Potassium Transport by Amino Acid Permeases in Saccharomyces cerevisiae*

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Deletion of the potassium transporter genes TRK1 and TRK2 impairs potassium uptake in Saccharomyces cerevisiae, resulting in a greatly increased requirement for the ion and the inability to grow on low pH medium. Selection for mutations that restored growth of trk1Δ trk2Δ cells on low pH (3.0) medium led to the isolation of a dominant suppressor that also partially suppressed the increased K⁺ requirement of these cells. Molecular analysis revealed the suppressor to be an allele of BAP2 that encodes a permease for branched chain amino acids. The suppressor mutation (BAP2-1) converts a phenylalanine codon, highly conserved among the amino acid permease genes, to a serine codon in a region predicted to lie within the sixth membrane-spanning domain. Generation of the analogous mutation in the histidine permease produced an allele, HIP1-293, that similarly suppressed the low pH sensitivity of trk1Δ trk2Δ cells. Suppression of trk1Δ trk2Δ phenotypes by BAP2-1 or HIP1-293 was correlated with increased Rb⁺ uptake. The presence of the substrate amino acids enhanced but was not essential for suppression of trk1Δ trk2Δ phenotypes and increased Rb⁺ uptake. The conserved site altered by the suppressor mutations appears to be important; his4 HIP1-293 cells show an increased requirement for histidine compared with his4 HIP1 cells.

Potassium transport in Saccharomyces cerevisiae is primarily dependent on the product of the TRK1 gene. Mutations in TRK1 lead to a significant increase in the requirement for potassium due to decreased uptake of the ion (1, 2). Cells in which TRK1 is deleted lose the ability to grow on medium containing micromolar concentrations of potassium but readily grow on medium containing millimolar concentrations of the ion due to the presence of a second, highly related transporter encoded by TRK2 (3, 4). Under conditions where transcription of TRK2 is genetically repressed, trk1Δ TRK2 cells regain the ability to grow on micromolar potassium (5, 6). Consistent with the ability of Trk2 under these conditions to suppress the trk1Δ phenotype, kinetic analysis showed that cells expressing either TRK1 or TRK2 exhibit high affinity K⁺ uptake compared with trk1Δ trk2Δ cells (7). Thus, the dual affinity potassium uptake activities in S. cerevisiae identified by Rodriguez-Navarro and Ramos (8) appear to require Trk1 and Trk2 for high affinity transport and other, unidentified pathways for low affinity transport.

Proteins highly related to Trk1 have been identified in Saccharomyces uvarum (9), Schizosaccharomyces pombe (10), and Neurospora, but few details have been discerned regarding their mechanism of transport. The Trk proteins do not contain sequences that are conserved in ion-transporting ATPases, nor do they contain the signature sequences of K⁺ channels. Recent electrophysiological analysis has addressed the participation of Trk proteins in K⁺ transport more directly. Whole cell patch-clamp experiments with wild-type S. cerevisiae cells detected Trk-dependent inward K⁺ currents in response to hyperpolarizing membrane potentials (11). These results strongly suggest that K⁺ transport via the Trk system is a passive process driven by the membrane potential. Trk-dependent K⁺ currents were not detected until the membrane potential was increased beyond −100 mV (11), which is consistent with previous estimates of the membrane potential in S. cerevisiae (12, 13).

Disruption of TRK1 and TRK2 significantly impairs the ability of the cell to take up K⁺, resulting in a 1,000-fold increase in the K⁺ requirement for growth (3). The hyperpolarization-dependent inward K⁺ currents observed with wild-type cells by whole cell patch clamp analysis were essentially abolished in trk1Δ trk2Δ cells (11). However, the membrane potential that appears to be the driving force for K⁺ uptake is intact. This is supported by the functional expression of Arabidopsis K⁺ channels in trk1Δ trk2Δ cells. Expression of Kat1 restores the ability of trk1Δ trk2Δ cells to grow on very low concentrations of potassium (14). Because the inward Kat1-dependent K⁺ channels are also activated by hyperpolarization beyond −100 mV (11, 15), the membrane potential in trk1Δ trk2Δ cells is probably at least of this magnitude and thus can provide sufficient driving force for the uptake of K⁺ ions provided a portal of entry is available.

Such routes of monovalent cation transport have been previously described for bacterial systems. Dosch et al. (16) showed that expression of the wild-type tetracycline/H⁺ antiporter resulted in partial suppression of a K⁺ transport-defective Escherichia coli mutant by increasing K⁺ uptake. More recently, it was shown that expression of the wild-type Ca²⁺/H⁺ antiporter gene cheA from E. coli and the wild-type tetracycline/H⁺ antiporter gene tetA(L) from Bacillus subtilis restore Na⁺ transport activity in Na⁺/H⁺ antiport-defective mutants of E. coli (17, 18). By selecting for suppressors in trk1Δ trk2Δ cells that restore the ability to grow on low potassium medium, we have obtained dominant mutations in genes encoding glucose trans-

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1 A. Rodriguez-Navarro, personal communication.
porters (19), the galactose transporter,2 and one of the inositol transporters3 among such revertants.

A second phenotype of trk1Δ trk2A cells is their hypersensitivity to low pH. Whereas wild-type cells can grow quite well on medium adjusted below pH 3.0, growth of trk1Δ trk2A cells is severely inhibited below pH 4.5. This phenotype appears to be the consequence of the impaired potassium uptake because high concentrations of K+, but not sodium or sorbitol, restore growth of trk1Δ trk2A at low pH (4). Further support of this interpretation is provided by the observation that K+ uptake by the K+-selective channel Kat1 fully suppresses the low pH sensitivity of trk1Δ trk2Δ cells (14). In this report, we describe the isolation and analysis of mutations at a highly conserved site in the amino acid permease genes BAP2 and HIP1 that suppress the low pH hypersensitivity and, to a lesser degree, the potassium requirement of trk1Δ trk2Δ cells by conferring increased K+ (Rb+) uptake. We present evidence that suggests wild-type amino acid permeases are also somewhat permeable to K+ ions.

MATERIALS AND METHODS

Strains and Media—S. cerevisiae and E. coli strains used in this study are listed in Table I.YPD, YNB, sporulation medium, and routine genetic manipulations are described by Sambrook (4). YEPD and YP media were made by adding the appropriate amount of filter-sterilized D-glucose to water. All amino acid permeases were made by omitting ammonium sulfate from the recipe and adding the appropriate amount of filter-sterilized amino acids as nitrogen sources were made by omitting ammonium sulfate and were made as described previously (1, 2). YNB media containing amino acids in the growth medium were prepared by bacterial transformations were performed by electroporation (21).

DNA Manipulations—Yeast genomic DNA, miniprep DNA, restriction endonuclease analysis, and gel electrophoresis were performed as described. DNA hybridization analysis was performed using Quikhyb (Stratagene) according to the directions of the manufacturer. DNA probes were prepared by incorporation of [α-32P]dCTP by random priming of denatured DNA fragments isolated by gel electrophoresis (23). Random hexamers were obtained from Pharmacia Biotech Inc. (23). [α-32P]dCTP was obtained from DuPont NEN.

Cloning and DNA Sequence Analysis of BAP2 and RPD4-1 (BAP2-1)—A S. cerevisiae genomic library was constructed by methods previously described (24) from a trk1Δ trk2A RPD4-1 (BAP2-1) mutant strain (MS40). Basically, size-selected (10–15-kb), 4 partially Sau3AI-digested genomic DNA fragments were cloned into the yeast centromeric shuttle vector pRS116 (25), and the ligated reaction was transformed directly into a trk1Δ trk2A recipient strain (CY152; Table 1). Transforms were selected on permissive medium (pH 5.9) lacking uracil and then replica-plated to identify potential RPD4-1 (BAP2-1) clones by their ability to suppress the negative growth phenotype of trk1Δ trk2A cells on low pH medium (pH 4.0). A single plasmid, pGM200, was obtained that conferred suppression of the low pH sensitivity of trk1Δ trk2A cells. pGM200 was rescued by transformation in E. coli and was tested to confirm that its reintroduction into trk1Δ trk2A cells by transformation led to suppression of the low pH sensitivity. A 2.6-kb EcoRV-HindIII fragment from pGM200 was subcloned into pRS316 (25) producing pKM19-1, which was capable of suppressing the trk1Δ trk2A phenotype. The sequence of both strands of this cloned fragment was determined using synthetic oligonucleotide primers (Beckman Oligo 1000 DNA Synthesizer Inc.) and Sequenase kit (U. S. Biochemical Corp.).

The wild-type allele of the suppressor gene was recovered by an integration and excision experiment with plasmid pMW131. pMW131 was constructed by subcloning a 745-bp HindIII-ClaI fragment that encompassed the 5′ end of RPD4 (BAP2) from pMKM2-1 into the integrative plasmid pRS306 (25). Only about 60 bp of the subcloned fragment overlapped with the coding region of RPD4 (BAP2). pMW131 was linearized with SpolI and used to transform strain CY152 to Ura-. Genomic DNA from one transformant was prepared, digested with XhoI, recircularized with T4 DNA ligase, and used to transform E. coli to ampicillin resistance. The DNA sequence of the 2.6-kb EcoRV-HindIII region of the cloned fragment in the resulting plasmid (pMW245) was determined using the same panel of oligonucleotide primers used for the RPD4-1 (BAP2-1) allele. DNA sequence analyses and comparisons were performed with the DNA Inspec II software program (Textco), Geneworks (Intelligenetics), and the package of programs of the Genetics Computer Group (26).

Construction of Strains Containing a Null Allele of BAP2—A deletion of 1.25 kb of the BAP2 coding region was made by the integration-replacement method (gamma deletion) (25). A plasmid capable of disrupting BAP2 (pMW133) was created by subcloning the 745-bp HindIII-ClaI fragment described above and a 450-bp XbaI-HindIII fragment corresponding to the region immediately 3′ to the BAP2 coding region into the yeast integrative vector pRS306 (25). Plasmid pMW133 was linearized by HindIII digestion and used to transform both diploid (MW156) and haploid (CY152 and MS40) recipient strains to Ura-. About half of the trk1Δ trk2A BAP2-1 Ura+ transformants lost the suppressor phenotype, indicating that they contained a disruption of the BAP2 locus. The other half had become His+ evidently due to recombination between pMW133 and the resident plasmid at the trk2Δ::HIS3 locus resulting in replacement of the HIS3 marker with URA3. The presence of the bap2Δ deletion in individual transformants was verified by Southern blot analysis (data not shown).

The bap2Δ::URA3 haploid strains MW158 and MW159 are pMW133 integrants of strains CY152 and MS40, respectively (Table 1).

Construction of Prototropic BAP2 Strains—To determine if suppression of the trk1Δ trk2A phenotypes by RPD4-1 (BAP2-1) required amino acids in the growth medium, trk1Δ trk2Δ bap2 strains lacking further auxotrophic requirements were generated. Briefly, strain MW158 was crossed with strain CY152, and meiotic segregants were dissected yielding strains MW79A-2D and MW79A-3B, which require only tryptophan (Table 1). Selectable marker replacement (27) was performed to convert bap2Δ::URA3 alleles to bap2Δ::TRP1 alleles by transformation of MW79A-2D and MW79A-3B with HindIII-digested plasmid pRS304 (25). All Trp+ transformants were tested to identify those that had become Ura- and had increased the BAP2::URA3 locus. This strategy yielded strains MW178 and MW179 that are able to grow on yeast minimal medium after transformation with URA3-containing plasmids. MW178 and MW179 were transformed with BAP2-1 and BAP2-1 alleles carried by centromeric (pKM22-1 and pMW136) and multicopy (pMW137 and pMW138) plasmids.

Construction of the HIP1-293 Allele—The 2.2-kb HindIII-XhoI fragment of plasmid pPL241 containing the functional HIP1 gene (kindly provided by P. Ljundahl and G. Fink) was subcloned into plasmid pSelect1 (Promega), and oligonucleotide-directed mutagenesis was performed according to the suggested protocol. The oligonucleotide 5′-CGAATAAAGAGGGAGCCGGTTG-3′, which is complementary to the HIP1 coding strand, was used to introduce a single T to C transition in the second nucleotide of amino acid codon 293. The presence of the mutation was verified by determining the DNA sequence of this region. Both the wild-type BAP2-1 allele and the mutant HIP1-293 allele were subcloned into the yeast integrative vector pRS316 (25) and pRS426 (28).

Rb+ Uptake Assays—Cells were grown in either YNB and uracil or YNB and uracil with all amino acids supplemented with 30 mM KCl. After 20 h of incubation, cells were harvested by centrifugation and suspended in uptake buffer (10 mM MES adjusted to pH 5.8–6.0 with Ca(OH)2, containing 0.1 mM MgCl2, 2% glucose, and either uracil alone or with a mixture of all amino acids (totaling 2%). After 2–3 min of incubation in uptake buffer, RbCl (100 mM) was added, and the velocity of uptake was determined during the first 7–10 min. Samples of cells were harvested by filtration, washed with 20 mM MgCl2, and solubilized in HCl. The rubidium content of each sample was analyzed by atomic absorption spectrophotometry.

RESULTS

Isolation of the RPD4-1 Mutant—Mutations that suppress the low pH hypersensitivity of trk1Δ trk2Δ cells were isolated by selecting for spontaneous revertants able to grow on YPD medium supplemented with 100 mM KCl but adjusted to pH 4.0. Genetic analysis of these mutants indicated the presence of both recessive (to be reported elsewhere) and dominant suppressors. The dominant suppressors also partially suppressed the increased potassium requirement conferred by the trk1Δ trk2A mutations and were thus designated RPD4 for their reduced potassium requirement. Recombination tests between

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the dominant suppressors revealed a single representative, RPD4-1, of the locus described here. Tetrad analysis of meiotic segregants from a cross between the trk1Δ trk2Δ RPD4-1 mutant and a compatible trk1Δ trk2Δ strain revealed that the suppressor mutation segregated as a single Mendelian factor (not shown).

Cloning of RPD4-1 and RPD4—To identify the RPD4 gene, a library of genomic DNA fragments made from the dominant RPD4-1 strain MS40 was screened for clones that could suppress the low pH sensitivity and potassium requirement of trk1Δ trk2Δ cells. A single suppressor clone was obtained, and a smaller subclone (pKM22-1) that conferred suppression of the trk1Δ trk2Δ phenotypes was chosen for molecular analysis. The wild-type RPD4 gene, recovered on a 2.6-kb HindIII fragment by an integration and excision experiment (see "Materials and Methods"), was subcloned onto both single copy and multi-copy plasmids (pMW136 and pMW137) and transformed into a trk1Δ trk2Δ recipient (CY152). Both plasmids failed to suppress the trk1Δ trk2Δ phenotypes, confirming that the cloned fragment contained the wild-type allele of the suppressor locus.

RPD4-1 Is an Allele of BAP2—DNA sequence analysis of the 2.6-kb HindIII-EcoRV fragment that contained the functional suppressor revealed a single long open reading frame of 609 amino acid codons, which predicts a protein of 67 kDa. Hydrophyly analysis (29) of the protein sequence revealed 12 putative membrane-spanning domains (M1–M12). A search of the S. cerevisiae genome revealed that the open reading frame encoding the RPD4-1 allele was essentially identical to open reading frame YBR068c discovered through the genomic sequencing project (30) and independently identified as BAP2, an amino acid permease capable of transporting branched chain amino acids (31). A comparison with related sequences revealed that Rpd4, henceforth referred to as Bap2, shares greatest sequence identity with the putative amino acid permease Pap1 (78%) (32) and least sequence identity (32%) with the proline permease, Put4 (33).

Effects of bap2Δ Mutations—The ability of trk1Δ trk2Δ cells to grow on K+–supplemented medium suggests that plasma membrane proteins other than Trk1 or Trk2 must be capable of mediating K+ uptake. To determine if Bap2 plays a major role in non-Trk-mediated potassium uptake, a null allele of BAP2 containing a 2.5-kb deletion of the open reading frame (bap2Δ) was generated in trk1Δ trk2Δ ura3-52 BAP2 and trk1Δ trk2Δ ura3-52 BAP2-1 recipient cells (strains CY152 and MS40; see "Materials and Methods" for details). As predicted, deletion of the BAP2 sequences in the trk1Δ trk2Δ BAP2-1 host abolished suppression of the trk1Δ trk2Δ phenotype. The trk1Δ trk2Δ bap2Δ cells exhibited no apparent growth defects compared with the pretransformed cells confirming that BAP2 is nonessential (31). In addition, the potassium requirement of trk1Δ trk2Δ bap2Δ cells, assessed by testing for growth on medium containing various concentrations of the ion, was not increased compared with the isogenic trk1Δ trk2Δ BAP2 cells (data not shown). Thus, wild-type Bap2 does not provide a major average of K+ uptake in trk1Δ trk2Δ cells and is not responsible for their ability to grow on 100 mM K+.

The shr3 Mutation Is Epistatic to BAP2-1—The product of the SHR3 gene has been shown to be localized within the endoplasmic reticulum and is believed to be required for the processing of most, if not all, newly synthesized amino acid permeases in S. cerevisiae (34). Although amino acid permeases are retained in the endoplasmic reticulum in shr3 mutants, other membrane proteins, including the H+-ATPase, are apparently unaffected. Thus, the role of Shr3 appears to be specific for the processing and/or targeting of amino acid permeases. To assess whether or not processing of Bap2 is dependent on SHR3, an shr3 null allele was generated in a trk1Δ trk2Δ BAP2-1 strain to test for epistasis, i.e. loss of the BAP2-1 suppressor phenotype. The shr3Δ mutation abolished the ability of trk1Δ trk2Δ BAP2-1 cells (strain MW210) to grow on low pH and on low potassium medium but only modestly increased the K+ requirement of trk1Δ trk2Δ cells expressing the wild-type BAP2 allele (strain MW211; data not shown). In addition, the K+ requirement of trk1Δ trk2Δ cells containing the shr3Δ mutation (strain MW236) was indistinguishable from that of trk1Δ trk2Δ cells harboring the wild-type SHR3 gene (strain MW235; data not shown), suggesting that the activity of Shr3 is not required for maturation of Trk-related proteins.

Suppression of trk1Δ trk2Δ Phenotypes by BAP2-1 Does Not Require the Presence of Amino Acids—In S. cerevisiae amino acid permeases apparently function as H+/amino acid-coupled symporters. To test whether or not the BAP2-1 mutation might have altered the specificity of an ion-binding site while leaving the amino acid transport coupling mechanism unaffected, the ability of BAP2-1 to suppress trk1Δ trk2Δ phenotypes was assessed in the absence of amino acids (Fig. 1), indicating that suppression of the trk1Δ trk2Δ phenotypes is not dependent on the uptake of amino acids. Thus, if the suppressor mutation in BAP2-1 alters

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Table I

| Strain                | Genotype†                                      |
|-----------------------|-----------------------------------------------|
| E. coli               |                                               |
| DH5a                  | F−, ΔphoA, ΔlacZAM15, endA1, recA1, hsdS17, supE44 thi1, gryA96, relA1, ΔlacZYA-argF, U169, r−, m−, relA1, supE44, Δlac-proAB [P]+, traΔ36, proAB, ilvG [ΔZAM15] |
| JM109                 |                                               |
| S. cerevisiae         |                                               |
| MS40                  | MATa trk1Δ hisΔ200 his4Δ15 trk1Δ trk2Δ::HIS3 |
| CY152                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| CY152                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW156                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW158                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW159                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW178                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW179                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW210                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW211                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW235                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |
| MW236                 | MATa trk1Δ hisΔ200 lys9 trk1Δ trk2Δ::HIS3    |

† All strains were generated in this laboratory.
FIG. 1. Suppression of trk1Δ trk2Δ phenotypes by BAP2-1 is not dependent on the presence of amino acids. The strains are MW179 (Table I) transformed with plasmids pRS316, pKM22-1 (BAP2-1 single copy), and pMW138 (BAP2-1 high copy). Cells were grown on permissive medium and then replicated to YNB without amino acids containing either 100 μM or 7 μM K⁺ at permissive pH (5.9) or 100 μM K⁺ at pH 3.0.

The putative proton translocation capability of the permease to allow potassium uptake, this new capability is not obligatorily coupled to amino acid symport.

The BAP2-1 Suppressor Mutation Alters a Highly Conserved Site—The DNA sequence of the 2.6-kb HindIII fragment of pMW243 containing the wild-type BAP2 gene was determined and compared with the BAP2-1 sequence. A single difference between the two sequences was found: a T (BAP2) to C (BAP2-1) transition of the second nucleotide of codon 299 resulting in a phenylalanine to serine substitution in the deduced amino acid sequence. This site is located within the putative sixth transmembrane domain of the transporter and occurs at a site highly conserved among S. cerevisiae amino acid permeases. All but one of the members of the amino acid permease gene family in S. cerevisiae contain phenylalanine at this site (Fig. 2). In the case of Tat2, the tyrosine permease, a conservative substitution to tyrosine is present at this position. The conservation of this site suggests that it plays an important role in the structure/function of amino acid permeases.

The Analogous F → S Mutation in HIP1 Suppresses trk1Δ trk2Δ Phenotypes—If the conserved phenylalanine residue that is changed to serine in BAP2-1 plays an important role in transport, an equivalent mutation in another amino acid permease might also suppress the trk1Δ trk2Δ phenotypes. To test this hypothesis, the analogous mutation was made in the histidine permease gene, HIP1 (Fig. 2). Codon 293 was converted from a phenylalanine to a serine codon by site-directed mutagenesis. The resulting allele (HIP1-293) was subcloned onto both centromeric and multi-copy plasmids (pRS316 and pRS426). The HIP1-293 plasmids were able to suppress both the sensitivity to low pH and the potassium requirement of a trk1Δ trk2Δ recipient (Fig. 3A). Like BAP2-1, suppression of the trk1Δ trk2Δ low potassium and low pH phenotypes could be detected on medium lacking amino acids, indicating that suppression could occur independently of histidine transport. Cells harboring the HIP1-293 allele on the multi-copy plasmid exhibited slightly stronger suppression of the trk1Δ trk2Δ potassium requirement compared with cells that expressed the suppressor from the centromeric plasmid (Fig. 3A), suggesting that transport of K⁺ by Hip1-293 was rate-limiting for growth when expressed as a single copy gene.

Although expression of the wild-type HIP1 allele from a centromeric plasmid did not suppress either of the trk1Δ trk2Δ phenotypes, expression from a multi-copy plasmid modestly suppressed the low pH phenotype (Fig. 3A), suggesting that wild-type amino acid permeases may transport small amounts of potassium. In further support of this hypothesis, deletion of SHR3 in trk1Δ trk2Δ cells resulted in slightly weaker growth on medium containing 100 mM potassium compared with congenic trk1Δ trk2Δ SHR3 cells. This was not due to a K⁺ transport-independent effect on growth because deletion of SHR3 in TRK1 cells had no effect on growth even under potassium-limiting conditions.

BAP2-1 and HIP1-293 Increase Rb⁺ Uptake in trk1Δ trk2Δ Cells—To determine if BAP2-1 and HIP1-293 increase potassium uptake, Rb⁺ uptake assays were performed using the trk1Δ trk2Δ recipient strain that was transformed with either the vector (pRS316) or plasmids expressing the wild-type or suppressor alleles of the amino acid permeases. Because Bap2 transports branched-chain amino acids and the presence of these substrates in the growth medium results in greatly increased BAP2-1 transcription (35), Rb⁺ uptake assays were performed using cells grown either in the presence or the absence of amino acids in the growth medium and in the uptake assay bath. The velocity of Rb⁺ uptake in trk1Δ trk2Δ cells expressing the BAP2-1 allele was indistinguishable from trk1Δ trk2Δ cells harboring the wild-type allele when the cells were cultured in minimal medium supplemented only with uracil (to satisfy their auxotrophic requirement). In contrast, the rate of Rb⁺ uptake increased approximately 2–3-fold in trk1Δ trk2Δ BAP2-1 cells when cultured under inducing conditions (Table II). Increased Rb⁺ uptake was not observed with trk1Δ trk2Δ cells expressing the wild-type BAP2 gene under these conditions. The presence of amino acids in the assay buffer slightly increased Rb⁺ uptake by trk1Δ trk2Δ BAP2-1 cells.

The expression of HIP1-293 in trk1Δ trk2Δ cells conferred increased Rb⁺ uptake compared with cells expressing the wild-type HIP1 allele (Table II). The ability to detect this increase was again dependent on culturing the cells in the presence of

| Potassium (mM) | 100 | 100 | 7 | 7 | 100 | 100 |
|---------------|-----|-----|---|---|-----|-----|
| pH            | 5.9 | 5.9 | 5.9 | 5.9 | 3.0 | 3.0 |

| Amino acids | + | - | + | - | + | - |

Fig. 2. Alignment of the sixth putative membrane-spanning domain of S. cerevisiae amino acid permeases with known substrates. The arrow indicates the site of mutation to serine in BAP2-1 and HIP1-293. Sites completely conserved are indicated by uppercase letters in the consensus line. Sites in which a single substitution has been observed are indicated by lowercase letters in the consensus. Swissprot accession numbers are indicated to the right of the sequences.

5 M. B. Wright and R. F. Gaber, unpublished results.
amino acids and again enhanced slightly by the addition of amino acids (or histidine) to the bath during the assay. The greatest effect was obtained when histidine was added in the absence of other amino acids (Table II), suggesting that amino acid transport increased K<sup>+</sup> uptake by the methods used in our assays.

The requirement of amino acids in the growth medium to detect Bap2-1 or Hip1-1-mediated Rb<sup>+</sup> uptake is in contrast with the ability of BAP2-1 or HIP1-1 to suppress the trk1Δ trk2Δ phenotype on minimal medium. Evidently the threshold for increased growth of trk1Δ trk2Δ cells on potassium-limiting or low pH medium is lower than that required to detect an increase in Rb<sup>+</sup> uptake by the methods used in our assays.

*DISCUSSION*

The loss of high affinity K<sup>+</sup> transport, mediated by the products of the TRK1 and TRK2 genes, confers strong negative phenotypes including a thousand-fold increase in potassium required to support growth and the inability to grow on medium below pH 4.5. The negative phenotypes associated with trk1Δ or trk1Δ trk2Δ cells have allowed several genetic approaches to be taken toward understanding K<sup>+</sup> transport. These include the molecular and functional analysis of heterologous proteins that restore K<sup>+</sup> uptake and the analysis of suppressor mutations. The first approach has led to the identification of K<sup>+</sup> transporters from other yeasts (9, 37) and wheat (38) and voltage-gated K<sup>+</sup> channels from Arabidopsis (14, 39), whereas the second approach is revealing that a variety of S. cerevisiae membrane proteins, including sugar transporters (19),<sup>6</sup> the inositol transporter,<sup>3</sup> and amino acid transporters (this report) can be genetically altered to confer increased potassium transport.

Here we describe a dominant, spontaneous mutation in the amino acid permease gene BAP2, isolated as a suppressor of the low pH-sensitive phenotype of trk1Δ trk2Δ cells. Consistent with previous observations that either high concentrations of potassium in the growth medium (3) or the expression of a heterologous K<sup>+</sup> transporter (14) can completely suppress this phenotype, we show that Bap2-1 confers a 3–4-fold increase in the rate of K<sup>+</sup> uptake by trk1Δ trk2Δ cells. Thus, suppression of the low pH sensitivity by Bap2-1 appears to reflect the acquisition of an alternative K<sup>+</sup> transport pathway in these cells. A mutation analogous to BAP2-1 was generated in the histidine permease (HIP1), and it similarly suppressed trk1Δ trk2Δ phenotypes by conferring a severalfold increase in the rate of K<sup>+</sup> uptake, suggesting that the ability of a single amino acid substitution to convert amino acid permeases into proteins capable

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<sup>6</sup>C. Ko and H. Liang, unpublished results.

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**Table II**

| Strain                  | Growth medium<sup>a</sup> | Assay bath | Rb<sup>+</sup> uptake<sup>b</sup> |
|-------------------------|---------------------------|------------|----------------------------------|
| BAP2 (CEN)              | –                         | –          | 6.0 ± 0.5                        |
|                         | –                         | +          | 6.5 ± 0.6                        |
|                         | +                         | +          | 5.8 ± 0.7                        |
|                         | +                         | +          | 6.6 ± 0.5                        |
| BAP2–1 (CEN)            | –                         | –          | 5.7 ± 1.0                        |
|                         | +                         | +          | 6.0 ± 0.6                        |
|                         | +                         | +          | 13.0 ± 1.7                       |
| HIP1 (CEN)              | –                         | –          | 5.2 ± 0.9                        |
|                         | –                         | +          | 5.5 ± 0.6                        |
|                         | –                         | –          | 5.0 ± 0.8                        |
|                         | –                         | +          | 5.5 ± 0.7                        |
|                         | +                         | +          | 5.2 ± 0.5                        |
|                         | –                         | –          | 5.4 ± 0.9                        |
| HIP1–293 (2μ)           | –                         | –          | NT                               |
|                         | –                         | +          | NT                               |
|                         | +                         | +          | 11.0 ± 0.8                       |
|                         | +                         | +          | 12.7 ± 1.4                       |
|                         | +                         | +          | 13.9 ± 0.8                       |
|                         | +                         | +          | 14.0 ± 1.0                       |
|                         | +                         | +          | 15.4 ± 1.9                       |
|                         | +                         | +          | 18.0 ± 0.8                       |

<sup>a</sup> Cells were grown in YNB supplemented with uracil only (–) or with uracil with amino acids (+; see "Materials and Methods" for details).

<sup>b</sup>Velocity of Rb<sup>+</sup> uptake is mm/mg cells (dry weight)/min. NT, not tested.

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**Fig. 3. Effects of the HIP1–293 mutation.** A, the HIP1–293 mutation confers suppression of the low pH sensitivity and K<sup>+</sup> requirement of trk1Δ trk2Δ cells. The yeast strain is CY152 (trk1Δ trk2Δ) containing a plasmid alone (pRS316), BAP2-1 (pKM22-1), and wild-type HIP1 and the mutant HIP1–293 genes in the plasmids pRS316 (CEN) or pRS426 (2μ). All strains were grown on permissive medium (100 mM K<sup>+</sup>, pH 5.9) and then replica-plated to low K<sup>+</sup> (7 mM K<sup>+</sup>, pH 5.9) and low pH (100 mM K<sup>+</sup>, pH 3.0) medium. B, the HIP1 and HIP1–293-containing plasmids were transformed into a his4-15 hip1-1 strain R770 (Table I) and tested for their ability to suppress the histidine requirement by replica-plating to a series of medium containing different concentrations of histidine.
of transporting K⁺ ions may be a general property of this family of transporters.

The BAP2-1 and HIP1-293 mutations substitute serine for a highly conserved phenylalanine predicted to reside within the sixth membrane-spanning domain. We have shown that this site is important for amino acid transport in the case of the histidine permease. The suppressor mutations replace a large, hydrophobic side-chain of amino acid for a small polar one. The increased permeability to K⁺ conferred by this mutation could be a consequence of the disruption of closely packed transmembrane helices or a change in the polarity at this position. The ability of calcium to inhibit suppression of trkΔΔ transport capability. An attractive possibility is that H⁺/amino acid symporters may not have an absolute requirement for H⁺ as a co-substrate and may be capable of coupling K⁺ ions to drive amino acid uptake. If so, the analysis of mutations that confer increased K⁺ uptake by amino acid symporters may identify residues or domains important for the ion-coupled symport mechanism.

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Potassium Transport by Amino Acid Permeases in *Saccharomyces cerevisiae*

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