NOTES

TRK2 Is Not a Low-Affinity Potassium Transporter in *Saccharomyces cerevisiae*

JOSE RAMOS,1 RAFAEL ALIO,1 ROSARIO HARO,2 AND ALONSO RODRIGUEZ-NAVARRO2*

Departamento de Microbiologia, Escuela Técnica Superior de Ingenieros Agrónomos, 14071 Córdoba,1 and Departamento de Microbiologia, Escuela Técnica Superior de Ingenieros Agrónomos, 28040 Madrid,2 Spain

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TRK1 and TRK2 encode proteins involved in K+ uptake in *Saccharomyces cerevisiae*. A kinetic study of Rb+ influx in trk1 and trk2 mutants reveals that TRK2 shows moderate affinity for Rb+. K+ starved trk1trk2 cells show a low-affinity component accounting for almost the total $V_{max}$ of the influx and a moderate-affinity component exhibiting a very low $V_{max}$. Overexpression of TRK2 in trk1Δ TRK2 cells increases the $V_{max}$ of the moderate-affinity component, and this component disappears in trk1Δ trk2Δ cells. In contrast, the low-affinity component of Rb+ influx in trk1Δ TRK2 cells is not affected by mutations in TRK2.

Consistent with the different levels of activity of the moderate-affinity Rb+ influx, trk1Δ TRK2 cells grow slowly in micromolar K+, trk1Δ trk2Δ cells grow rapidly, and trk1Δ trk2Δ cells fail to grow. The existence of a unique K+ uptake system composed of several proteins is also discussed.

K+ uptake in fungi and plants is mediated by transport systems different from those mediating equivalent processes in animal cells or in prokaryotic organisms. But despite the work devoted to these systems, a comprehensive model of K+ transport in cell-walled eukaryotic cells is still elusive, with various models proposed. In part, this reflects the diversity of the organisms studied, but it is also due to the lack of basic genetic studies. However, the molecular genetics of K+ transport in *Saccharomyces cerevisiae* has developed rapidly during the last years (5).

*S. cerevisiae* cells can grow in media with K+ concentrations extending from 2 to 4 μM K+ (11, 17) to 2.1 M K+ (16). Growth rates and the K+ contents are almost constant in this wide range of K+ concentrations (11, 14, 17), and consequently, the K+ uptake system must adapt to provide almost constant net K+ uptake. Furthermore, the system also provides rapid K+ replenishment in K+-depleted cells (14). To meet these requirements, the $K_{m}$ of K+ influx can take any value from approximately 15 μM K+ in K+ starved cells to 5 mM in cells growing at low-millimolar K+ concentrations (11, 14, 17), and it may reach values exceeding 50 mM K+ in cells grown at 0.5 M K+ (15). This broad capacity of adaptation is affected by mutations in two genes, *TRK1* and *TRK2*. *TRK1* is clearly involved in high-affinity K+ uptake because *trk1*Δ cells do not grow at micromolar K+ concentrations and do not exhibit K+ influx $K_{m}$s in the micromolar range (6, 11). In *trk1*Δ cells, disruption of *TRK2* increases notably the K+ requirement, and its overexpression restores a wild-type K+ requirement (20–22). In contrast, disruption of *TRK2* does not have an appreciable effect on *TRK1* cells. To account for these observations, it has been proposed that *TRK2* may encode a low-affinity K+ transporter, dispensable when *TRK1* is functional (8, 9). Both *TRK1* and *TRK2* are putative membrane proteins (55% identical), but their exact function has not been demonstrated and cannot be deduced from their putative amino acid sequences (6, 9).

On the basis of the K+ requirements and the K+ and Na+ uptake kinetics of the K+ uptake mutants, two hypotheses have been proposed to explain the organization of the K+ uptake system of *S. cerevisiae*: (i) a complex system with different binding sites for K+ or Rb+ and for Na+ (11) and (ii) two independent transporters, a high-affinity system encoded by *TRK1* and a low-affinity system encoded by *TRK2* (5, 8, 9).

Here we report a kinetic study in different mutants of the K+ uptake system. The results indicate that the product of *TRK2* does not mediate low-affinity K+ uptake and suggest that *TRK1* and *TRK2* are two components of a complex system composed of several proteins.

*S. cerevisiae* DBY746 (*MATa ura3 leu2 his3 trpl1*) was used as the wild type. Strain RH2.2, containing a deletion within the *TRK1* gene in DBY746, was constructed by the one-step gene disruption method (18). Plasmid pRH22 containing the *TRK1* gene was isolated from a yeast genomic library constructed in the shuttle vector YCP50. The bacterial clone harboring this plasmid was identified by in situ hybridization with a 23-mer oligonucleotide reproducing the sequence of a DNA fragment of *TRK1* (starting at position 1000 of the sequence published in reference 6). The 2.3-kb *XbaI* fragment in pRH22 was removed and replaced by the 3.0-kb *BglII* fragment of plasmid YEp13 containing the *LEU2* gene, and then the 4.2-kb *SacI* fragment of the new plasmid was used for transforming DBY746. Disruption of *TRK1* in several *Leu*+ transformants was confirmed by Southern blot analysis. Strains R1155 (*MATa ura3 lys9 his4 trk1Δ*), M469 (*MATa ura3 leu2 his3 trpl1 trk1Δ trk2::pCK64*), and A105 (*MATa ura3 his4 lys9 trk1Δ TRK2Δ*), with different levels of expression of *TRK2* (6, 9, 20), were also used. Standard protocols (19) and manufacturers’ recommendations were followed for routine DNA manipulations. Ammonium phosphate medium, free of K+ and Na+, and arginine phosphate medium, free of ammonium, K+, and Na+, were prepared as described previously (17). These media contained approximately 2 μM K+ and were supplemented
with KCl to obtain the required K⁺ concentration. Cells were normally grown in the medium with arginine because ammonium greatly increases the K⁺ requirement (11, 12), with 3 mM K⁺ for TRK1 strains and with 30 mM K⁺ for trklΔ strains. These K⁺ concentrations saturated the growth rate response of the strains to K⁺, and the cells in these media (normal-K⁺ cells) contained approximately 500 nmol of K⁺ mg⁻¹. K⁺-starved cells, containing 250 nmol of K⁺ mg⁻¹ and a maximal capacity for K⁺ uptake, were prepared by incubating normal-K⁺ cells in the medium with arginine and without K⁺ for 5 h (14). Azide-treated cells with different K⁺ contents were prepared as described previously (14). Dry weights are used in all references to weights of cells.

Uptake experiments were carried out in 10 mM morpholineethanesulfonic acid brought to pH 6.0 with Ca(OH)₂, containing 0.1 mM MgCl₂ and 2% glucose (incubation buffer). The methods for the cell analyses have been described previously (14, 17). Briefly, normal-K⁺ cells or K⁺-starved cells were harvested by centrifugation, washed with water, and suspended in incubation buffer. Rb⁺ or Li⁺ was added to the suspension of cells at time zero, samples were removed by filtration at intervals, and the cation contents were determined in acid extracts by atomic absorption or by atomic emission spectrophotometry. The initial rates of uptake were determined from the time courses of the cellular cation contents. Low rates were constant for long periods of time (17), but sampling time was kept as short as possible and was never longer than 15 min. Technical reasons made it difficult to carry out experiments at Rb⁺ concentrations exceeding 100 mM, and Kₚ,s in the 50 to 100 mM Rb⁺ range calculated in different experiments showed appreciable variability. Reported figures are from representative experiments, and reported data are means from at least five experiments. The standard errors of these means were lower than 20% of the corresponding mean. Kₚ,s and Vₘₐₓ of two-component kinetics were calculated by a computer-assisted nonlinear regression program, assuming that the actual rate resulted from the addition of two Michaelis-Menten equations. The legitimate use of Rb⁺ as a K⁺ analog has been demonstrated (17).

Growth rate constants were calculated from the semilogarithmic plots of the absorbance of cultures versus time. Tubes were inoculated with less than 10⁶ cells ml⁻¹, starting the recording of the absorbance when the culture reached approximately 0.04 absorbance units.

Rb⁺ influx kinetics in trklΔ cells. trkl1 mutants grow well at high K⁺, although both growth rates and K⁺ uptake are considerably lower than in the wild-type, when the external K⁺ is low. This finding suggests that these mutants have only a low-affinity K⁺ transporter (6, 11). However, careful study of the reported data shows that the reduction of the K⁺ uptake capacity is, surprisingly, more severe than the reduction in the ability to grow at low K⁺. A mutant in which K⁺-starved cells exhibited a K⁺ influx Kₚ,m of 11 mM and Vₘₐₓ of 11 nmol mg⁻¹ min⁻¹ grew in 100 μM K⁺ with a growth rate constant of 0.05 h⁻¹ (11). Even if the cells growing at 100 μM K⁺ were K⁺-starved, with minimal K⁺ content and maximum K⁺ uptake capacity, they would need a net K⁺ uptake of 14 nmol mg⁻¹ h⁻¹ to keep a K⁺ content of 280 nmol mg⁻¹ (11), but they contained only 65 nmol mg⁻¹ if the kinetic data are correct. New experiments with this trkl1 mutant revealed that a minor component (Vₘₐₓ of 0.6 nmol of K⁺ mg⁻¹ min⁻¹) of apparent high affinity was still present in K⁺-starved cells. This finding suggested that either the mutant retained a low TRK1 activity or K⁺ uptake in trkl1 mutants could not be explained simply by a low-affinity system.

To exclude the possibility of a residual function of TRK1, we constructed a mutant strain (RH2.2) carrying a trkl1Δ null allele and studied the K⁺ requirement and the Rb⁺ influx kinetics of the mutant. Like the trkl1Δ mutant previously reported (11), RH2.2 required a higher K⁺ concentration than the wild type for maximum growth rate, still grew in low K⁺ (growth rate constant of 0.99 h⁻¹ at 50 μM K⁺), and showed complex kinetics of Rb⁺ influx: in normal-K⁺ cells, Rb⁺ influx exhibited a typical Michaelis-Menten kinetics of low affinity (Kₚ,m of 65 mM and Vₘₐₓ of 16 nmol mg⁻¹ min⁻¹), but in K⁺-starved cells, it exhibited two kinetic components, a low-affinity component of the same Kₚ,m and Vₘₐₓ as in normal-K⁺ cells and a minor component of moderate affinity (Kₚ,m of 0.4 mM and Vₘₐₓ of 1.2 nmol mg⁻¹ min⁻¹), compared with the Kₚ,s of the high-affinity (80 μM) and low-affinity (6 mM) modes of Rb⁺ uptake in wild-type cells (11, 14, 17). The existence of the moderate-affinity component could explain the growth of the trklΔ strain at low K⁺ concentrations (Rb⁺ uptake was strongly inhibited by K⁺, but an exact calculation of the Vₘₐₓ was technically impossible because of the low Vₘₐₓ of the system).

TRK2 exhibits moderate affinity for K⁺. Disruption of TRK2 increases the K⁺ requirement of trklΔ cells (8, 9). This finding indicated that either (i) TRK2 somehow governs both the low- and moderate-affinity components of Rb⁺ influx in trklΔ cells or (ii) TRK2 governs only a low-rate system of moderate affinity for Rb⁺ and there is a separate major system of high rate and low affinity. To test these two possibilities, the Rb⁺ influx kinetics were compared in trklΔ trk2ΔA, trklΔ trk2ΔB, and trklΔ trk2ΔB strains (M469, R1155, and A105; null, normal, and overexpressed TRK2, respectively) [8, 9, 20]. In normal-K⁺ cells of the three strains, Rb⁺ influx followed a Michaelis-Menten kinetics of low affinity (Kₚ,m of 65 mM and Vₘₐₓ of 8.5 nmol mg⁻¹ min⁻¹), and an identical kinetics (i.e., no moderate-affinity component) was observed in K⁺-starved cells of the trklΔ trk2ΔA double mutant. In contrast, in K⁺-starved cells of the other two strains, trklΔ TRK2 and trklΔ TRK2B, Rb⁺ influx had two kinetic components, one of low affinity, with Kₚ,m of 65 mM as in normal-K⁺ cells, and a moderate-affinity component with Kₚ,m of 0.3 mM. This component had Vₘₐₓ of 0.7 nmol mg⁻¹ min⁻¹ in the TRK2 strain and 7 nmol mg⁻¹ min⁻¹ in the TRK2B strain (Fig. 1). Thus, the moderate-affinity influx of Rb⁺ varied with the expected

![Figure](http://jb.asm.org/fig1.jpg)
FIG. 2. Edie-Hofstee plots of the initial rates of Rb uptake in tryk1Δ tryk2Δ cells (strain A105) treated with 2 mM azide for 5 min (●) or with 10 mM azide for 15 min (■). The K^+ contents were 350 and 250 nmol mg^{-1}, respectively. Experimental data can be explained by the addition of two independent processes following Michaelis-Menten equations.

level of the tryk2Δ product, indicating that tryk2Δ was required for moderate-affinity but not for low-affinity Rb uptake.

In cells of the tryk1Δ tryk2Δ^0 strain growing at 30 mM (high) K^+, the moderate-affinity component of Rb^+ uptake was not detected, although this component presented a high V_{max} in K^+-starved cells. This finding suggested the possibility that the activity of tryk2Δ might be regulated, e.g., by the internal K^+ and by the internal pH, like tryk1Δ (13). To test this possibility, we measured Rb^+ influx in normal-K^+ treated cells with azide to produce a rapid decrease of the K^+ content (14). This treatment triggered the appearance of the moderate-affinity Rb^+ influx, whose kinetic characteristics depended on the resulting K^+ content (Fig. 2): cells initially containing 500 nmol of K^+ mg^{-1} and no moderate-affinity component of Rb^+ uptake contained 350 nmol of K^+ mg^{-1} after a 5-min treatment with 2 mM azide and showed a moderate-affinity component of Rb^+ influx of K_{m} of 1.25 mM and V_{max} of 5.5 nmol mg^{-1} min^{-1}, and cells treated for 15 min with 10 mM azide, containing 250 nmol of K^+ mg^{-1}, showed a K_{m} of 0.76 mM and a V_{max} of 7.0 nmol mg^{-1} min^{-1}. Although the latter K^+ content is at the lower limit attainable either by azide treatment or K^+ starvation (13, 14), the Rb^+ influx K_{m} of azide-treated cells was significantly higher than that found in K^+-starved cells (compare Fig. 1C and 2). An analogous difference in K_{m} for the two types of treatment is also known for tryk1Δ-mediated Rb^+ influx (14), suggesting that the regulation of tryk1Δ and that of tryk2Δ are similar. However, for tryk1Δ, Rb^+ uptake is activated by decreasing the internal pH with permeant acids (13, 17), and no such activation occurred in tryk1Δ tryk2Δ^0 cells (experiments like those presented in Fig. 2 performed in the presence of 10 mM butyric acid at pH 5.0).

The notable V_{max} of the tryk2Δ system in K^+-starved tryk2Δ^0 cells enabled the calculation of the K^+ K_{0} of this system. Experiments as in Fig. 1C but also including different added external K^+ concentrations showed that K^+ inhibited Rb^+ uptake competitively, with a K_{0} of 35 μM (the K_{0} of K^+ in the low-affinity system could not be calculated analogously because experiments of Rb^+ uptake were not performed at concentrations higher than 100 mM Rb^+). Remarkably, in K^+-starved cells, the ratio between the K_{m} of Rb^+ and the K_{0} of K^+ in tryk2Δ was not significantly different from that found in tryk1Δ (17), i.e., 8.6 and 6.6, respectively.

There are several examples of repeated genes encoding transport systems in S. cerevisiae yeast (7, 10), and tryk2Δ seems to be a copy of tryk1Δ, perhaps a defective copy. However, its unusually large promoter region as well as its transcriptional regulation (9, 21, 22) suggest that tryk2Δ may also perform physiological functions unknown at this moment.

**K^+ requirements of tryk1Δ tryk2Δ and tryk1Δ tryk2Δ cells.** The growth of tryk1Δ strains at low K^+ was the consequence of the activity of tryk2Δ, a tryk1Δ tryk2Δ strain should present very modest affinity for K^+ (1). Growth experiments at 1 mM K^+ showed that the tryk1Δ tryk2Δ strain (M469) doubled in 15 h (growth rate constant of 0.05 h^{-1}) and contained approximately 300 nmol of K^+ mg^{-1}, whereas the tryk1Δ tryk2Δ strain (R1155) showed a doubling time of 4.5 h (growth rate constant of 0.15 h^{-1}) and contained 450 nmol of K^+ mg^{-1}. Because K^+ influxes must be sufficient to maintain the K^+ content of the cells, they must have minimal values of 15 nmol mg^{-1} h^{-1} in tryk2Δ cells and 68 nmol mg^{-1} h^{-1} in tryk2Δ cells, and these values can be accounted for by the described kinetic characteristics of the strains. The K^+ uptake capacity at 1 mM K^+ is 17 nmol mg^{-1} h^{-1} in tryk2Δ cells (K^+ influxes can be calculated from the Rb^+ influx kinetics, assuming the same V_{max} for K^+ and Rb^+ and that the ratios between the Rb^+ and K^+ K_{m} are 1 in the low-affinity mode [11, 17]) and 59 nmol mg^{-1} h^{-1} in tryk2Δ cells (17 nmol mg^{-1} h^{-1}) through the low-affinity component plus 42 nmol mg^{-1} h^{-1} through the moderate-affinity component; the latter influx is calculated assuming the same V_{max} for K^+ and Rb^+, 0.7 nmol mg^{-1} min^{-1}, and that the K_{0} of K^+ for Rb^+ influx, 35 μM, is the K_{0} of K^+ influx (17)]. At 30 μM K^+, tryk1Δ tryk2Δ cells did not show appreciable growth, and tryk1Δ tryk2Δ cells doubled in 8 h (growth rate constant of 0.09 h^{-1}) and contained 300 nmol of K^+ mg^{-1}. This growth requires a net K^+ uptake of 26 nmol mg^{-1} h^{-1}, and the difference between this uptake and that expected from the kinetics of tryk2Δ is within the limits of experimental error (5 nmol mg^{-1} min^{-1}) and a K_{m} of 35 μM K^+ provides 20 nmol mg^{-1} h^{-1} at 30 μM K^+.

The capacity reported here of the tryk1Δ tryk2Δ and tryk1Δ tryk2Δ mutants to grow at low K^+ is higher than previously reported for the same strains (9), but the two sets of data are consistent. The differences may be explained by the different nitrogen sources of the testing media; ammonium medium used in the previous report is known to increase the K^+ requirement by comparison with arginine used in present work (11, 17); in addition, growth rate constants were not reported previously (9).

**The number of the K^+ uptake systems.** The proposal that tryk1Δ is a high-affinity K^+ transport system and tryk2Δ is a low-affinity transporter (8, 9) is not consistent with the actual K_{m} value of Rb^+ and K_{0} of K^+ in tryk2Δ. It could be proposed that yeast cells have three K^+ uptake systems. The real low-affinity transporter would be the system mediating K^+ and Rb^+ uptake in tryk1Δ tryk2Δ cells, tryk1Δ, the high-affinity system, and tryk2Δ, a system of moderate affinity. However, in this hypothesis, the physiological function of the low-affinity transporter in wild-type cells is not clear: kinetic evidence of the presence of the transporter has not been found (14, 17), and it would not be related to the low-affinity mode of K^+ uptake (K_{m} of Rb^+ influx of 65 and 6 mM [11], respectively). Furthermore, with a multiple K^+ uptake system, it is difficult to explain that K^+, Rb^+, Na^+, and Li^+ are taken up through the same transport system in wild-type strains (2-4, 11) and that a trkl-l mutation affects K^+ or Rb^+ uptake much more than...
Na⁺ uptake (11), a differential effect also observed in \textit{trk}IΔ mutants (the \( K_m \) of Li⁺ increased only twofold, from 50 to 100 mM, in K⁺-starved cells of RH2.2).

These results can be explained if there is a single K⁺ uptake system composed of one or several unknown proteins in addition to TRK1 and TRK2, the latter being present in a small number of transporters in normal conditions. The main function of TRK1 would be the regulation of K⁺ influx in response to the K⁺ content of the cell and to the cell pH (13, 14, 17). Interestingly, glucose activation of the K⁺ uptake system (12) would occur in one of the yet unknown proteins because activation is normal in \textit{trk}IΔ \textit{trk}2Δ cells (1).

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REFERENCES