Molecular Microbiology (2000) **37**(3), 671–679

Individual functions of the HAK and TRK potassium transporters of *Schwanniomyces occidentalis*

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Summary

We have cloned the gene encoding the TRK transporter of the soil yeast *Schwanniomyces occidentalis* and obtained the *HAK1 trk1Δ* and the *hak1Δ TRK1* mutant strains. Analyses of the transport capacities of these mutants have shown that (i) the HAK1 and the TRK1 potassium transporters are the only transporters operating at low and medium K^+ concentrations (< 1 mM); (ii) the HAK1 transporter is functional at low pH but fails at high pH; and (iii) the TRK1 transporter functions at neutral and high pH and fails at low pH. At neutral pH, both transporters are functional, but HAK1 is not expressed, except at very low K^+ concentrations (< 50 μM) where HAK1 is very effective. TRK1 is also involved in the control of the membrane potential.

Introduction

Potassium is an important cellular constituent, which is normally maintained at concentrations ranging from 50 mM to 500 mM in the cytoplasm of all living cells. Because of this requirement, K^+ must be taken up from the external medium, in which K^+ may be at a fairly high and constant concentration (1–5 mM), as in sea water and in the milieu bathing animal cells, or at low and highly variable concentrations, as in many terrestrial environments. In animal cells, K^+ uptake has been studied extensively, but much less is known about this process in eukaryotic non-animal cells. *Saccharomyces cerevisiae* was the first fungal species in which the molecular basis of K^+ uptake was completely clarified (Ko and Gaber, 1991; Gaber, 1992). This clarification was an important breakthrough. However, *S. cerevisiae* is adapted to fruit products with high K^+ contents, and its K^+ transporters may be different from those existing in other fungi adapted to the more nutritionally limiting life in soil. Probably because of its environmental adaptation, *S. cerevisiae* is furnished exclusively with transporters of one sole type, TRK1 and TRK2 (Rodríguez-Navarro, 2000). The same exception applies to *Schizosaccharomyces pombe*, in which the K^+ transporters also seem to be of the TRK type only (Calero *et al.*, 2000).

Soil is probably the environment in which the K^+ concentration varies most, as do the concentrations of other cations, such as H^+, Na^+ and Ca^{2+}, which affect K^+ uptake in fungi and plants. Probably as a means of adaptation to these complex conditions, K^+ uptake in plants and in soil fungi is mediated by at least three different types of transport systems, TRK-HKT and HAK transporters and K^+ channels (Rodríguez-Navarro, 2000). *Neurospora crassa* is the soil fungus in which K^+ uptake has been studied most extensively. *N. crassa* is furnished with a TRK transporter similar to the *S. cerevisiae* transporters, but also with a HAK transporter, which belongs to a family of K^+ transporters that are apparently better adapted than the TRK transporters to operate at low K^+ concentrations (Haro *et al.*, 1999). In *Arabidopsis thaliana* and probably in other plants, root K^+ uptake is thought to be mediated by HAK and HKT transporters (HKT transporters belong to the TRK family) co-existing with inward-rectifying K^+ channels (Rodríguez-Navarro, 2000). Although the genes and cDNAs encoding these transporters have been cloned, expressed in heterologous systems and studied quite extensively, the understanding of the overall process of K^+ uptake in plants and soil fungi is still conditioned by incomplete knowledge of the contribution of each transport system to the overall process.

To establish each individual contribution, the disruption of the genes encoding the transporters is the most powerful approach. These disruptions have been attained in *S. cerevisiae*, *S. pombe* and *Arabidopsis thaliana*, but a general picture of K^+ uptake in fungi and plants has not yet been deduced from these experiments, except in *S. cerevisiae*. Making the problem even more difficult, in all the cases studied so far, the disruption of K^+ transporters resulted in a hyperpolarization of the cells, both in fungi (Madrid *et al.*, 1998; Calero *et al.*, 2000) and in *A. thaliana* (Hirsch *et al.*, 1998; Spalding *et al.*, 1999).

Schwanniomyces occidentalis was the first eukaryotic organism in which a HAK K^+ transporter was identified (Bañuelos *et al.*, 1995). The HAK transporter of *S. occidentalis* is very active and apparently makes the

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