

## Towards an understanding of the molecular basis of plants K<sup>+</sup> transport: Characterization of cloned K<sup>+</sup> transport cDNAs

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### Abstract

Recently, two K<sup>+</sup>-transport cDNAs, *KATI* and *AKTI*, were cloned in *Arabidopsis thaliana*. These cDNAs had structural similarities to K<sup>+</sup> channel genes in animals, and also conferred the ability for growth on micromolar levels of K<sup>+</sup> when expressed in K<sup>+</sup> transport-defective yeast mutants. In this study, we examined the possibility that *KATI* encodes the high-affinity K<sup>+</sup> transport system that has been previously characterized in plant roots, by studying the concentration-dependent kinetics of K<sup>+</sup> transport for *KATI* expressed in *Xenopus* oocytes and *Saccharomyces cerevisiae*. In both organisms, the K<sup>+</sup> transport system encoded by *KATI* yielded Michaelis-Menten kinetics with a high K<sub>m</sub> for K<sup>+</sup> (35 mM in oocytes, 0.6 mM in yeast cells). Furthermore, Northern analysis indicated that *KATI* is expressed primarily in the *Arabidopsis* shoot. These results strongly suggest that the system encoded by *KATI* is not a root high-affinity K<sup>+</sup> transporter.

### Introduction

Potassium arguably plays the most important role of any macronutrient cation in plant growth and development, as K<sup>+</sup> is the most abundant cellular cation and is required for many processes central to growth and development (Läuchli and Pflüger, 1979). Despite the importance of this ion, the processes by which K<sup>+</sup> is transported at the cellular, organ, and whole plant level remain poorly understood (for a review, see Kochian and Lucas, 1988). Plant roots absorb K<sup>+</sup> over a wide range of soil K<sup>+</sup> concentrations. The pioneering tracer-flux studies for root K<sup>+</sup> uptake conducted by Epstein and coworkers (Epstein et al., 1963) indicated that at least two K<sup>+</sup> transport mechanisms exist, one mediating high-affinity K<sup>+</sup> uptake and the other low-affinity uptake. Several labs have suggested that the high-affinity system is a thermodynamically active transporter, and is possibly a K<sup>+</sup>-H<sup>+</sup> or K<sup>+</sup>-Na<sup>+</sup> co-transport. The

low-affinity system most likely involves inward-directed K<sup>+</sup> channels.

Recent collaborative work that involved our laboratories has resulted in the cloning of the first plant K<sup>+</sup> transport cDNA, *KATI*, from *Arabidopsis thaliana* (Anderson et al., 1992). *KATI* was cloned by complementation of a K<sup>+</sup> transport-defective mutation in yeast with *Arabidopsis* cDNA; expression of *KATI* in the yeast mutants allowed growth on 200 μM K<sup>+</sup> whereas mutants require 100 mM K<sup>+</sup>. Injection of *KATI* mRNA into *Xenopus* oocytes conferred the expression of an inward K<sup>+</sup> current that was characterized as an inwardly-rectifying channel with a high selectivity for potassium ions (Schachtman et al., 1992). Simultaneous to the Anderson et al. publication, Sentenac and co-workers reported the isolation of another K<sup>+</sup> transport cDNA, *AKTI*, from *Arabidopsis* (Sentenac et al., 1992). *KATI* and *AKTI* have extensive sequence similarity, but are not allelic.

This suggests that plant  $K^+$  transport is mediated by a multigene family. *KAT1* and *AKT1* were found to share structural features with the Shaker family of voltage-activated  $K^+$  channels in *Drosophila* and related genes in vertebrates and invertebrates, including a putative voltage-sensing region and a region thought to line the pore of the channel.

Because expression of these putative  $K^+$  channel cDNAs in yeast allows growth on micromolar levels of  $K^+$ , the possibility exists that both high and low affinity  $K^+$  transport could be mediated by  $K^+$  channels. In this study, we use expression of *KAT1* cDNA in two heterologous systems, *Xenopus* oocytes and  $K^+$  transport-defective mutants of *Saccharomyces cerevisiae*, to conduct a more-detailed biophysical and physiological characterization of the  $K^+$  transport system encoded by *KAT1*.

## Materials and methods

### Expression of *KAT1* in *Xenopus* oocytes

For a detailed description of the methods employed to investigate the  $K^+$  transport characteristics of *KAT1* expressed in *Xenopus* oocytes, see Schachtman et al. (1992). *KAT1* mRNA was synthesized *in vitro* from the cloned *KAT1* cDNA. Oocytes were injected with 50 ng of *KAT1* mRNA, and after 5 days,  $K^+$  transport function was examined using conventional two-microelectrode, voltage clamp techniques.

### Expression of *KAT1* in $K^+$ Transport-Defective Yeast Mutants

A  $K^+$  transport-defective strain of *Saccharomyces cerevisiae* (strain CY162) and CY162 cells

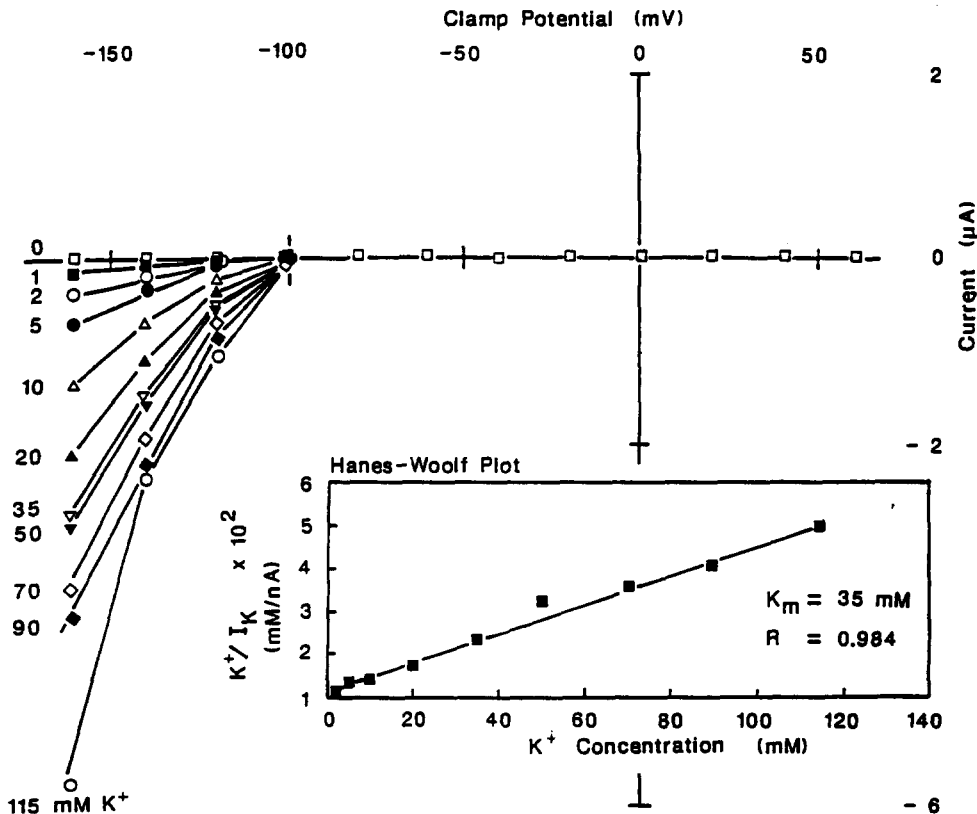


Fig. 1. *KAT1*-mediated expression of inward  $K^+$  currents in oocytes bathed in solutions ranging in  $K^+$  concentration from 1 to 115 mM. Oocytes were injected with *KAT1* mRNA 5 days prior to the experiment. When inward current,  $I_{K+}$ , is plotted as a function of external  $K^+$  concentration, saturating Michaelis-Menten kinetics were observed. The insert depicts a Hanes-Woolf plot for the kinetic data; the straight line generated by this kinetic transformation is indicative of Michaelis-Menten kinetics.

expressing *KAT1* were generously provided by Dr. R.F. Gaber, Northwestern University. Cells were grown in a low-salt medium (see Ramos et al., 1985, for media composition) to mid-log phase, centrifuged, and resuspended in -K<sup>+</sup> media for 3 h prior to measurement of unidirectional K<sup>+</sup> influx with radiotracers (<sup>42</sup>K<sup>+</sup> and <sup>86</sup>Rb<sup>+</sup>). Radiotracer influx experiments were conducted in 2 mL volumes of aerated yeast suspensions with a cell density of approximately 10<sup>7</sup> cells/mL. K<sup>+</sup> uptake was initiated by introduction of KCl and radiotracer, and terminated after a 2 min uptake period by pipetting a 1 mL subsample of reaction solution into 10 mL of desorption solution (10 mM KCl + 5 mM CaCl<sub>2</sub>) on top of a 0.6 μm pore cellulose acetate filter. Desorption solution was rapidly removed by vacuum filtration, the cells were washed twice more with desorption solution, and radiolabel was measured via liquid scintillation counting.

## Results and discussion

Potassium uptake into plant roots has been suggested to be mediated by two or more distinct systems (Epstein et al., 1963; Kochian and Lucas, 1988). At low external K<sup>+</sup>, there appears to be a high-affinity system with a very low K<sub>m</sub> for K<sup>+</sup> (2–20 μM), which is highly electrogenic (depolarizing), and is presumed to mediate active K<sup>+</sup> transport. If this system is thermodynamically active, this would preclude a role for K<sup>+</sup> channels in high-affinity transport. K<sup>+</sup> uptake at higher external K<sup>+</sup> levels has been presumed to be mediated by inwardly-directed K<sup>+</sup> channels. However, the distinction between high- and low-affinity systems is now muddled, based on recent information gleaned from the cloning of the first K<sup>+</sup> transport cDNAs in plants, *KAT1* and *AKT1* (Anderson et al., 1992; Sentenac et al., 1992). Both of these transport-cDNAs share structural similarities with Shaker-like voltage-gated cation channels in animals. Additionally, Schachtman et al. (1992) showed that when *KAT1* was expressed in *Xenopus* oocytes, it conferred the expression of a hyperpolarization activated K<sup>+</sup> current that was selective for K<sup>+</sup> over other monovalent cations, and was blocked by the known K<sup>+</sup>-channel blockers, tetraethylammonium and Ba<sup>2+</sup>.

When *KAT1* and *AKT1* are expressed in K<sup>+</sup> transport-defective yeast mutants that require high K<sup>+</sup> (100 mM) for growth, the cells can then grow on micromolar levels of K<sup>+</sup>. Thus, the question arises as to whether K<sup>+</sup> channels can mediate both high- and low-affinity K<sup>+</sup> uptake into roots.

In this study, we addressed this question by investigating the concentration-dependent kinetics of K<sup>+</sup> uptake in both *Xenopus* oocytes and yeast cells expressing *KAT1*, in order to compare the kinetic characteristics of the transport system encoded by *KAT1* with the high-affinity system we have previously characterized in maize roots. The data depicted in Figure 1 are for a biophysically-based characterization of K<sup>+</sup> transport via *KAT1* in oocytes. There was no K<sup>+</sup> current in uninjected or water-injected oocytes (data not shown), while oocytes injected with *KAT1* mRNA exhibited a strong inward K<sup>+</sup> current in response to hyperpolarizing voltages. The current-voltage relationships for this system at external K<sup>+</sup> concentrations ranging from 1 to 115 mM were studied; when the K<sup>+</sup> currents are

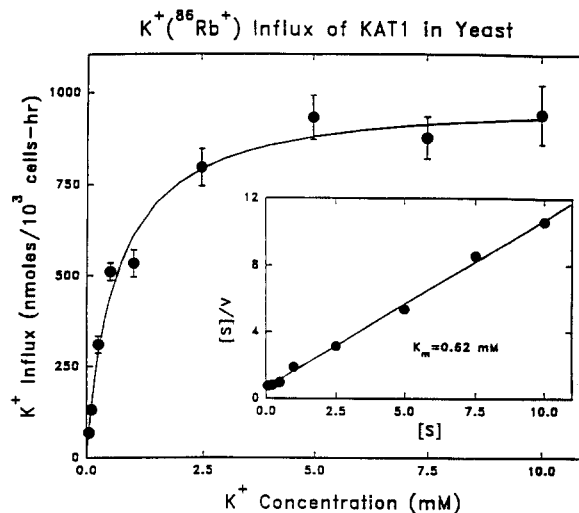


Fig. 2. *KAT1*-mediated expression of K<sup>+</sup> influx in K<sup>+</sup> transport-defective mutants of *Saccharomyces cerevisiae*. The concentration-dependence of unidirectional K<sup>+</sup> influx was measured using radiotracer flux techniques. The insert depicts a Hanes-Woolf kinetic transformation of the transport data, demonstrating that the kinetics are Michaelis-Menten in nature, with a K<sub>m</sub> of 0.62 mM.

plotted as a function of external  $K^+$  concentration, a saturating, Michaelis-Menten relationship is observed, with a  $K_m$  of approximately 35mM (see insert, Figure 1). These results are important, for they suggest that *KATI* does not encode a high affinity  $K^+$  transport system similar to the one we previously characterized in maize roots (Newman et al., 1987; Kochian et al., 1989).

We have also studied the kinetics of *KATI*-mediated  $K^+$  transport in another heterologous system,  $K^+$  transport mutants of *Saccharomyces cerevisiae* that express *KATI*. We developed protocols to measure unidirectional  $K^+$  influx in these yeast cells, using  $^{42}K^+$  and  $^{86}Rb^+$  as radiotracers. As shown in Figure 2, the concentration-dependent kinetics for  $K^+$  transport by *KATI* in yeast cells also yielded Michaelis-Menten kinetics; however, the  $K_m$  for this system was approximately 0.6 mM.

The results of the  $K^+$  transport studies in the two heterologous systems raise an important point. First, it is clear from the kinetic studies of *KATI* in oocytes and yeast that it does not encode for the high-affinity  $K^+$  transport system observed in plant roots. This hypothesis is bolstered by the results of recent Northern analysis which found that *KATI* was expressed primarily in the shoot and was expressed weakly in roots of *Arabidopsis* (data not shown). We have also recently cloned *AKT1* by PCR-based techniques; Northern analysis of *AKT1* indicated that it is expressed very strongly in the root. Thus, we are now attempting to study the

transport function of *AKT1* in oocytes and yeast in order to determine if it shows similarities to root high affinity  $K^+$  uptake.

In summary, the cloning of these two  $K^+$  transport-cDNAs has opened up a number of new possible avenues of research aimed at better understanding the mechanisms and regulation of plant  $K^+$  transport at the molecular, cell, and whole plant levels. Future research in our labs will be directed at more-detailed studies on the developmental regulation of *KATI* and *AKT1* in plants, as well as gaining a better understanding of the transport mechanisms of the proteins encoded by these cDNAs.

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