenush, J. Ariño, J. Ramos, M. Kschischo, Potassium starvation in yeast: mechanisms of homeostasis revealed by mathematical modeling, PLoS Comput. Biol. 8 (2012) 1–11.
[16] J.P. Brunelli, M.L. Pall, A series of yeast/*Escherichia coli* lambda expression vectors designed for directional cloning of cDNAs and cre/lox-mediated plasmid excision, Yeast 12 (1993) 1309–1318.
[17] C. Navarrete, S. Petrezsélyova, L. Barreto, J.L. Martínez, J. Zahrádka, J. Ariño, H. Sychrová, J. Ramos, Lack of main K^+ in *Saccharomyces cerevisiae* cells affects yeast performance in both potassium-sufficient and potassium-limiting conditions, FEMS Yeast Res. 10 (2010) 508–517.
[18] J. Ramos, R. Haro, A. Rodríguez-Navarro, Regulation of potassium fluxes in *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1029 (1990) 211–217.
[19] L. Marešová, E. Urbánková, D. Gášková, H. Sychrová, Measurement of plasma membrane potential changes in *Saccharomyces cerevisiae* cells reveal the importance of the Tok1 channel in membrane potential maintenance, FEMS Yeast Res. 6 (2006) 1039–1046.
[20] S. Petrezsélyova, J. Zahrádka, H. Sychrová, *Saccharomyces cerevisiae* BY4741 and W303-1A laboratory strains differ in salt tolerance, Fungal Biol. 114 (2010) 144–150.
[21] D. Gášková, B. Brodská, P. Heřman, J. Večeř, J. Malínský, K. Sigler, O. Benada, J. Plášek, Fluorescent probing of membrane potential in walled cells: diS-C_3(3) assay in *Saccharomyces cerevisiae*, Yeast 14 (1998) 1189–1197.
[22] R. Haro, L. Sainz, F. Rubio, A. Rodríguez-Navarro, Cloning of two genes encoding potassium transporters in *Neurospora crassa* and expression of the corresponding cDNAs in *Saccharomyces cerevisiae*, Mol. Microbiol. 31 (1999) 511–520.
[23] A. Rivetta, K.E. Allen, C.W. Slayman, C.L. Slayman, Coordination of K+ transporters in *Neurospora*: TRK1 is scarce and constitutive, while HAK1 is abundant and highly regulated, Eukaryot Cell 12 (2013) 684–696.
[24] R. Ke, P.J. Ingram, K. Haynes, An integrative model of ion regulation in yeast, PLoS Comput. Biol. 9 (2013) 1–14.
[25] T.M. Yi, Y. Huang, M.I. Simon, J. Doyle, Robust perfect adaptation in bacterial chemotaxis through integral feedback control, Proc. Natl. Acad. Sci. 97 (2000) 4649–4653.
[26] S. Lecchi, C.J. Nelson, K.E. Allen, D.L. Swaney, K.L. Thompson, J.J. Coon, M.R. Sussman, C.W. Slayman, Tandem phosphorylation of Ser-911 and Thr-912 at the C terminus of yeast plasma membrane H+-ATPase leads to glucose-dependent activation, J. Biol. Chem. 282 (2007) 35471–35481.
[27] G. Amoroso, L. Morell-Avrahov, D. Müller, K. Klug, D. Sültemeyer, The gene NCE103 (YNL036w) from *Saccharomyces cerevisiae* encodes a functional carbonic anhydrase and its transcription is regulated by the concentration of inorganic carbon in the medium, Mol. Microbiol. 56 (2005) 549–558.