The presumed potassium carrier Trk2p in *Saccharomyces cerevisiae* determines an H^+-dependent, K^+-independent current

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Abstract Ionic currents related to the major potassium uptake systems in *Saccharomyces cerevisiae* were examined by whole cell patch-clamping, under K^+ replete conditions. Those currents have the following properties. They (1) are inward under all conditions investigated, (2) arise instantaneously with appropriate voltage steps, (3) depend solely upon the moderate affinity transporter Trk2p, not upon the high affinity transporter Trk1p. They (4) appear to be independent of the extracellular K^+ concentration, (5) are also independent of extracellular Ca^{2+}, Mg^{2+} and Cl^- but (6) are strongly dependent on extracellular pH, being large at low pH (up to several hundred pA at −200 mV and pH 4) and near zero at high pH (above 7.5). They (7) increase in proportion to $\log[H^+]_o$, rather than directly in proportion to the proton concentration and (8) behave kinetically as if each transporter cycle moved one proton plus one (high pH) or two (low pH) other ions, as yet unidentified. In view of background knowledge on K^+ transport related to Trk2p, the new results suggest that the K^+ status of yeast cells modulates both the kinetics of Trk2p-mediated transport and the identity of ions involved. That modulation could act either on the Trk2 protein itself or on interactions of Trk2 with other proteins in a hypothetical transporter complex. Structural considerations suggest a strong analogy to the KtrAB system in *Vibrio alginolyticus* and/or the TrkH system in *Escherichia coli*.

Key words: Yeast; Patch-clamp; Potassium carrier; Proton current; Inward rectifier; *TRK2*; Transporter complex; TrkH; *Escherichia coli*

1. Introduction

High affinity uptake of potassium by the yeast *Saccharomyces cerevisiae*, originally described by Rodriguez-Navarro and Ramos [1] following growth under K^+-limited conditions, was ascribed to the protein Trk1p after specific deletion of the gene *TRK1* had been shown to disrupt the ability of yeast cells to extract net K^+ out of sub-millimolar solutions [2]. Mutagenesis and subsequent heat enrichment screening of *trk1*Δ cells revealed a second gene, *TRK2*, involved in the K^+ uptake [3], but apparently with a lower affinity. The *TRK2* gene product predicted a protein similar to Trk1p but lacking ∼50% of a large hydrophilic segment (650 amino acids) present in the first half of Trk1p [4]. Apart from the deleted span, Trk2p aligns on Trk1p with nearly 55% sequence identity and another 15% conservative replacement [4]. However, the regulation of both K^+ transporter systems has proven complex and flux studies on yeast strains deleted of either *TRK1* or *TRK2* have indicated the apparent $K_{0.5}$ of each, for K^+, to vary with conditions, both with the actual level of intracellular potassium ($[K^+]_i$) and with the manner in which potassium depletion is produced [5,6].

Previous patch-clamp measurements on yeast, carried out mainly to characterize native and heterologous K^+ channels in the yeast membrane [7,8], have assigned a small inward current to the TRK proteins. That current, amounting to ∼50 pA at a membrane voltage of −200 mV, rose instantaneously (time constant < 5 ms) with negative voltage steps, which was taken to be consistent with carrier-type ion conduction. The current was not affected by simple deletion of the *TRK1* gene but disappeared upon deletion of *TRK2* and thus could be attributed to Trk2p or Trk1p+Trk2p, but not to Trk1p alone. This seemed an odd conclusion, since Trk1p have been identified as the high affinity K^+ transporter in yeast and since the high affinity K^+ transporter in *Neurospora crassa* (a related ascomycete fungus) have previously been described as a strong inward current carrier [9,10]. However, since the actual expression levels of the two TRK proteins have not been monitored or carefully controlled, in the yeast experiments, there was considerable room for flexibility in the interpretation of the results.

The present experiments were undertaken to define more carefully the structure and nature of the inward current, seen in ordinary patch-clamp experiments with yeast. To our great surprise, the fast inward current observed in the presence of millimolar (or higher) $[Ca^{2+}]_o$ proved not to be carried by K^+ or by other common ions (Mg^{2+}, Cl^-) but apparently by protons. Since that current was abolished, again, by deletion of *TRK2*, but not by the separate deletion of *TRK1*, it is difficult to avoid the conclusion that Trk2p somehow confers a proton carrying capability upon the assembled potassium transport system(s) in *Saccharomyces*. A similar conclusion has been reached recently by Madrid et al. [11] on the basis of specific H^+-modulated, TRK-driven dye signals thought to be related to membrane depolarization.

2. Materials and methods

Whole cell recording from *Saccharomyces* was carried out as previously described [12], comparing four yeast strains: Pmr1Δ (K837: *MAT*a *ade2-1 his3-11,15 leu2-3,112 trp1-1 pmr1::HIS3*, haploid from K. Cunningham), Trk1Δ (AB541: *MAT*a/α *ura3-52 trk1Δ1* $Trk2^{D-5}$, homozygous diploid [13]), Trk2Δ (NY39: *MAT*a/α *ura3-52 his3-Δ200 trk2Δ::HIS3*, homozygous diploid [4]) and TrkΔΔ (CY152/CY162: *MAT*a/α *ura3-52 his3Δ200 trk1Δ1 trk2Δ::HIS3*; diploid [4]). In all of these strains, the activity of the outward rectifying channel, Duk1p, provided as an internal control for proper behavior of the

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Abbreviations: MES, 2-(*N*-morpholino) ethanesulfonic acid; Tris, tris-(hydroxymethyl)aminomethane

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membrane in response to various experimental tests (see, e.g. legends to Figs. 1 and 2).

To prepare protoplasts, late log-phase cells were treated briefly with zymolyase-20T (0.01–0.03%, w/v) in 50 mM K-phosphate buffer (pH 7.2), augmented with 0.2% β-mercaptoethanol as an antioxidant and with 1.2 M sorbitol as an osmoprotectant. The suspended protoplasts and cells were harvested by centrifugation, then washed and stabilized in saline (220 mM KCl) buffered with Tris/MES (1–5 mM, pH 7.2) and supplemented with 10 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.05% (w/v) glucose.

Patch pipettes were pulled from Kimax-51 borosilicate glass (Kimble/Kontes number 38500) to a tip I.D. of ~1 μm, fire-polished and usually filled with buffer containing 175 mM KCl, 5 mM $MgCl_2$, 4 mM K-ATP and 1 mM EGTA, titrated to pH 7.0 (with KOH) and pCa 7.0 (with $CaCl_2$), total $[K^+]_{pipette} = 195$ mM. The reference recording solution was made up with 1–5 mM MES, titrated to ~pH 7.5 with Tris base and contained the following salts: 150 mM KCl, 10 mM $CaCl_2$ and 5 mM $MgCl_2$.

After tight seals (5–15 Gohm) had been formed by the usual procedures [12,14], the underlying membrane patch was broken by simultaneous application of light suction and a brief high voltage pulse (~900 mV, 100 μs). The main voltage-clamp protocol used in these experiments was a series of 2.5 s voltage steps, ranging from +100 mV to −200 mV, in 20 mV increments, and the resultant current traces have always been displayed super-imposed (Figs. 1 and 2). A more rapid voltage-clamp protocol, used in auxiliary, was a 5 s descending ramp, from +100 mV to −200 mV. In almost all cases the direct I-V diagram thus obtained was essentially identical to that obtained, under comparable test conditions, from the steady state pulse data. Data were pre-filtered at 3 kHz, sampled at 1 kHz and digitally filtered at 100 Hz, unless indicated otherwise.

Except as specifically noted (see especially Fig. 1), all current data are corrected for likely non-specific leakage by subtraction of the ohmic currents (linear, through the origin) from total measured currents, as demonstrated in Fig. 1.

3. Results

3.1. The inward currents depend on TRK2 but not on TRK1, at normal expression levels

The assumption that TRK-related currents in *S. cerevisiae* would be dependent on the Trk1 protein (see Section 1, above) was shaken by the observations of Bertl et al. [7,8], working with TrkΔΔ and the PC-1 strain [15] bearing a defective allele of *TRK1* but the normal *TRK2* gene. In those experiments, the inward currents were essentially identical between wild-type yeast and PC-1, but were missing in TrkΔΔ, implying that Trk2p, but not Trk1p, was responsible. Refinements and extensions of this observation are demonstrated in Fig. 1, from records made under standard conditions, except for the lowered extracellular pH ($pH_o = 5.5$). The four different strains bore the four combinations of normal and disrupted TRK genes. Evidentially, substantial inward currents were detected in cells harboring the wild-type *TRK2* gene independent of the presence of a TRK1 gene. In contrast, no significant inward currents were detected with *trk2*Δ cells (beyond the ohmic 'leak' currents), regardless of the status of the *TRK1* locus. Thus, *TRK1* disruption had no effect on these currents.

These records also demonstrate the quantitative effect of ionic leakage. The left column (Fig. 1A–D), obtained with the standard voltage-pulse protocol, displays current traces already corrected for the ohmic leakage. The right column (Fig. 1a–d) displays, for each of the four strains, an I-V curve obtained directly from an adjacent test, via the voltage-ramp protocol, on the same protoplast. The gray traces represent original data and the black traces the leak-corrected results. In both cases with intact *TRK2*, the corrected inward current at

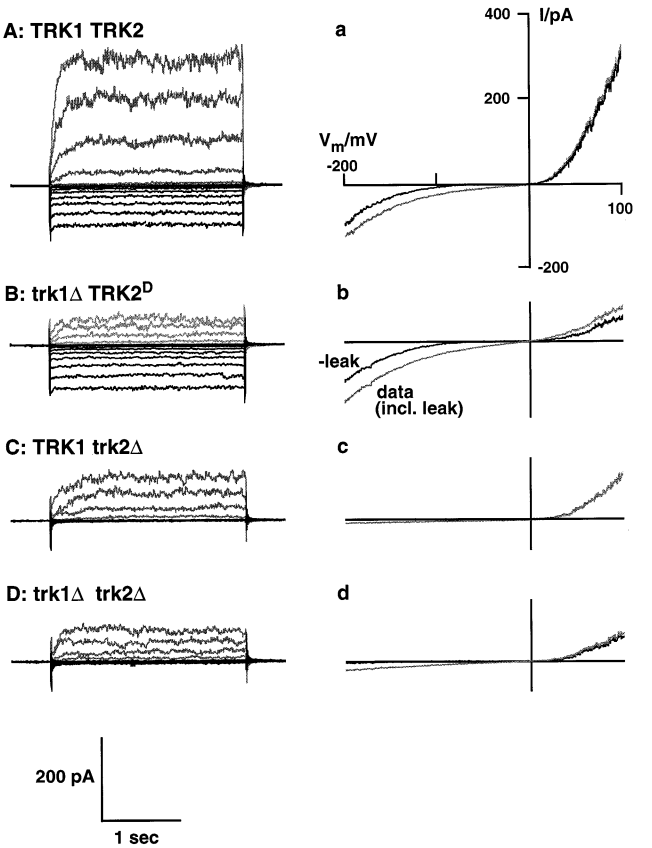

Fig. 1. Demonstration that TRK2, but not TRK1, underlies normal inward currents. Whole cell patch-clamp, using yeast strains with four different genotypes (critical genes indicated only). Left column: superimposed current records for the standard pulse protocol (see Section 2), corrected for the ohmic leak: 180 pS, 250 pS, 25 pS and 90 pS in A→D, respectively. Right column: I-V plots obtained directly by the standard ramp protocol. Gray traces: original data; Black traces: corrected by subtraction of the ohmic leak from the gray traces (slope through the origin): 190 pS, 250 pS, 30 pS and 85 pS in a→d, respectively. A, a: Pmr1Δ: *TRK1 TRK2 DUK1 pmr1*Δ (haploid). B, b: Trk1Δ: *trk1*Δ *TRK2 DUK1 PMR1* (diploid). C, c: Trk2Δ: *TRK1 trk2*Δ *DUK1 PMR1* (homozygous diploid). D, d: TrkΔΔ: *trk1*Δ *trk2*Δ *DUK1 PMR1* (homozygous diploid). Reference (standard) recording solution, but buffered at pH 5.5 (1 mM MES titrated with Tris) in all cases.

−200 mV (the largest tested membrane voltage (V_m)) was ~70% of the total inward current. With *TRK2* disrupted, no significant residual non-lineary inward current was observed. Another noteworthy aspect of these records is the large outward currents in the haploid yeast strain Pmr1Δ (A: *TRK1 TRK2*), compared with those in the other strains. Part of the discrepancy derives from the fact that Pmr1Δ protoplasts are often larger than protoplasts of other yeast strains (larger, for example, than many diploid and tetraploid protoplasts) and part may come from a legitimate down-regulation of *DUK1* expression in strains (Fig. 1B–D) with missing or defective TRK transporters.

3.2. Inward currents are enhanced by lowering the extracellular pH

The haploid yeast strain Pmr1Δ, with its Golgi resident calcium pump disrupted [16], has proven very durable in patch-clamp experiments, often permitting whole cell record-

ing for 2 h or more. This has allowed a thorough analysis of the effects of pH_o, among other parameters, on the Trk2p-dependent inward currents. In a typical experiment, lasting 75 min, 45 I-V tests were run, mixing the standard voltage-pulse protocol with the (faster) voltage-ramp protocol. Sets of several tests were run at each pH: 7.5 (control), 6.0 and 4.5, with each set bracketed by measurements at the control pH. Representative current traces from pulse tests at the three pH values are displayed in Fig. 2. The Trk2p-dependent inward currents (downward) were increased several-fold upon lowering the pH_o from 7.5 to 4.5.

The intrinsic experimental control afforded by the behavior of Duk1p channels was otherwise evident in this experimental sequence. In agreement with previous observations, changing extracellular pH in the range of 7.5–4.5 had little effect upon the steady state amplitude of the outward currents, [17].

This latter fact, as well as a direct comparison of Trk2p currents at the different levels of pH_o is emphasized in Fig. 3, which displays interspersed I-V curves obtained via the voltage-ramp protocol, from the same experiment as in Fig. 2. The pH-independence of the outward currents is obvious, with near super-position of the traces, positive to −40 mV, at the three values of pH_o: 7.5, 6.0 and 4.5. The clear pH-dependence of inward currents is demonstrated by the splay of the same three curves at voltages negative to −40 mV. Replotting of the data in Fig. 3 (black traces, lower left quadrant) reveals that the Trk2p-dependent inward current increased roughly linearly with a decreasing pH. The logarithmic, rather than linear, dependence of current upon the proton concentration suggests a role for the chemical potential (or electrochemical potential) of protons, rather than for simple chemical mass action of protons. The detailed dependence of Trk2p currents upon pH_o and membrane voltage will be described elsewhere (Bihler et al., in preparation).

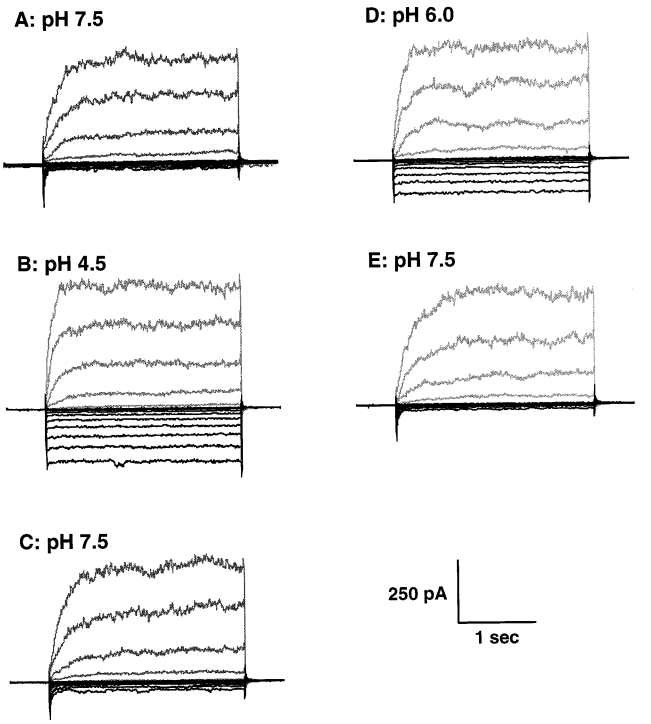

Fig. 2. Trk2p-related (inward) currents increase with lowered pH_o but Duk1p-mediated (outward) currents do not change significantly. Superimposed currents from the standard pulse protocol, with the Pmr1Δ strain (*TRK1 TRK2 DUK1 pmr1*Δ). Scans were run (in order, A–E) at ∼4.5 min, 13.5, 18.5, 24.5 and 33.5 min after the start of whole cell recording. Each scan was made after stabilization of the pH shift. All records were corrected for the ohmic leak. Within the pH range tested here, Trk2p-related currents (inward, downward) increased monotonically with decreasing pH. Reference recording solutions with pH_o varied by the Tris/MES ratio.

3.3. *Trk2p-dependent currents are not modulated by extracellular K^+*

Surprisingly, the Trk2p-dependent inward currents are insensitive to changes of the extracellular $[K^+]$. Fig. 4A shows leak-corrected I-V curves, obtained from a single Pmr1Δ protoplast tested in 150 mM $[K^+]_o$ and in 10 mM, 1 mM and 0 K^+, with potassium replaced by choline (voltage-ramp protocol). No significant reduction of the inward current was observed upon lowering the external K^+ from 150 mM to 10 mM or 1 mM. A minor change of the inward current can be detected upon the complete removal of external K^+. However, a slight increase of the inward current was evident, rather than a decrease, as expected for a conductance carried by K^+. Similar results were obtained at all pH_o values tested, 5.5 in the experiment of Fig. 4 and 4.5, 6.0 and 7.5 in separate experiments. This behavior probably reflects a small shift in the liquid junction potential between the bath and the reference electrode.

The dramatic reduction of the outward current upon removal of extracellular K^+ in these experiments provided, again, a useful check on procedures. It has been known for some time [18] that under certain conditions, the functional gating voltage for Duk1p shifts with E_K, the Nernst potential for the permeant ion, so that lowering the $[K^+]_o$ should increase the open probability for the channel at any given (positive) voltage. Recently, however, it has been discovered (H. Bihler, unpublished experiments) that reducing the $[K^+]_o$ to the range of 10 μM or below actually shuts down Duk1p. Thus, the small amplitude and upward convexity of the curves for outward current in 300 mM sorbitol certify that extracellular K^+ actually is reduced at least to the micromolar level, well below the expected range of affinities for K^+ binding to Trk2p [5,6].

There was also a systematic, though small, difference between tests run by replacing extracellular KCl with choline chloride and tests replacing extracellular KCl with sorbitol, with inward currents in the latter case appearing to be ∼10% smaller than in the former, as shown by comparison of the two 0-K^+ traces in Fig. 4B. As in the case of experiments with normal extracellular $[K^+]$ (see Section 4 of chloride removal in the next section, below), this discrepancy could be described as a slight negative shift of the whole I-V curve, produced by the reduction of extracellular chloride. The super-posability of chloride and sorbitol I-V curves, upon axial shifting, is demonstrated in Fig. 4C.

3.4. *Summary of additional characteristics: dependence on ATP, independence of extracellular Mg^{2+}, Ca^{2+} and Cl^-*

We had previously adopted the addition of 4 mM ATP (and Mg^{2+}) to pipette solutions for whole cell recording from *Saccharomyces* cells, because of the stabilizing effect of ATP on outward currents (through Duk1p [18,19]). This approach has also proven essential to the investigation of the Trk2-dependent inward currents. Indeed, whole cell record-

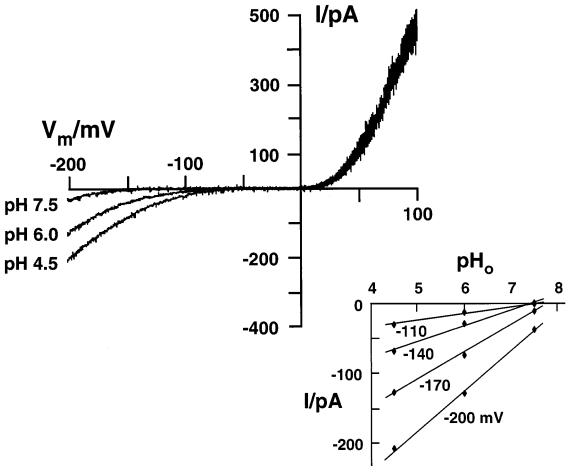

Fig. 3. Quantitative demonstration of the pH_o-dependence of inward currents. Representative I-V plots generated by interspersed voltage-ramp protocols in the experiment of Fig. 2, after subtraction of the ohmic leak. Data were filtered at 1 kHz. Note the systematic and stable increase of inward current at pH 6.0 and 4.5, compared with pH 7.5. Inset: parametric plot of the measured currents versus pH_o at several voltages, the straight lines confirm the similarity in shape of the three I-V curves.

ings with ATP-free pipettes showed no non-linear inward current and no enhancement of the inward currents with lowered pH_o.

The standard extracellular recording solutions for whole cell patch-clamp studies on *Saccharomyces* contain K^+, Mg^{2+}, Ca^{2+} and Tris as deliberately added cations. Changing $[Ca^{2+}]_o$, over the range 10 mM–100 mM did not influence the inward current. Testing lower extracellular concentrations of Ca^{2+}, however, was complicated by the fact that reducing the $[Ca^{2+}]_o$ below 1 mM clearly activated a non-selective, inwardly rectifying channel, NSC1 [20], passing very large inward currents (~nA). Fortunately, the latter currents proved to be blocked by a low extracellular pH ($pH_o = 4.0$), where the effect of reducing $[Ca^{2+}]_o$ to 100 nM or below could then be checked on the Trk-dependent currents. Although the ohmic (leakage) component of the inward current increased significantly with calcium removal (perfusion of the recording chamber with buffer containing 1 mM EGTA), no significant effects were seen on the Trk2p-specific inward currents

Mg^{2+} removal from extracellular solutions, either separately or simultaneously with Ca^{2+} removal, also had no measurable effect on the Trk2p-dependent currents. Finally, replacement of Tris with KOH also did not affect the inward currents, at least in the presence of millimolar Ca^{2+}.

Because a small chloride-dependent inward current across the yeast membrane, ~10 pA extrapolated to −200 mV has been reported (for haploid cells with $pH_o = 5.5$ [21]), we investigated whether the Trk2p-dependent inward currents described above might also be related to chloride. Removal of extracellular chloride ions, by replacement with gluconate, systematically reduced the inward currents slightly by ~15% at pH 5.5. In contrast, outward (Duk1p) currents were increased slightly by chloride lowering or removal and the overall effect, of reduction to 0, 1 or 10 mM, was a negative displacement of the entire membrane current-voltage curve by 8–12 mV. This was the same phenomenon observed in response to KCl removal during the low K^+ tests (Fig. 4B and C) and is most simply attributed to a small change in the liquid junction potential between the bath and the reference electrode.

3.5. Protons conduct the Trk2p-dependent inward current

The strong implication of the experiments described above, especially those represented in Fig. 2, is that the Trk2 protein somehow mediates a flow of protons into the yeast cytoplasm, which is reflected in the measured inward currents. A prominent feature of the pH-dependence curves, which was flagged by the linear parametric plots in Fig. 3 (inset), is that the I-V curves for different values of pH_o all have the same shape. In fact, as demonstrated in Fig. 5, the leak-corrected I-V curves at different pH's (for any one preparation) are super-imposable by a simple shift along the voltage axis. The required shift is rather precise: 50 mV in the direction of the changed E_H (reversal potential for the diffusion of H^+ ions) for each unit pH change over the range pH 7.5→6.0 and 31 mV over the range pH 6.0→4.5. These numbers are close to the expectation for a hypothetical carrier process moving two net

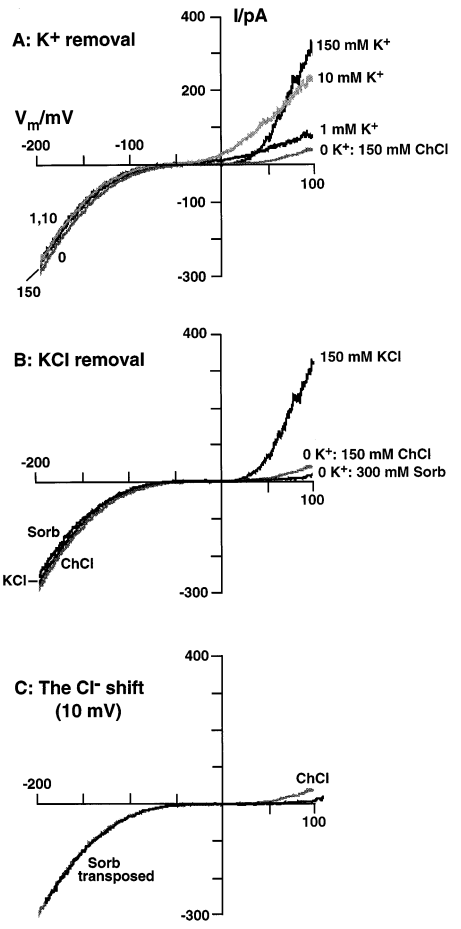

Fig. 4. Inward currents are not influenced by extracellular potassium. I-V plots via the voltage-ramp protocol on the strain Pmr1Δ, with A: varied extracellular K^+ concentration and B: varied $[K^+]_o$, with and without chloride. C: Superposition of 0-K^+ curves with KCl replaced by 150 mM choline chloride or 300 mM sorbitol (180 mM or 30 mM Cl^-, respectively) by a 10 mV positive shift of the latter, compensating a presumed small shift in the liquid junction potential at the reference electrode. (Small differences in inward currents between control and low K^+ tests (A) were not significant in cell to cell comparisons, either with choline replacement of $[K^+]_o$ or sorbitol replacement of $[KCl]_o$). The test pH_o was 5.5 in all three cases.

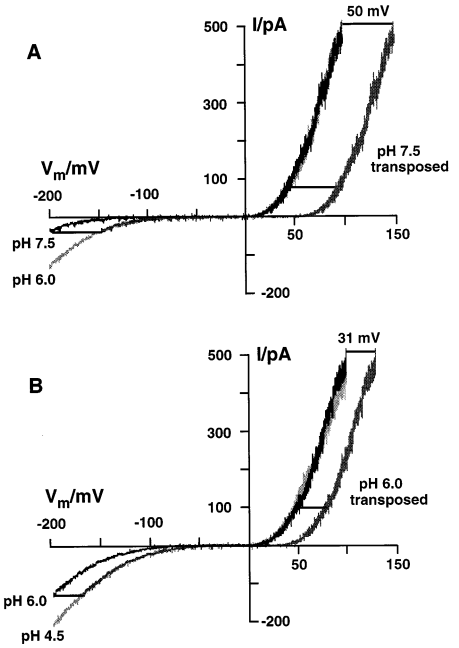

Fig. 5. Inward currents at different pH_o super-impose by a simple voltage shift. I-V curves from Fig. 3 re-plotted and transposed to demonstrate their super-posability. Because the outward (Duk1) current was not appreciably affected by pH_o, the voltage shift required for super-position of the inward currents was easily measured by the horizontal displacement of outward currents. Data were filtered at 1 kHz. Light gray traces and black traces: data curves re-plotted from Fig. 3. Dark gray traces: pH 7.5 trace transposed positive by 50 mV (A), pH 6.0 trace transposed positive by 31 mV (B). Horizontal bars indicate the displacements. See text for interpretation.

charges inward with each proton, over the higher pH range, and moving three net charges inward with each proton, over the lower pH range. (That is, 50 mV ≈ 44 mV = (1.5 × 58.5 mV)/2 and 31 mV ≈ 29 mV = (1.5 × 58.5 mV)/3). However, since the same thermodynamic argument would exclude protons as the second and third ion, there must be at least one missing ionic species. A primary task then, toward serious understanding the functioning of Trk2p, is to identify that missing ion.

4. Discussion

4.1. The relation of TRK2 currents to potassium transport

As outlined in the Section 1, the notion that the Trk2 protein is involved primarily with potassium uptake in yeast is based on three results. (i) Its sequence similarity with the yeast Trk1 protein, (ii) the effects of its deactivation and reactivation upon yeast growth phenotypes and (iii) its effects on direct K^+ flux measurements. In order to preserve this notion in the face of our present inability to demonstrate significant K^+-dependence of Trk2p currents, we must suppose that the potassium nutritional status of yeast (i.e. repletion versus starvation) modulates either the transport properties of Trk2p itself or the interaction of Trk2p with other K^+ transporter proteins. A detailed understanding of the situation would demand not only repeating the present kinds of experiments on potassium-starved cells, but also carefully comparing the K^+ influx (isotopic) and net H^+ flux with the measured electric currents.

Historically, indeed, the demonstration of K^+-H^+ symport during high affinity K^+ uptake by *Neurospora* [9] depended critically upon the comparison of three distinctly different measures of the transport process: current (net charge movement), dpH/dt (yielding net proton flux) and the unidirectional K^+ influx. If conditions exist in which either Trk1p or Trk2p operates as a K^+-H^+ symporter and operates against the background of proton efflux pumping by the plasma membrane H^+ ATPase, Pma1p, then the ratios of current:H^+ flux:K^+ flux should equal 2:1:1, with the (net) proton flux being outward. Superficially, then, the combined system of PMA1 and TRK should look like a 1:1 exchanger for protons and potassium.

On the basis of present data, however, we can address only the possibilities. Under the assumption that potassium starvation might alter the properties of Trk2p itself, proton transport via Trk2p as analyzed above (K^+ replete growth conditions) could arise in three distinctly different ways: either as a normal function, with Trk2p working as a K^+-H^+ symporter but with the K^+ concentration not influencing the current for kinetic reasons or as an alternative to K^+ transport, with Trk2p working as a uniport unable to bind K^+ or finally, as an abnormal or 'idling' function, when Trk2p is not functionally needed for the K^+ uptake. In the latter circumstance, the H^+ flux could arise as occasional channel-type activity, affecting only a small fraction of the Trk2p molecules present or it could arise as steady state carrier activity affecting a large fraction of Trk2p molecules in the membrane.

4.2. Relationship to other microbial K^+ transporters

Under the assumption that potassium starvation might alter the interaction of Trk2p with other proteins, the normal function of Trk2p would be to transport protons inward, but not coupled to other ionic processes during K^+ repletion. Then K^+ starvation would lead to interaction between Trk2p and other transporter molecules such as Trk1p (if that is present) to create K^+-H^+ cotransport, or Pma1p (the yeast proton pump) or even yet undiscovered K^+ transporter proteins (see below), thus modifying the properties of both to produce net K^+-H^+ countertransport.

In fact, sequence comparisons which have emerged from the rapidly expanding gene databases have pointed to strong structural similarities between the yeast Trk1 and Trk2 proteins and three other groups of membrane proteins. (a) Other fungal and plant K^+ transporters, including Trk and Trkh in *Schizosaccharomyces pombe* [22], Hkt1 in *Triticum aestivum* (wheat [22]) and T9A4 in *Arabidopsis thaliana* (GenBank Accession number AF096373), (b) bacterial K^+ transporters like TrkH and TrkG in *Escherichia coli* [23,24] and KtrB in *Vibrio alginolyticus* [24,25] and finally, (c) a series of bacterial K^+ channels, which include KcsA in *Streptomyces lividans*, YugO in *Bacillus subtilis*, Aq303 in *Aquifex aeolicus* and Hp0490 in *Helicobacter pylorii* [22,24]. (More specifically, all three groups of proteins align in a simple fashion to reveal a core sequence resembling that of bona fide K^+ channels, two hydrophobic (transmembrane) helices bounding a hydrophilic P-loop containing a glycine signature motif for K^+ channels (GYG in the channels, usually GLS or GFS in the carriers). While the bacterial channels, typical of K^+ channels generally, each comprise a single core and assemble as a homotetramer to form the functional protein, most of the carriers contain a string of four cores within a single polypeptide. This view of the Trk1 and Trk2 sequences would revise the predicted num-

ber of membrane-spanning segments downward from 12 [2,4] to eight [24]. Explicit folding data are available only for the single protein which has been crystallized, the KcsA channel [26]).

The implied structural similarities between the yeast TRK transporters and the bacterial TrkH and KtrAB systems, in particular, are unlikely to be limited to the folding of a single polypeptide molecule, but almost certainly extend to intermolecular associations and functions. In *E. coli*, then, the TrkH system functions as a certified hetero-oligomer, bound to a smaller NAD^+-binding peptide (TrkA) and an ATP-binding peptide (TrkE) which belongs to the ABC class of regulatory proteins [23]. In *V. alginolyticus*, KtrB, the main transporter protein, is also bound to a small NAD^+-binding accessory protein, KtrA. These bacterial systems may provide a useful starting point for identifying other protein components of the yeast TRK transporters as well as for exploring how the state of potassium starvation or repletion modulates K^+ transport functions in *Saccharomyces*.

4.3. Chemiosmotic balance

Regardless of its role in potassium transport, per se, the discovery that Trk2p mediates substantial inward proton currents through the yeast plasma membrane appears to resolve a long standing major problem in the general, and now well-accepted, view that fungi and plants fuel most of the membrane transport with proton currents driven ultimately by their plasma membrane proton pump.

The problem has been that resting membrane conductances in these organisms are usually high enough to require pumped outward proton currents to be much larger than observed net H^+ effluxes. In mature hyphae of *Neurospora crassa*, for example, the normal membrane conductivity is $\sim 10^{-4}$ S/cm^2 and the resting voltage is circa -200 mV, requiring an outward proton current of ~ 20 μA/cm^2 (reviewed in Slayman et al. [27]). Translated for the surface/volume ratio of the cells (*Neurospora* hyphae are cylinders ~ 15 μm in diameter), this means a proton flux of roughly 35 mmol/kg cell water/min or about 10-fold the steady state proton release rate observed under standard laboratory conditions (respiring on glucose and ammonium at pH 6), and is approximately equal to the proton release rate observed at high pH_o ($\sim$pH 9).

The easy resolution to this problem has always been that there must be a large proton re-entry current, which in the absence of explicit coupling to required nutrient flux (sugars, amino acids, K^+, etc.) is normally wasteful, from a strictly energetic point of view. The Trk2-dependent currents in yeast, as reported above, are of approximately the correct size to mediate this required steady state H^+ influx.

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