Trk1 and Trk2 Define the Major K^+ Transport System in Fission Yeast

Fernando Calero, Néstor Gómez, Joaquín Ariño and José Ramos


Updated information and services can be found at:
http://jb.asm.org/content/182/2/394

These include:

This article cites 29 articles, 10 of which can be accessed free at:
http://jb.asm.org/content/182/2/394#ref-list-1

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»
Trk1 and Trk2 Define the Major K⁺ Transport System in Fission Yeast

FERNANDO CALERO,† NÉSTOR GÓMEZ,‡ JOAQUÍN ARÍÑO,§ AND JOSÉ RAMOS†

Departamento de Microbiología, Escuela Técnica Superior de Ingenieros Agrónomos y Montes, 14080 Córdoba,† and Departamento de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra 08193, Barcelona, Spain‡

Received 13 August 1999/Accepted 25 October 1999

The trkI⁺ gene has been proposed as a component of the K⁺ influx system in the fission yeast *Schizosaccharomyces pombe*. Previous work from our laboratories revealed that trk1 mutants do not show significantly altered content or influx of K⁺, although they are more sensitive to Na⁺. Genome database searches revealed that *S. pombe* encodes a putative gene (designated here trk2⁺) that shows significant identity to trk1⁺. We have analyzed the characteristics of potassium influx in *S. pombe* by using trk1 trk2 mutants. Unlike budding yeast, fission yeast displays a biphasic transport kinetics. trk2 mutants do not show altered K⁺ transport and exhibit only a slightly reduced Na⁺ tolerance. However, *trk1 trk2* double mutants fail to grow at low K⁺ concentrations and show a dramatic decrease in Rb⁺ influx, as a result of loss of the high-affinity transport component. Furthermore, *trk1 trk2* cells are very sensitive to Na⁺, as would be expected for a strain showing defective potassium transport. When *trk1 trk2* cells are maintained in K⁺-free medium, the potassium content remains higher than that of the wild type or trk single mutants. In addition, the *trk1 trk2* strain displays increased sensitivity to hygromycin B. These results are consistent with a hyperpolarized state of the plasma membrane.

An additional phenotype of cells lacking both Trk components is a failure to grow at acidic pH. In conclusion, the Trk1 and Trk2 proteins define the major K⁺ transport system in fission yeast, and in contrast to what is known for budding yeast, the presence of any of these two proteins is sufficient to allow growth at normal potassium levels.

In cell-walled eukaryotic cells, the intracellular concentration of K⁺ is quite constant (in the range of 10⁻¹ M), whereas the concentration of Na⁺ varies from insignificant (less than 10⁻⁷ M) to values, in saline environments, very close to those of K⁺. In terrestrial environments, however, the levels of K⁺ and Na⁺ are highly variable; the norm is that potassium in the external media is several orders of magnitude less concentrated than inside the cell, whereas sodium is several times more concentrated. To maintain these asymmetric ionic distributions across the plasma membrane, different types of potassium transporters have evolved in cell-walled eukaryotic cells, all of them driven by the membrane potential created by the H⁺ pump ATPase (29).

Two different families of potassium transporters responsible for the uptake of the cation have been found in fungi. Transporters of the HAK type are present in mycelial fungi (10) and for the uptake of the cation have been found in fungi. Trans-

*Corresponding author. Mailing address: Departamento de Bioquímica i Biologia Molecular, Facultat de Veterinària, Ed. V, Universitat Autònoma de Barcelona, Bellaterra 08193, Barcelona, Spain. Phone: 34-93-5812182, Fax: 34-93-5812006 E-mail: J.Arino@cc.uab.es.

†Departamento de Microbiología, Escuela Técnica Superior de Ingenieros Agrónomos y Montes, 14080 Córdoba.

‡and Departamento de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra 08193, Barcelona, Spain.

§ phone: 34-93-5812182, Fax: 34-93-5812006 E-mail: J.Arino@cc.uab.es.

The trkI⁺ gene has been proposed as a component of the K⁺ influx system in the fission yeast *Schizosaccharomyces pombe*. Previous work from our laboratories revealed that trk1 mutants do not show significantly altered content or influx of K⁺, although they are more sensitive to Na⁺. Genome database searches revealed that *S. pombe* encodes a putative gene (designated here trk2⁺) that shows significant identity to trk1⁺. We have analyzed the characteristics of potassium influx in *S. pombe* by using trk1 trk2 mutants. Unlike budding yeast, fission yeast displays a biphasic transport kinetics. trk2 mutants do not show altered K⁺ transport and exhibit only a slightly reduced Na⁺ tolerance. However, *trk1 trk2* double mutants fail to grow at low K⁺ concentrations and show a dramatic decrease in Rb⁺ influx, as a result of loss of the high-affinity transport component. Furthermore, *trk1 trk2* cells are very sensitive to Na⁺, as would be expected for a strain showing defective potassium transport. When *trk1 trk2* cells are maintained in K⁺-free medium, the potassium content remains higher than that of the wild type or trk single mutants. In addition, the *trk1 trk2* strain displays increased sensitivity to hygromycin B. These results are consistent with a hyperpolarized state of the plasma membrane.

An additional phenotype of cells lacking both Trk components is a failure to grow at acidic pH. In conclusion, the Trk1 and Trk2 proteins define the major K⁺ transport system in fission yeast, and in contrast to what is known for budding yeast, the presence of any of these two proteins is sufficient to allow growth at normal potassium levels.
equally to potassium influx and that they define the major potassium uptake system in fission yeast.

MATERIALS AND METHODS

Media and growth of E. coli and S. pombe strains. E. coli NM522 cells were grown at 37°C in Luria-Bertani medium containing 50 mg of ampicillin per ml for plasmid selection. S. pombe cells were grown at 28°C in YES medium (0.5% yeast extract, 3% glucose, 225 mg each of adenine, uracil, and leucine per ml) or essential minimal medium supplemented with the necessary requirements (18). The pH of the medium was buffered at 5.5 with 20 mM MES (2-(morpholinooethanesulfonic acid). In some experiments mineral medium (containing 30 mM ammonium phosphate and 8 mM ammonium sulfate) with slight modifications (5× vitamins), supplemented with the auxotrophic requirements, was used (1). All S. pombe strains described in this report derive from the wild-type strain 117 (h2 ade6-M210 ura4-D18 leu1-32).

Recombinant DNA techniques and gene disruptions. E. coli cells were transformed by the standard calcium chloride method (28). S. pombe cells were transformed by a modification of the lithium acetate method (19). Standard recombinant DNA techniques were performed essentially as described elsewhere (28).

The construction of strain LB9 (trk1::LEU2) has been described previously (3).

FIG. 1. Sequence comparison of the S. pombe Trk1 and Trk2 putative potassium transporters. Pairwise alignment of the Trk1 and Trk2 amino acid sequences (accession no. P47946 and Q10065, respectively) were performed by the Clustal W method (open gap penalty, 10; extended gap penalty, 0.2). Identical amino acids are boxed and highlighted. The residue number for each protein is indicated at the right. Asterisks denote putative transmembrane domains.

VOL. 182, 2000 K TRANSPORT IN FISSION YEAST 395

<table>
<thead>
<tr>
<th>Trk1</th>
<th>Trk2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQLSGFSTNG SGRASVPCKE KLLFKHNF... SQQSDPECK MLKVL</td>
<td>46</td>
</tr>
<tr>
<td>TQFSTTSHV YDAFALASSA TVECSVLA INSISTITCC ISLYDAYAIVT</td>
<td>89</td>
</tr>
<tr>
<td>GSS... NLXG IDAFLASSA CVQCEFYY ITQSFALCD TILLCPFLST</td>
<td>93</td>
</tr>
<tr>
<td>LAVXEPSIF TRK1 TRK2 KONQNRTR KPQCONQL MKSHE...</td>
<td>136</td>
</tr>
<tr>
<td>TTVYKLGLF FYVQTDYK YKYPN... KNTNTY TYHTVRST</td>
<td>156</td>
</tr>
<tr>
<td>HEGVGYD TK VRYPLPYLS LRSFSLQEP SRFDTQSNP NYPYDPNP</td>
<td>185</td>
</tr>
<tr>
<td>FCRPKVIXK XQTVQGONQ NRWRAPEPT AEEAQE PDK KMRHHR</td>
<td>185</td>
</tr>
<tr>
<td>TADLSDMS... YVKLYELE KSDTLDENG VWMTFDYD PEISRRH...</td>
<td>229</td>
</tr>
<tr>
<td>MCFPASFG AFRKNTAF LPSVAYSQ RPQISRPQ AKEQSGT...</td>
<td>235</td>
</tr>
<tr>
<td>YEG... SGHE SADDYKEMD DLDLRLKGD SISS... VN KNTNDRR</td>
<td>273</td>
</tr>
<tr>
<td>SLDKRVCCV YQANARNFVQ YIQESFEK PCTCFIPE SFQGQUN</td>
<td>285</td>
</tr>
<tr>
<td>LSEGLVQRQ LPMAYESDT MLYSVNQF LLQDDNLFPE CGLP FAPNQ</td>
<td>323</td>
</tr>
<tr>
<td>QLHHRGBS S... HSHN PSETAEGCN EYSSBSNFI STQAGDD</td>
<td>330</td>
</tr>
<tr>
<td>GINCLUSSS S LSDKISPSQ PQQEIAFPG IRRKOFFER N LKGQKRKR</td>
<td>371</td>
</tr>
<tr>
<td>PHVGQSWON NQFQHQAAT SQAKBNQVRV GSAAFTAPT PFRMNGH</td>
<td>380</td>
</tr>
<tr>
<td>FVYYKLRLSRF SKPHELFT WQTVSPSNRR NITL... ... YVSASFAK</td>
<td>414</td>
</tr>
<tr>
<td>KADDINGAQ KREPLAFGQ GRGRPLAPV TPATENRS MSYFPFQAT</td>
<td>430</td>
</tr>
<tr>
<td>TCSRKLAC VCMSVYTVI IFPHAAFTL IVGVSQTVQVR VQVSVYDLR</td>
<td>505</td>
</tr>
<tr>
<td>ATEYRLHI LTVLIVYVL FHKLIGAV LHTYHAKV KVQTVGDDWN</td>
<td>530</td>
</tr>
<tr>
<td>ROWNQCCS S NQDLNPMQ IPEVRPMNE MLILLSSL HPAQVDT</td>
<td>555</td>
</tr>
<tr>
<td>RDCWCSNS S KGDLNPMQ NQDLNPMQ AMEPGLGTL LEPP</td>
<td>560</td>
</tr>
<tr>
<td>CFOVRTPWT YKLIFEPFKE KFIATLAFR PRPFTILF TGAVWYLFV</td>
<td>605</td>
</tr>
<tr>
<td>IPEAIRFFM IRTRFPFNF CGQLYFEPF PRPFTILF SKTQWLVL</td>
<td>620</td>
</tr>
<tr>
<td>LALWIDLV LTVYFYSEK ASPASLQ WNPAPQCV VTPACGFAA</td>
<td>655</td>
</tr>
<tr>
<td>NFAKPASFF FQWPSWSF QKPRNKA FAAPQCV VTPACGFAA</td>
<td>680</td>
</tr>
<tr>
<td>DCRPADVY SKPHELTVI KVAYPMNM YASEYCRV TR... TR</td>
<td>702</td>
</tr>
<tr>
<td>LSFQPAVW TPQTPMIS AFTVYPMNM YASEYCRV TR... TR</td>
<td>730</td>
</tr>
<tr>
<td>G... SFQK DBKLYEFTQ TPQTPMIA TPQTPMIA</td>
<td>728</td>
</tr>
<tr>
<td>NINNNBNNND MTPKCVL MLQKVLDPQL HNYQAKME</td>
<td>780</td>
</tr>
<tr>
<td>NLFYTDISRL TVLTYFAY VTFCSSQGL SSCKIQE RXTISKLLE</td>
<td>797</td>
</tr>
<tr>
<td>SEAEPPFQH AIYKFTYTV TVCGSFQCK ... NPKLPPFQPKRKLKV</td>
<td>828</td>
</tr>
<tr>
<td>ALRGHRMIL ERPBDPAF LEDQNNKLE KEDORRNF SIDAAGSIA</td>
<td>837</td>
</tr>
<tr>
<td>AQVRGHRMIL ERPBDPAF LEDQNNKLE KEDORRNF KNTAAGDPVP</td>
<td>878</td>
</tr>
</tbody>
</table>

The construction of strain LB9 (trk1::LEU2) has been described previously (3).
Gene disruptions were made by using the one-step gene disruption method (27). Disruption of the gene trk2* was made as follows. A 4.76-kbp fragment containing the gene was amplified from genomic DNA obtained from strain 117 by PCR with oligonucleotides 5'GCTAGCATTCTTTCTCTAAATAG-3' and 5'CATATAAGCATCATTCCCCAAATCG-3' and cloned into plasmid pUR18 (Promega) via restriction digestion with EcoRI and NheI, which remove residues 77 to 446 of the trk2* coding region. To construct an ura4+ disruption cassette, the ura4+ gene was amplified by PCR from plasmid pUR18 (5) with oligonucleotides 5'-GCTAGCATTCTTTCTCTAAATAG-3' and 5'-CCATGTGATTTCATTACATTC-3' (added NheI and NcoI sites, respectively, are underlined) and cloned into pGEM-T (Promega). The 1.6-kbp marker was then released by digestion with NheI and NcoI and cloned into these sites of the above-mentioned trk2* construct. The disruption cassette (4.4 kbp) was recovered with ClaI/PvuII (which yields the ura4+ marker flanked by 0.463 and 2.581 kbp of trk2* sequences) and used to transform wild-type and LB9 cells to generate strains NG1 (trk2::ura4+) and NG2 (trk1::LEU2 trk2::ura4+), respectively.

To construct a LEU2 deletion cassette for trk2, a plasmid harboring the LEU2 marker (2) was cleaved with NcoI, blunt ended with the Klenow fragment, cleaved with NheI, and then ligated into the EcoRV/NheI sites of the above-mentioned trk2* plasmid. The disruption cassette (3.7 kbp) was recovered with AccI (which yields the LEU2 marker flanked by 1.143 and 0.951 kbp of trk2* sequences) and used to transform wild-type cells to yield strain NG3.

In all cases, positive clones were selected by plating in essential minimal medium plates lacking the appropriate supplement, and disruptions were verified at the molecular level by PCR analysis.

Determination of sodium sensitivity of yeast strains. Sensitivity to sodium chloride was tested in liquid cultures by inoculating 96-well microtiter plates containing medium with different salt concentrations at an initial OD550 of 0.05. Cells were grown for about 20 h with shaking, and growth rate determined by measuring the absorbance at 550 nm (OD550).

Uptake experiments and cation contents of the cells. K+ starved cells were obtained by suspending cells with a normal K+ content in K+-free ammonium phosphate medium for 5 h (5). To study uptake of Rh1+ (used as an analog of K+), K+ starved cells were resuspended in uptake buffer [10 mM MES brought to pH 5.5 with Ca(OH)2, containing 0.1 mM MgCl2, and 2% glucose]. RhCl3 (0.1 to 100 mM) was added at time zero; at different times, samples of cells were taken, filtered, and treated for determination of intracellular Rh1+. Uptake was linear with time up to 15 min, and the initial uptake rate was obtained from the slope of the line. Kinetics parameters were calculated from Edle-Hofstee plots of the experimental data.

Potassium loss was determined in cells grown in ammonium phosphate medium supplemented with 50 mM KCl up to an optical density at 550 nm (OD550) of 0.5. Cell were recovered by centrifugation and resuspended in the same medium lacking added KCl. Samples of cells were taken at different times, filtered, and treated for intracellular potassium content determination.

The intracellular cation (Rh1+, K+, and Na+) content of the cells was determined as previously described (21, 25). Briefly, samples of cells were filtered, washed with 20 mM MgCl2, and treated with HCl, and the cations were analyzed by atomic absorption spectrophotometry.

RESULTS

S. pombe contains a gene encoding a protein structurally related to trk1*. The finding that S. pombe cells lacking a functional trk1* gene did not show an evident impairment in potassium uptake or altered potassium requirements prompted us to examine the S. pombe genomic database maintained at the Sanger Center. A BLAST search using the entire trk1*-encoded protein revealed the existence of a putative gene, located at chromosome I (accession no. Z68136), that codes for a 880-residue protein 34% identical (almost 50% similar) to the trk1* gene product. A more detailed analysis (Fig. 1) revealed a number of features that supported the possibility that this putative gene, here called trk2*, might be a homolog of trk1*.

In essence, both genes are roughly of the same size and display very similar hydrophobic profiles (not shown). Similarly to S. pombe Trk1 and S. cerevisiae Trk1 and Trk2, 12 transmembrane domains can be predicted for S. pombe Trk2. These elements are placed essentially at the same positions as those predicted for fission yeast Trk1 and define two regions within the Trk proteins: three transmembrane domains lie within the first 150 residues, whereas the remaining are grouped within the second half of the polypeptide. In fact, these two regions display the highest levels of identity between fission yeast Trk1 and Trk2 (45 and 51%, respectively).

Lack of Trk1 and Trk2 results in increased potassium requirements. The striking similarities between fission yeast Trk1 and Trk2 led us to isolate trk2* and to disrupt this gene in wild-type and trk1Δ cells. Here we present a detailed analysis of the growth characteristics of wild-type, trk1Δ, trk2Δ, and trk1 trk2Δ strains at different potassium concentrations in the medium. Figure 2A shows that under the conditions tested, the single mutants grew similarly to the wild-type strain, whereas growth of the double mutant was severely affected at low potassium. None of the strains were able to grow at external KCl concentrations as low as 1 mM (not shown). To confirm these observations, we performed experiments in liquid media con-
However, in the case of the double mutant, the characteristics of K\(^+\) transport were completely different. Rb\(^+\) influx was hardly observed at micromolar concentrations, and the kinetics of transport were monophasic, with \(V_{\text{max}}\) of 17 nmol/mg/min and \(K_{0.5}\) of 17 mM for the first phase; \(V_{\text{max}}\) of 17 nmol/mg/min, and \(K_{0.5}\) of 6.8 mM for the second phase). However, in the case of the double mutant, the characteristics of Rb\(^+\) transport were completely different. Rb\(^+\) influx was hardly observed at micromolar concentrations, and the kinetics of transport were monophasic, with \(V_{\text{max}}\) of 17 nmol/mg/min and \(K_{0.5}\) of 17 mM. Therefore, the lack of growth at low K\(^+\) concentrations observed for the trk1 trk2 strain can be explained on the basis of a failure to take up potassium when the external levels of this cation are too low.

**trk1 trk2 mutants are highly sensitive to sodium ions.** Previous work on characterization of the trk1 mutant (3) identified a phenotype of increased sensitivity to Na\(^+\) ions. We have extended this study to the whole set of trk mutants (Fig. 4). Under the conditions tested in this work, the wild-type strain shows a 50% inhibitory concentration (IC\(_{50}\)) for NaCl of about 140 mM. This value is only slightly reduced (128 mM) in the trk1::LEU2 cells (strain NG1). The use of the marker ura4\(^+\) instead of LEU2 for disruption of trk2 (strain NG3) gave essentially the same results (not shown). The change observed is somewhat less prominent than that observed upon disruption of trk1 (IC\(_{50}\) of 110 mM). Interestingly, the double mutant was very sensitive to Na\(^+\) (IC\(_{50}\) of 62 mM), indicating that the lack of both Trk components results in a dramatic effect on growth under sodium stress. This growth defect is in keeping with the observation that cells lacking both trk1 and trk2 genes fail to maintain a proper Na\(^+\)/K\(^+\) intracellular ratio. This has been evaluated by growing wild-type and double mutant NG2 cells in the presence of 10 mM KCl plus 100 mM NaCl. Under these conditions, the intracellular levels of sodium and potassium in wild-type cells were 35 ± 6 and 430 ± 30 nmol/mg of cells, respectively. Under the same conditions, NG2 cells accumulated 95 ± 8 nmol of sodium per mg of cells, with an intracellular concentration of potassium ions of only 160 ± 15 nmol/mg of cells.

**Lack of Trk transporters might result in hyperpolarization of the plasma membrane.** Wild-type, trk1, trk2, and trk1 trk2 cells contained similar amounts of internal potassium when grown at nonlimiting potassium concentrations (around 400 nmol of K\(^+\)/mg of cells). When these cells are suspended in K\(^+\)-free medium, potassium is immediately lost; after 3 to 5 h, the equilibrium is reached and internal potassium levels become stable (Fig. 5). In these conditions the cells were viable, and interestingly, wild-type and single mutants retained less potassium than the double mutant. This result could be explained on the basis of a failure to take up potassium when the external levels of this cation are too low.
explained on the basis of a hyperpolarized state of the plasma membrane of the \textit{trk1 trk2} strain. The resistance of yeast cells to the antibiotic hygromycin B has been also related to their membrane potential. If our hypothesis were correct, NG2 cells would display an altered tolerance to this compound. We tested (Fig. 6) hygromycin B tolerance in wild-type and \textit{trk} mutants and found that the \textit{trk1 trk2} double mutant was clearly more sensitive to the antibiotic. Interestingly, whereas \textit{trk2} cells behaved like the wild-type strain, \textit{trk1} cells appeared to be somewhat more sensitive to the drug. It must be noted that these effects cannot be attributed to an altered function of the H\textsuperscript{+}-ATPase, since measurement of proton efflux yielded essentially identical results (30 \pm 2 and 28 \pm 4 nmol of H\textsuperscript{+}/mg of cells/min for wild-type and NG2 cells, respectively).

Low pH sensitivity requires the absence of both \textit{trk1} and \textit{trk2}. It has been reported that in budding yeast, a phenotype associated with lack of the \textit{TRK1} gene (but not of \textit{TRK2}) is the hypersensitivity to low pH. This phenotype appears to be the consequence of the impaired potassium uptake, because high concentrations of K\textsuperscript{+} restore growth of these cells under low-pH conditions (11). We have tested the effect of acidic pH on growth of cells lacking the Trk components. The results (Fig. 7) indicate that in \textit{S. pombe}, the lack of a single \textit{trk} gene does not impair growth even at a pH as low as 3. However, the \textit{trk1 trk2} double mutant fails to grow even at pH 4.5 when the amount of potassium ions in the medium is relatively low (30 mM). Our data indicate that these cells cannot grow at pH 3 even in the presence of 100 mM KCl. Therefore, in contrast with the evidence described for budding yeast, lack of both \textit{trk} genes is necessary to confer low pH sensitivity to fission yeast cells.

**DISCUSSION**

Our recent finding that disruption of the \textit{trk1} gene in \textit{S. pombe} did not result in significant changes in potassium requirements or in potassium influx (3) indicated that despite the ability of the Trk1 protein to behave like a potassium transporter in a heterologous system, alternative potassium uptake systems should exist in fission yeast. These observations drew our attention to a related, uncharacterized gene (\textit{trk2}) found during the \textit{S. pombe} systematic sequencing project. We show here that deletion of \textit{trk2} does not result in changes in potassium requirements or in potassium influx. Furthermore, sodium tolerance in \textit{trk2} mutants decreases only marginally. In fact, deletion of this gene fails to significantly alter sodium tolerance in the sodium-hypertolerant \textit{pzh1} (2) background (not shown). Interestingly, simultaneous deletion of both \textit{trk1} and \textit{trk2} results in a strong requirement for potassium in the medium, defective rubidium uptake, and dramatically increased sodium sensitivity. These results indicate that in \textit{S. pombe} the Trk1 and Trk2 proteins have largely equivalent functions, since deletion of both genes is needed to observe a clear-cut mutant phenotype. This situation is different from what has been described for budding yeast, where, as mentioned above, the role of one of the Trk proteins (Trk1) is largely predominant. On the other hand, the strong Na\textsuperscript{+} sensitivity observed in \textit{trk1 trk2} mutants provides further support for the notion that in fission yeast, an efficient potassium transport is an important factor for sodium tolerance, a circumstance that has been previously documented for budding yeast (8).
We have analyzed the kinetics of rubidium transport in *S. pombe* and observed that this is a biphasic process, with a high-affinity and a low-affinity component. This is again different from the situation described for *S. cerevisiae*, which under the same conditions shows a high-affinity, monophasic potassium transport (24, 26), or even for other yeast types such as *S. occidentalis* (5) or *Debaryomyces hansenii* (21). Interestingly, a biphasic uptake process is well documented in fungi (22) and higher plants (14). Our data clearly show that trk1 and trk2 are similarly responsible for the high-affinity uptake in fission yeast, since this component is fully present in the single mutants and completely absent in cells lacking both genes. It should be stressed that our data do not rule out the possibility that the low-affinity process observed in trk1 trk2 mutants is different in nature from that observed in wild-type cells.

A remarkable observation is that after K\(^+\) starvation, trk1 trk2 cells retain more potassium than do wild-type cells. This could be explained if one assumes that the defect in potassium influx results in a hyperpolarization of the plasma membrane. This effect has been recently documented for *S. cerevisiae* (15) and is supported by our observation that *S. pombe* trk1 trk2 cells are more sensitive to hygromycin B, an aminoglycoside drug for which the resistance of the cells depends on their membrane potential (16, 20).

In conclusion, in this report we show that the kinetics of rubidium transport in fission yeast is more similar to that of higher plants than to that of other yeast cells such as *S. cerevisiae*, on the basis that it exhibits a biphasic process. The high-affinity component of this process is driven by the Trk1 and Trk2 transporters, which define the major uptake system in fission yeast. However, and in contrast to what has been described for budding yeast, the roles of the fission yeast Trk proteins are essentially equivalent.

**ACKNOWLEDGMENTS**

Nestor Gómez and Fernando Calero contributed equally to this work.

The contribution of L. Balcells at the earliest stage of this work as well as the skillful technical help of Anna Vilalta and Mircea Zugrăvere are acknowledged. We thank Alonso Rodríguez-Navarro for fruitful discussion and the Sanger Center for maintaining and allowing free access to the *S. pombe* genome data bank.

This work was supported by grants PB98-0565-C4-02 and PB98-1036 (Dirección General de Investigación Científica y Técnica, Spain) to J.A. and J.R., respectively, by an Ajunt de Suport als Grups de Recerca de Catalunya (SGR97-127) from the Generalitat de Catalunya to J.A., and by grant BIO4-CT97-2210, from the European Union to J.R.

**REFERENCES**