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Potassium Uptake Through the TOK1 K^+ Channel in the Budding Yeast

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Abstract. The current through TOK1 (YKC1), the outward-rectifying K^+ channel in *Saccharomyces cerevisiae*, was amplified by expressing *TOK1* from a plasmid driven by a strong constitutive promoter. *TOK1* so hyper-expressed could overcome the K^+ auxotrophy of a mutant missing the two K^+ transporters, TRK1 and TRK2. This *trk1*Δ *trk2*Δ double mutant hyperexpressing the *TOK1* transgene had a higher internal K^+ content than one expressing the empty plasmid. We examined protoplasts of these *TOK1*-hyperexpressing cells under a patch clamp. Besides the expected K^+ outward current activating at membrane potential (V_m) above the K^+ equilibrium potential (E_{K^+}), a small inward current was consistently observed when the V_m was slightly below E_{K^+}. The inward and the outward currents are similar in their activation rates, deactivation rates, ion specificities and Ba^{2+} inhibition, indicating that they flow through the same channel. Thus, the yeast outwardly rectifying K^+ channel can take up K^+ into yeast cells, at least under certain conditions.

Key words: TOK1 — YKC1 — K^+ channel — Yeast — Patch clamp

Introduction

Ion channels are best known for their roles in excitable tissues; conducting ion currents during membrane excitation and enabling the rapid propagation of action potentials in neurons and muscles (Hille, 1992; Armstrong & Hille, 1998). However, channels are found in all cells including nonexcitable ones where they participate in sensory reception, secretion, membrane-potential (V_m) regulation, and other functions. Though traditionally studied in animals, ion channels are found in plants and microbes as well. For example, AKT1, a K^+ channel found in the root cells of *Arabidopsis*, appears to play a role in the uptake of K^+ (Serrano, 1991; Hirsch et al., 1998). Patch-clamp examination of the plasma membrane of budding yeast reveals the activities of a K^+-specific channel (Gustin et al., 1986) whose gene was then cloned and manipulated (below).

Biophysical characterization and molecular cloning have allowed us to sort ion channels into different families. The superfamily of K^+ channels is probably the most thoroughly studied and the knowledge gained from these studies illustrates how delicately each variety of channel is tuned to a particular function (Hille, 1992). A voltage-gated K^+ channel of the *Shaker* superfamily is a filter-fitted pore enclosed by four subunit proteins. Each subunit has the motif of $S_{1–5}$-P-S_6, where the Ss are transmembrane (TM) domains and P is the ''P-loop'' containing the canonical TXGYGD sequence that forms the K^+ filter in the tetramer. Inwardly rectifying K^+ channels are similar, but with only two TMs arranged as S_1-P-S_2 [6]. Recently Doyle et al. (1998) solved the crystal structure of a two-TM K^+ channel from the bacterium *Streptomyces lividans* and revealed its architecture at atomic resolution.

The genome sequence of the budding yeast, *Saccharomyces cerevisiae*, revealed an open reading frame (ORF) (Miosga, Witzel & Zimmermann, 1994) which conceptually corresponds to a channel peptide with eight TMs and two P loops, arranged as $S_{1–5}$-P_1-$S_{6–7}$-P_2-S_8. This ORF is called *TOK1* (Ketchum et al., 1995), also known as *YKC1* (Zhou et al., 1995), *DUK1* (Reid et al., 1996) and *YORK* (Lesage et al., 1996). Deletion of *TOK1* removes the native yeast K^+ current observed under patch clamp; overexpression of *TOK1* enhances it (Zhou et al., 1995; Reid et al., 1996). *TOK1* can be expressed in *Xenopus* oocytes and the heterologously expressed K^+ current has most of the characteristics of the K^+ current native in yeast (Ketchum et al., 1995; Lesage et al., 1996; Vergani et al., 1997; Loukin et al., 1997). TOK1 currents show a clear outward rectification above the equilibrium potential of K^+ (E_{K^+}); i.e. when the

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V_m is more positive than the E_{K^+}, the outward K^+ current increases disproportionately, indicating increased channel open probability. This was observed in the K^+ current native to yeast (Gustin et al., 1986; Zhou et al., 1995; Reid et al., 1996; Bertl, Slayman & Gradmann, 1993; Bertl et al., 1998) as well as in TOK1 heterologously expressed in oocytes (Ketchum et al., 1995; Lesage et al., 1996; Loukin et al., 1997). No clear inward current through this channel has been reported previously. Whether this channel can serve as a conduit for K^+ influx under growth conditions (membrane polarized and interior negative) is open to speculation. For example, Serrano (1991) suggested that TOK1 channels may mediate the uptake of K^+ in energized, hyperpolarized cells, and the efflux of K^+ in depolarized cells. The possibility of uptake through the TOK1 K^+ channel was again raised recently by Bertl et al. (1998). Unlike animal cells, the membrane potential of fungi and plants is largely determined by the electrogenic H^+ pump. Though direct measurement from yeast is not practical (Bertl et al., 1998), the V_m of yeast is thought to be very internally negative (some −100 to −200 mV). If V_m is more negative than E_{K^+}, a small inward K^+ current through TOK1 should allow seepage of K^+ into the cell, and, integrating over the cell cycle, even a small seepage may become significant. Wild-type yeast has two powerful active K^+ uptake mechanisms encoded by *TRK1* (Gaber, Styles & Fink, 1988; Ko, Buckley & Gaber, 1990) and *TRK2* (Ko & Gaber, 1991). The possible passive influx of K^+ through TOK1 would ordinarily be overshadowed by the strong active uptake by TRK1 and TRK2. To maximize the chances of detecting this passive influx, we examined yeast cells hyperexpressing *TOK1* but deleted of *TRK1* and *TRK2*. We studied the growth patterns of this and other yeast cells on plates and in liquid media of different $[K^+]_{out}$ (external concentration of K^+), measured their $[K^+]_{in}$ (internal K^+ content) by flame photometry, examined membrane K^+ currents under patch clamp and found evidence consistent with K^+ influx through TOK1 under certain conditions.

Materials and Methods

Plasmids and Strains

pSUFYKC1 (Zhou et al., 1995, Loukin et al., 1997) is a *URA3* yeast expression plasmid with a *TOK1* open reading frame inserted behind the strong constitutive promoter of the *SUF14* gene (Hill et al., 1986). pSUF000, a plasmid containing no insert was used as control. All *S. cerevisiae* strains used were derived from the ''wild-type'' W303, genotype *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1.* Strain ΔJ0911 is αW303 *tok1*::*URA3.* Both strains were gifts of T. Miosga (Institute fur Mikrobiologie, Darmstadt) (1994). Strain αKU8 was selected for its Ura^- auxotrophy from ΔJ0911 in the presence of 5-flouroorotic acid. SGY1529, genotype *MATa ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 trk1::HIS3 trk2::TRP1* was a gift of S. Kurtz, (Bristol-Myers Squibb, New Jersey) (Tang et al., 1995). This strain has disruptions in both its *TRK1* and *TRK2* K^+ transporters. It is referred to as the ''double knockout mutant'' and labeled as ''*trk1*Δ *trk2*Δ'' below. The ''triple knockout mutant'' or *trk1*Δ *trk2*Δ *tok1*Δ was generated by crossing ΔJO911 with SGY1529 giving the genotype *MAT*α *ade2–1, can1–100, his3–11/15, leu2–3/112, trp1–1, ura3–1, trk1::HIS3, trk2::TRP1, TOK1::URA3.* This triple mutant was then made Ura^- by transplacing (using electroporation) with a disrupted *URA3* gene (from Yep352ΔES, courtesy of Johan den Boon, University of Wisconsin, Madison; disrupted from the internal *EcoR1* site at bp415 to the *Stu1* site at bp663) and selecting for Ura^- auxotrophy. This strain, named CF1001, has the genotype *MAT*α *ade2–1, can1–100, his3–11/15, leu2–3/112, trp1–1, ura3–1, trk1::HIS3, trk2::TRP1, tok1::ura3*Δ*ES.*

Cell Cultures

SD, a synthetic medium having NH_4^+ as the nitrogen source and glucose as the energy source (Sherman, 1991), was used in preparing protoplasts for patch clamping. All other cultures were grown on modified arginine-based SL medium (Rodriguez-Navarro & Ramos, 1984), which has a basal $[K^+]$ of 2 to 5 μM K^+ and has the following composition: 10 mM L-arginine, 5.0 mM glutamic acid, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 2% glucose, 4.4 mM phosphate plus vitamins, amino acids, and trace elements. Here, 0.5, 1.0, 5.0, 20.0 or 80.0 mM KC1 was added to form the various test media. Colony-forming capability was tested by plating on 1.5% agar in various K^+-enriched SL media. Growth tests in liquid SL media were at 30°C and began with preculturing in 5 mM K^+ (SL with 5 mM KCl, a medium permissive to all strains). These overnight cultures were diluted with fresh 5-mM K^+ SL medium to an OD_{600} of 0.05, grown again, and were then used that evening to inoculate new cultures in the same medium at an OD_{600} of 0.05. This is to make sure that the inocula were in the logarithmic phase of growth and fully adapted to 5 mM SL. The changes in OD_{600} were monitored every 2 hr for a total of 12 h. Duplicate 1-ml samples were taken from the culture at the last time point (12 hr) and from the preculture half an hour before the inoculation (time −0.5 hr) for the analysis of K^+ content and 4-ml samples were taken at the same times for dry weight determination.

Determination of Dry Weight and Potassium Content

To determine dry weight, 4-ml samples from each of the above cultures were collected by centrifugation in predried and preweighed microfuge tubes, dried in an 80–90°C oven for >24 hr and then weighed again using a Mettler Toledo AG245 balance. Duplicate 1-ml aliquots of the same cultures were each filtered through a 25-mm diameter 0.22-μm nitrocellulose filter (Millipore) and washed with 4 ml of SL medium with no additional KCl. Filters were placed in a 50-ml beaker and dried at 80–90°C overnight. The filters were then resuspended in 4 ml of double-distilled water and assayed for potassium content using a flame photometer (PFP7 Buck Scientific) following the instructions of the manufacturer. Potassium content is expressed as nmole K^+ per mg dry weight.

Protoplast Formation and Patch-Clamp Methods

Yeast protoplasting was carried out as described (Gustin et al., 1986; Zhou et al., 1995; Bertl, Slayman & Gradmann, 1993) with minor modification. Briefly, cells were precultured overnight in 1 ml of SD

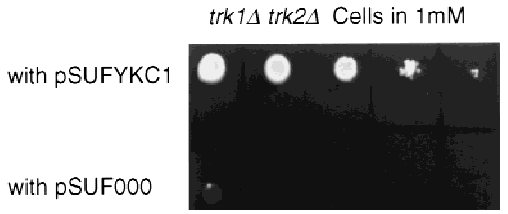

Fig. 1. Hyperexpressing *TOK1* overcomes the K^+ auxotrophy of a *trk1*Δ *trk2*Δ double mutant missing the two known K^+ transporters. Leftmost spots show patterns of growth after 3 days at 30°C after an inoculation with 5 μl of culture at 0.2 OD_{600}. Serial tenfold dilution of the first inocula are shown to the right. *Top row*: *trk1*Δ *trk2*Δ double mutant expressing the plasmid pSUFYKC1. *Bottom row*: same mutant expressing the empty plasmid pSUFOOO.

medium. A 0.1 ml aliquot was cultured the next morning in 1 ml of fresh SD medium at 30°C for 2 hr. Cells were washed with 50 mM KH_2PO_4, 40 mM β-mercaptoethanol, pH 7.2 (KOH), and then incubated in 0.5 ml of the same buffer at 30°C for 0.5 hr. 0.1 ml of cells were then digested in 1 ml of the same buffer containing 2 mg/ml zymolyase, 2 mg/ml glucuronidase, 30 mg/ml BSA and 2M sorbitol at 30°C for 1–2 hr, depending on strain and growth rate. Protoplasts were harvested and resuspended in 0.5 ml of 250 mM KCl, 5 mM $MgCl_2$ and 5 mM HEPES pH 7.2 (KOH). Patch-clamp examination was carried out in the whole-cell mode as previously described (Gustin et al., 1986; Zhou et al., 1995). Membrane currents were recorded using an EPC7 patch-clamp amplifier (List Medical System) at room temperature. The signal was filtered at 1 kHz before digitizing and analyzing with pCLAMP 6.0 software (Axon Instruments).

Results

TOK1 Can Support Colony Growth After Known K^+ Uptake Mechanisms Are Removed

Deletion of the K^+ channel, TOK1, in the wild-type background has little effect on yeast growth under ordinary laboratory conditions (Miosga et al., 1994; Zhou et al., 1995; Reid et al., 1996). However, its possible role in K^+ uptake can be tested more clearly in a K^+ auxotrophic yeast strain. The two major K^+ transporters, TRK1 and TRK2 (Serrano, 1991; Gaber et al., 1988; Ko et al., 1990) enable the wild-type cells to proliferate in the low micromolar K^+ inherent to SL medium. The *trk1*Δ *trk2*Δ double mutant, on the other hand, cannot form colonies on SL plates unless they are supplemented with 3 mM or more of KCl. We tested the effects of TOK1 by expressing plasmid-borne *TOK1* genes driven by the strong constitutive *SUF* promoter. The *TOK1* transgenes so expressed clearly support colony formation of *trk1*Δ *trk2*Δ on plates containing only 1 mM K^+ (Fig. 1*A*, top row). This is not due to a nonspecific effect of plasmid transformation because the control cells transformed with empty plasmids do not form colonies (bottom row). The difference between the control and experimental is robust, repeatedly observed in more than 20 experiments in different concentrations of added K^+. In permissive conditions, e.g., 5.0 mM K^+, the *TOK1* transformed cells form larger colonies sooner than the empty plasmid control. Note that all *trk1*Δ *trk2*Δ cells have a single chromosomal copy of *TOK1*, and the comparisons here are of the presence or absence of additional strongly promoted *TOK1* transgenes in multiple copy.

Table 1. Potassium content (nMol K^+ per mg dry weight) of *trk1*Δ *trk2*Δ yeast cells cultured in different media

	*trk1*Δ *trk2*Δ cells transformed with pSUFYKC1	pSUFOOO
Precultured in 5.0 mM K^+	432 ± 77*	184 ± 8062
Cultured in 5.0 mM K^+	361 ± 37*	151 ± 36
Cultured in 0.5 mM K^+	54 ± 16	n.d.**

* $P < 0.05$ from the empty plasmid control, $n = 3$. ** Not determined because of cell death.

Growth of Mutants Correlates with Their K^+ Content

The plate phenotypes in Fig. 1 have their parallels in liquid cultures. The *trk1*Δ *trk2*Δ cells hyperexpressing *TOK1* in trans proliferate faster than the *trk1*Δ *trk2*Δ expressing the empty plasmid in liquid media containing permissive $[K^+]_{out}$ ranging from 3 to 20 mM K^+. For example, expression of *TOK1* transgene doubles the growth rate of the empty plasmid control cells in 5.0 mM K^+ (Fig. 2, filled symbols). Above 20 mM K^+ the growth-rate difference between the two cell types become obscure. Below 3 mM K^+, cells with *TOK1* transgenes grew slowly and the ones without did not grow. For example, in an SL medium containing 0.5 mM K^+, the former grows slowly, doubling only once in 10 hr, while the latter died, as gauged by colony forming units on plates of a permissive (10 mM K^+) SL medium (Fig. 2, open symbols). Growth became difficult to detect below 0.5 mM K^+, even for the *TOK1* hyperexpressing cells. This contrasts the robust growth in less than 10 μM K^+ of wild-type yeast with intact *TRK1* and *TRK2*.

To rule out the possibility that TOK1 supports growth of *trk1*Δ *trk2*Δ cells by means unrelated to K^+ we used flame photometry to directly measure the K^+ content, $[K^+]_{in}$, of yeast cells from the different cultures represented in Fig. 2. Table 1 shows that the hyperexpression of TOK1 is positively correlated with $[K^+]_{in}$. The inocula were cultured in the permissive SL medium with 5.0 mM K^+ (Row 1 and 2, Table 1) as were the cells in the first experiment (Row 2). While there is no difference between the K^+ content of the precultures (0.5 hr before inoculation) and culture (12 hr after inoculation), the K^+ content of cells transformed with *TOK1*-bearing plasmids is consistently more than twice that of control

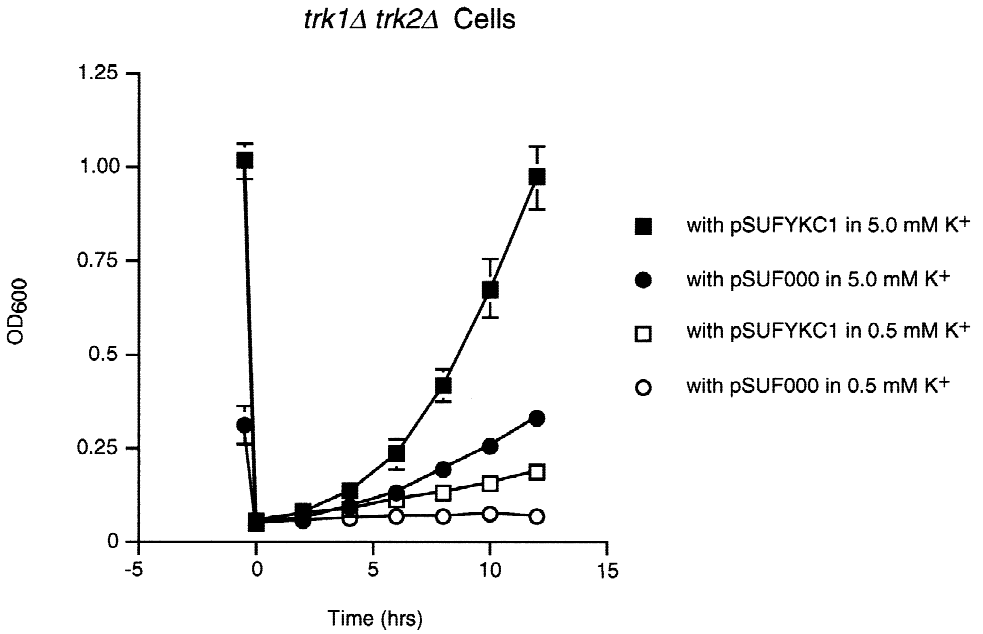

Fig. 2 Growth of *trk1*Δ *trk2*Δ cells in SL liquid media supplemented with 5.0 or 0.5 mM K^+. Cells were precultured starting with a concentration of 0.05 OD_{600} and grown for 12 hr. These cultures ($T = -0.5$ hr) were used to assay $[K^+]$ values and dry weights (Table) prior to redilution of cultures to 0.05 OD_{600} ($T = 0$ hrs) for a further 12-hr growth. In SL media with 5.0 mM K^+, a premissive concentration, *trk1*Δ *trk2*Δ cells with pSUFTOK1 (filled squares) grow faster than those with the empty plasmid (filled circles). In 0.5 mM K^+, a restrictive concentration, *trk1*Δ *trk2*Δ cells with pSUFTOK1 grow slowly (open squares); the *trk1*Δ *trk2*Δ cells containing the empty plasmid do not grow (open circles). Bars are mean ±SD when SD is larger than the symbol, $n = 3$.

cells with empty plasmids. Cells with *TOK1*-bearing plasmids inoculated into SL with 0.5 mM K^+ survive and grow slowly (Fig. 2, open square). Correlated with this slow growth is a very low $[K^+]_{in}$ measured after 12 hr of culturing. The $[K^+]_{in}$ of cells with empty plasmids in 0.5 mM K^+ cannot be reliably measured because these cells are dying, and by 12 hr, nearly all dead.

An Inward K^+ Current Through TOK1

Currents through the plasma membrane of individual yeast cells have been examined directly with patch clamp in the whole-cell recording mode (Gustin et al., 1986; Zhou et al., 1995; Reid et al., 1996) or excised inside-out mode (Bertl et al., 1993; Bertl et al., 1998). The TOK1 channel in its native setting is K^+ specific, activates at low voltages, and rectifies outwardly at voltages above E_{K^+}, the equilibrium potential of K^+ (Zhou et al., 1995; Reid et al., 1996). This current is evident in *trk1*Δ *trk2*Δ protoplasts with empty plasmids and with an intact single chromosomal copy of *TOK1* (Fig. 3*A*, second current trace). Deletion of *TOK1* erases this current (top current trace). The current is dramatically amplified when the yeast cell is expressing *TOK1* from a plasmid driven by the *SUF* promoter (9) (Fig. 3*A* bottom trace). As expected, this current activates strongly at voltages above the E_{K^+} which is at 0 mV here because of the symmetric test solutions diagramed by the inset of Fig. 3 (top). Below 0 mV, however, we found the membrane current to reverse and flow inwardly, peaking at about −10 mV and declining at more negative voltages (Fig. 3*A*, arrow). In all cases tested ($n > 20$), this smaller inward current always accompanies the large outward current previously determined to be carried by K^+ and through a channel encoded by *TOK1* (Zhou et al., 1995; Reid et al., 1996). Comparison of currents such as those shown in Fig. 3 clearly show that, like the outward current, this inward current is induced by the pSUFYKC1 plasmid and is therefore an expression of *TOK1*. To confirm that this inward current indeed flows through TOK1, we tested whether it changes with the electromotive force of K^+. Figure 3*B* shows a separate experiment that began as in *A* (symmetric 180 mM K^+ solutions) and the same currents are seen (the rising trace on the right in Fig. 3*B*). However, when the bath K^+ was reduced to 90 mM, both the outward and the inward current were found to be activated at more negative voltages. The leftmost trace in Fig. 3*B* shows that when the E_{K^+} is shifted by changing the bath solution, the reversal potential of the currents is shifted by −16 mV very close to the calculated E_{K^+} of −17 mV. In addition, the whole profile of the TOK1 current, including the inward current, shows a parallel left shift of −16 mV.

The kinetics of TOK1 expressed in oocytes have

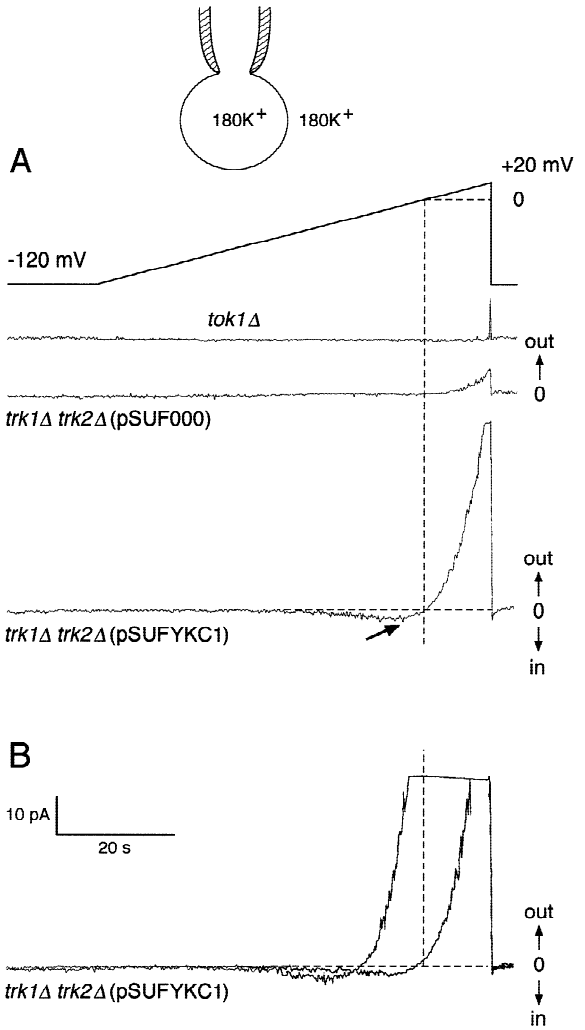

Fig. 3. Patch-clamp examination of various yeast protoplasts in whole-cell recording mode. (*A*) Whole-cell currents from three types of protoplasts at slow-ramp voltage. As diagrammed, both the pipette and the bath contain 180 mM K^+ solution. The *top trace* diagrams the command voltage from −120 to +20 mV applied. The *second trace* shows current from a *TOK1*Δ cell deleted of its TOK1 K^+-channel gene (strain αKU8). Neither inward nor outward current is observed at this resolution (only a brief current artifact). The *third trace* shows current from a *trk1*Δ *trk2*Δ mutant cells (with an intact chromosmal copy of *TOK1*) bearing the empty plasmid pSUFOOO. It shows an outwardly rectifying current when V_m is above 0 mV (the E_{K^+} of the symmetric solutions here). This current is similar to the *TOK1* current native to the wild type with a single copy of *TOK1* [9, 16]. Inward current is not evident at this magnification. The *bottom trace* shows the current from a *trk1*Δ *trk2*Δ mutant cell strongly expressing the *TOK1* current, due to the plasmid pSUFYKC1. A strong outward current is recorded above 0 mV as expected of *TOK1*'s outward rectification. However, at voltages just below 0 mV, an inward current is clearly evident (arrow). Each trace is typical of more than 20 protoplasts examined. (*B*) Parallel Shift of currents with E_{K^+}. As in (*A*), a pSUFTOK1-bearing *trk1*Δ *trk2*Δ mutant cell is first recorded in symmetric 180 mM K^+. The current is similar to that of the bottom trace of *A*, as expected. After the bath solution is replaced with 90 mM K^+ and 90 mM Na^+, both the outward and the inward current remain clearly discernable. However, the currents as well as the reversal potential shift to the left by about 16 mV in parallel. This shift indicates that both the inward and outward currents are K^+ selective. Typical of 3 experiments. Leakage current has been subtracted from both *A* and *B*.

been modeled as C1 - C2 - O where C1 and C2 represent closed states and O represents the open conformation. The slower departure from the closed state has a set of time constants on the order of hundreds of milliseconds depending on the K^+ driving force (Ketchum et al., 1995; Loukin et al., 1997). We found the outward and the inward K^+ currents in yeast spheroplasts activate at similar rates. In the experiment shown in Fig. 4*A*, the activation of the outward current has a time constant of about 380 msec; that of the inward current about 300 msec. The small difference is expected, since the test voltages are different. The deactivation rates of the two currents are also very similar (*data not shown*). That the ensemble kinetics of the outward and the inward currents are similar supports the notion that the same channel molecules underlie both currents.

TOK1 current can be discerned at the microscopic level even though unitary currents are small and flicker rapidly (Gustin et al., 1986; Zhou et al., 1995; Reid et al., 1996; Loukin et al., 1997). We make use of this behavior and let the coincidence of brief multiple openings indicate the direction of current flow. At very low positive voltages where the open probability is low, multiple unitary outward currents sum into a form that appears as upward spikes rising from the baseline closed level (Fig. 4*B*, upper traces). At −10 mV, however, these spikes point downward from the baseline closed level, indicating multiple unitary currents flowing inward (Fig. 4*B*, middle traces). At −40 mV, where channel open probability is very low and the coincidence of multiple openings rare, inwardly directed currents remain obvious but the amplitude of spike-like activity is small (Fig. 4*B*, bottom traces). These activities are comparable to those previously reported for TOK1 (Gustin et al., 1986; Zhou et al., 1995; Reid et al., 1996; Loukin et al., 1997) and are distinct from noises of the recording system, since the activities greatly diminish rather than increase when the voltage is lowered further from −10 to −40 mV. In experiments where $[K^+]_{out}$ is varied from 180 to 60 to 30 mM, the current reversed very near the expected E_{K^+}s. Like the outward K^+ current, the inward current is also blocked by 20 to 30 mM Ba^{2+} (*data not shown*).

Deletion of TOK1 Further Aggravates the K^+ Auxotrophic Mutant

The above experimentation uses yeast cells strongly expressing *TOK1* transgenes from plasmids. It is more difficult to demonstrate the possible role of the inward current through TOK1 channels expressed from single copy chromosomal *TOK1*. If TOK1 serves as a K^+-uptake pathway, one would expect its removal to aggregate the pathology of the *trk1*Δ *trk2*Δ double mutant. Indeed, when plated on K^+ limiting media, the triple knockout *tok1*Δ *trk1*Δ *trk2*Δ grows less well than does the double

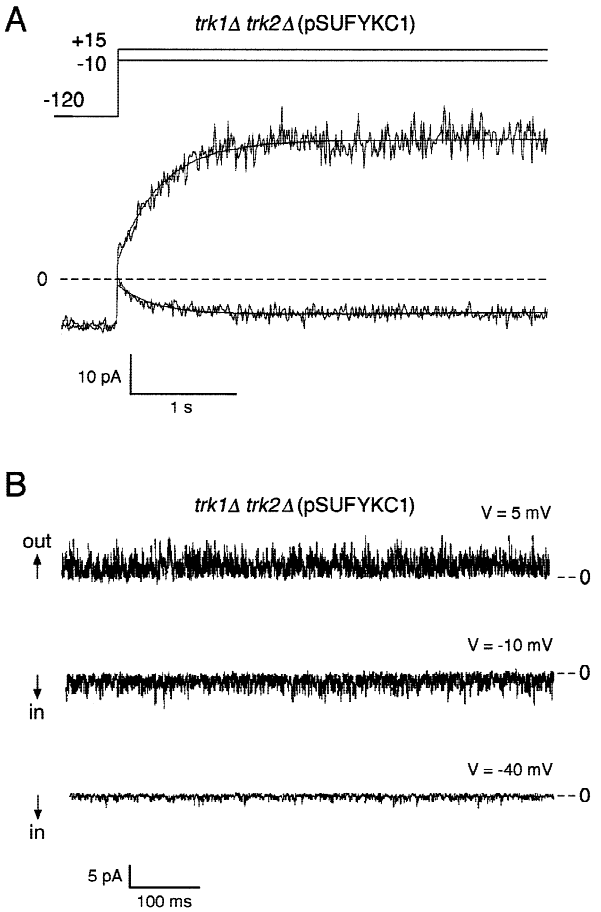

Fig. 4. The inward and the outward currents have similar kinetics. (*A*) Current activation kinetics of a pSUFYKC1-bearing *trk1*Δ *trk2*Δ mutant cell. Shown are activation of the outward current upon a step depolarization from −120 to +15 mV (the rising current trace) and the activation of the inward current upon a step depolarization from −120 to −10 mV (the falling current trace). Note that the two currents have similar activation rates. Single exponential fits resulted in time constants of 384 msec and 304 msec for the outward and inward currents, respectively. Typical of over 20 experiments. (*B*) Unitary current events observed at different voltages. *TOK1* microscopic current has the appearance of flickering rapidly when examined at fast time base. *Top trace* shows that, at +5 mV (above the 0 mV E_{K^+} here), spikelike multiple unit currents point outward. Middle trace shows that, at −10 mV, just below E_{K^+}, such currents point inward. At −40 mV (bottom trace) the less ''noisy'' trace indicates fewer multiple unitary events. Typical of over 20 experiments.

knockout, *trk1*Δ *trk2*Δ (Fig. 5). This difference was consistently observed over a range of limiting K^+ concentrations from 2 to 5 mM. We also found that when K^+ is critical (5 mM), the addition of NH_{4^+} to the SL medium inhibits growth of the triple knockout more than that of the double knockout.

Hyperexpression of TOK1 Can Also Be Deleterious

Though the hyperexpression of *TOK1* complemented the K^+-uptake defects of *trk1*Δ *trk2*Δ cells in limiting $[K^+]_{out}$ (e.g., 1 mM, Fig. 1), it is apparently detrimental when $[K^+]_{out}$ is high. For example, 80 mM $[K^+]_{out}$ is permission to these cells, as shown by cells expressing the empty plasmids (Fig. 6, bottom row). Expression of plasmid-borne *TOK1* inhibits the growth of these *trk1*Δ *trk2*Δ cells (top row). Similar results are found with wild-type cells (i.e., *TRK1 TRK2*) expressing the two plasmids (*data not shown*).

Discussion

We discovered that the yeast K^+ channel, TOK1, passes an inward current at membrane potential slightly below the equilibrium potential of K^+. This was found by magnifying the TOK1 current through the hyperexpression of *TOK1* genes from plasmids. Such transgenes rescue from auxotrophic death in K^+-poor media the *trk1*Δ *trk2*Δ double mutant lacking the two active K^+ uptake systems. At permissive but K^+-poor media, cells expressing the transgenes grow faster and accumulate more K^+ per weight than control cells. These results indicate that yeast can use its TOK1 K^+ channel to take up K^+ in vivo, at least in these circumstances.

K^+ Fluxes Through TOK1

The prominent outward rectification of a K^+ current was noticed in the very first patch-clamp examination of the yeast plasma membrane (Gustin et al., 1986). This rectification is also a prominent feature when the *TOK1*-encoded current was examined in *Xenopus* oocytes (Ketchum et al., 1995; Lesage et al., 1996; Loukin et al., 1997). Unlike strictly voltage-activated outward rectifiers TOK1 channel open probability depends upon both V_m and E_{K^+} and the channel activation profile shifts in parallel with E_{K^+} when $[K^+]_{out}$ is changed (Ketchum et al., 1995; Reid et al., 1996; Lesage et al., 1996; Loukin et al., 1997). A value of an inward current at a voltage below E_{K^+} is given in a nearly linear unitary-current *vs.* voltage plot by Bertl et al. (1995). However, because the yeast cell is small and the current density low, this inward current has been difficult to demonstrate directly in yeast. Our work using hyperexpression of *TOK1* from a plasmid driven by the constitutive *SUF14* promoter clearly demonstrates that this inward current indeed exists in yeast (Figs. 3, 4). This inward current is blockable by Ba^{2+} as is the K^+ outward current. The inward current is also continuous with the outward K^+ current in *I-V* plots reversing at E_{K^+}. That this inward current always accompanies the outward K^+ current, is proportional to it, and has both activation and deactivation kinetics similar to it, further indicates that both currents flow through the same channel protein, TOK1.

The Outward Rectification

Presumably, one of TOK1's functions is to reset the membrane potential as do other outward rectifiers. This

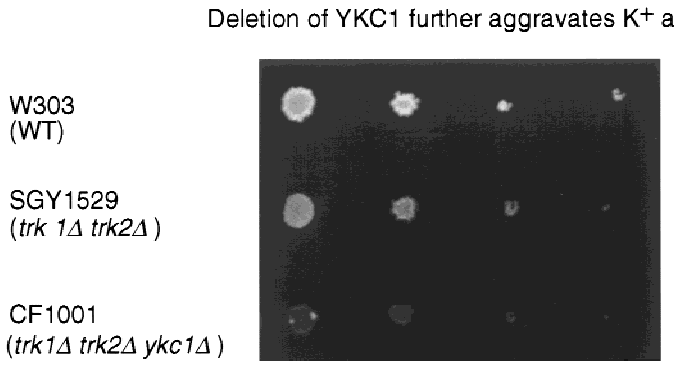

Fig. 5. Cells grown after 3 days at 30°C on LS media with 4 mM K^+ added. Shown are the wild type (*TRK1 TRK2 TOK1*, strain W303), the double mutant (*trk1*Δ *trk2*Δ *TOK1*, strain SGY1529) and the triple mutant (*trk1*Δ *trk2*Δ *tok1*Δ strain CF1001). Leftmost spots show 5μl of culture at OD_{600} of 0.2 with 10-fold dilutions to the right.

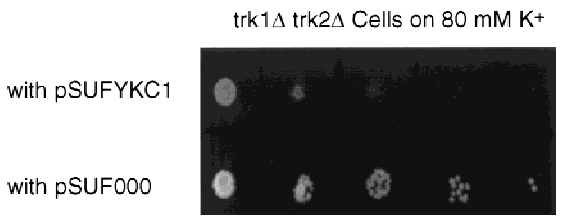

Fig. 6. Hyperexpression of *TOK1* in the *trk1*Δ *trk2*Δ double mutant can block its growth at high concentration of K^+. Shown are the growth pattern of cells with the *TOK1* plasmid or the empty plasmid on SL plates containing 80 mM K^+ after 3 days at 30°C. Leftmost spots show 5 μl of culture at OD_{600} of 0.2 with 10-fold dilutions to the right.

notion seems reasonable since free-living cells should have evolved mechanisms to recharge their membranes using a prestored K^+ gradient; for example, upon depolarization due to changes in the external milieu or after their membranes have been temporarily compromised. Because TOK1 is activated according to the K^+ motive force ($V_m - E_{K^+}$) and not just by the absolute V_m, TOK1 should prohibit a V_m drift away from the deep E_{K^+} regardless of $[K^+]_{out}$.

K^+ Influx Through TOK1 Can Support Growth

Although TOK1 channels mediate K^+ efflux in depolarized cells, Serrano (1991) and Bertl et al. (1998) suggest that these channels may mediate the uptake of K^+ under certain conditions. We have demonstrated the K^+ uptake they proposed. By bacteriological methods, we have shown that hyperexpression of *TOK1* increases $[K^+]_{in}$ and supports growth. By electrophysiological methods, we have also registered inward K^+ currents through the TOK1. Calculations show that this inward current is more than sufficient to provide the K^+ needed for yeast cell doubling. However, these experiments were carried out in a contrived situation: hyperexpressing *TOK1* in *trk1*Δ *trk2*Δ cells. Whether channels encoded by single copy *TOK1* under normal control and normal promotion takes up significant K^+ for yeast cell remains uncertain. K^+ uptake through TOK1 is certainly not necessary in the wild type, which is equipped with *TRK1* and *TRK2*. Further, deletion of *TOK1* does not result in K^+ auxotrophy. Although we found that the deletion of *TOK1* further aggravates the K^+ auxotrophy of the *trk1*Δ *trk2*Δ double mutant (Fig. 5), it is difficult to rule out the possible indirect pathological aggravation of a third mutation on a strain that already suffers two. That the triple mutant is more sensitive to NH_4^+ toxicity is consistent with such an aggravation.

Hyperexpression of TOK1 Can Be Deleterious

Although expressing the TOK1 transgene clearly rescues the *trk1*Δ *trk2*Δ double mutant (Fig. 1), such expression is not necessarily beneficial. Active K^+-uptake mechanisms such as TRK1 and TRK2 that are directly coupled to energy may be expected to slow down or stop when $[K^+]_{in}$ reaches a physiological limit (Ko & Gaber, 1991). Passive flows of K^+ and its analogs through TOK1 are apparently poorly controlled. The hyperexpression of TOK1 can block yeast cell growth when $[K^+]_{out}$ is increased (Fig 6). This may be due to excess amount of K^+ in the cytoplasm entered through the channel, although we cannot rule out the possible entry of other contaminating toxic ions.

The Possible Physiological Role of K^+ Influx Through TOK1

What TOK1 does ordinarily when expressed from a single copy gene in the wild type remains uncertain. In yeast, unlike animal cells, the V_m is largely determined by the action of the electrogenic H^+-ATPase, PMA1 (Serrano, 1991). Indirect experiments show that reduced-function *pma1* mutants seem to have lower V_m. It therefore seems unlikely that TOK1 is the major determinant of V_m in yeast, unlike in animal cells where V_m is largely determined by K^+ conductance. TOK1, native in yeast or expressed in oocytes, is clearly regulated by cytoplasmic pH (Lesage et al., 1996; Bertl et al., 1998; Zhou, *unpublished observations*). TOK1 channel activities are also strongly affected by Ca^{2+} concentrations (Bertl et al., 1993; Zhou, *unpublished observations*) and

require the presence of cytoplasmic ATP (Bertl et al., 1995). One could therefore speculate that TOK1 functions in certain physiological adjustments by integrating the status of V_m, $[K^+]_{in}$, $[K^+]_{out}$, Ca^{2+}, and cytoplasmic pH and [ATP]. How TOK1 may relate to K^+ channels in other organisms also requires further studies. The possible uptake of K^+ through TOK1 resembles the suggested function of *Arabidopsis'* AKT1 (Hirsch et al., 1998) which has the S_{1-5}-P-S_6 structural motif. At present TOK1 is the only known K^+-channel subunit protein that has a S_{1-5}-P_1-S_{6-7}-P_2-S_8 structure, although several ''two-pored'' channels of the S_1-P_1-S_{2-3}-P_2-S_4 motif have been reported in mammals and *Drosophila*, such as TWIK1 (Lesage et al., 1996), TREK1 (Fink et al., 1996), and ORK1 (Goldstein et al., 1996). The physiological roles of these diverse K^+ channels and their relationship await further studies. Like plant and animal channels, TOK1 can be studied through oocyte expression and site-directed mutageneses. Being native to yeast, TOK1 can also be subjected to forward genetic dissection using standard microbial genetic technique. For example, gain-of-function mutants selected after random mutageneses of TOK1 revealed that residues at the cytoplasmic ends of S_6 and S_8 are crucial in channel gating (Loukin et al., 1997). Thus, future genetic and other studies may help us further understand the role of TOK1 in vivo, including the role of its inward current.

In sum, we have shown unambiguously that TOK1 is capable of passing inward K^+ current in vivo. Under special circumstances this influx of K^+ can be used to support growth. However, the role of TOK1 in wild-type yeast cells remains unclear and requires further investigation.

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