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RESEARCH PAPER

# The high affinity $K^+$ transporter AtHAK5 plays a physiological role *in planta* at very low $K^+$ concentrations and provides a caesium uptake pathway in *Arabidopsis*

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## Abstract

**Caesium ($Cs^+$) is a potentially toxic mineral element that is released into the environment and taken up by plants. Although $Cs^+$ is chemically similar to potassium ($K^+$), and much is known about $K^+$ transport mechanisms, it is not clear through which $K^+$ transport mechanisms $Cs^+$ is taken up by plant roots. In this study, the role of AtHAK5 in high affinity $K^+$ and $Cs^+$ uptake was characterized. It is demonstrated that AtHAK5 is localized to the plasma membrane under conditions of $K^+$ deprivation, when it is expressed. Growth analysis showed that AtHAK5 plays a role during severe $K^+$ deprivation. Under $K^+$-deficient conditions in the presence of $Cs^+$, *Arabidopsis* seedlings lacking AtHAK5 had increased inhibition of root growth and lower $Cs^+$ accumulation, and significantly higher leaf chlorophyll concentrations than wild type. These data indicate that, in addition to transporting $K^+$ *in planta*, AtHAK5 also transports $Cs^+$. Further experiments showed that AtHAK5 mediated $Cs^+$ uptake into yeast cells and that, although the $K^+$ deficiency-induced expression of *AtHAK5* was inhibited by low concentrations of $NH_4^+$ *in planta,* $Cs^+$ uptake by yeast was stimulated by low concentrations of $NH_4^+$. Interestingly, the growth of the *Arabidopsis atakt1-1* mutant was more sensitive to $Cs^+$ than the wild type. This may be explained, in part, by increased expression of *AtHAK5* in the *atakt1-1* mutant. It is concluded that AtHAK5 is a root plasma membrane uptake mechanism for $K^+$ and $Cs^+$ under conditions of low $K^+$ availability.**

Key words: Caesium, plasma membrane, potassium, uptake.

## Introduction

Caesium ($Cs^+$) is a group I alkali metal with chemical properties similar to potassium ($K^+$). It is found naturally as the stable isotope $^{133}Cs$. Caesium is not required by plants and although $Cs^+$ can perturb cellular biochemistry by competing with $K^+$, it is rarely present at toxic concentrations in soil solutions (White and Broadley, 2000). Nevertheless, two anthropogenic radioisotopes of $Cs^+$ ($^{134}Cs$ and $^{137}Cs$) produced in nuclear reactors and thermonuclear explosions are of environmental concern (White and Broadley, 2000). These radioisotopes emit harmful β and γ radiation during their decay, have relatively long half-lives (2.06 and 30.17 years, respectively) and are incorporated rapidly into biological systems (White and Broadley, 2000). Efforts to reduce the entry of radiocaesium into the food chain through phytoremediation technologies and/or the development of 'safer' crops that accumulate less radiocaesium are,

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Abbreviations: HA, hemagglutinin; VICC, voltage-insensitive cation channels; GLR, glutamate receptors; CNGC, cyclic nucleotide gated channels; AKT, *Arabidopsis* potassium transporter; ER, endoplasmic reticulum; PM, plasma membrane.

therefore, a priority for contaminated land (White and Broadley, 2000; White *et al*., 2003).

Various studies on the fate of $Cs^+$ in a variety of ecosystems have been conducted (Avery, 1996; Delvaux *et al*., 2000*a*, *b*). Where $Cs^+$ is present in the soil solution, plant roots take it up through $K^+$ uptake pathways because of the chemical similarity between the two cations (Delvaux *et al*., 2000*b*; White and Broadley, 2000; Zhu and Smolders, 2000). Plants can be used to extract $Cs^+$ from contaminated sites, but these plants if eaten by animals are a potential source of $Cs^+$ in the food chain (White *et al*., 2003). To increase or reduce plant $Cs^+$ accumulation in a targeted fashion, it will be necessary to identify and characterize the precise mechanisms of $Cs^+$ influx.

Early studies found that plant roots could accumulate $Cs^+$ and it was proposed that $K^+$, $Rb^+$, and $Cs^+$ shared the same uptake mechanisms: high affinity mechanisms at micromolar range and low affinity mechanisms in the millimolar range (Collander, 1941; Epstein and Hagen, 1952). In the past decade, significant progress has been made in identifying proteins responsible for both high and low affinity $K^+$ uptake in *Arabidopsis* (Maser *et al*., 2001; Very and Sentenac, 2003; Ashley *et al*., 2006). The wealth of insertional mutants available in *Arabidopsis* (Krysan *et al*., 1999; Alonso *et al*., 2003) and the knowledge of the molecular identities of $K^+$ transporters provide a platform for elucidating the $Cs^+$ uptake mechanisms in plants (Hampton *et al*., 2005).

Multiple mechanisms for $K^+$ uptake have been characterized in plants, but it is not known which of these transport mechanisms provide routes for $Cs^+$ uptake. The *Arabidopsis* inward-rectifying $K^+$ channel AKT1 is a major pathway for $K^+$ uptake into plant root cells (Hirsch *et al*., 1998; Spalding *et al*., 1999; Reintanz *et al*., 2002; Gierth *et al*., 2005; Xu *et al*., 2006). The transport of $K^+$ through the AKT1 channel expressed in yeast is inhibited by $Cs^+$ (Bertl *et al*., 1997), but the *Arabidopsis akt1* mutant does not have reduced $Cs^+$ influx or accumulation (Broadley *et al*., 2001). These data suggest that AKT1 is a $Cs^+$-sensitive $K^+$ channel, but probably not a main $Cs^+$ uptake pathway.

Based on theoretical models and pharmacological studies, voltage-insensitive cation channels (VICCs) are thought to mediate most $Cs^+$ influx into root cells when plants are $K^+$ replete (White and Broadley, 2000; Hampton *et al*., 2005). In *Arabidopsis*, VICCs are encoded by homologues of animal ionotropic glutamate receptors (*AtGLR*s) and cyclic nucleotide gated channels (*AtCNGC*s) (Demidchik *et al*., 2002; White *et al*., 2002). AtGLRs are suggested to be glutamate-gated non-selective cation channels that conduct $Ca^{2+}$, $K^+$, and $Cs^+$ (Dennison and Spalding, 2000; Demidchik *et al*., 2004; Qi *et al*., 2006). Expression of two genes encoding AtGLRs (*AtGLR1.2* and *AtGLR1.3*) significantly increases in roots of plants grown at high $Cs^+$ concentrations (Hampton *et al*., 2004). Some AtCNGCs conduct $K^+$, $Ca^{2+}$, and $Cs^+$ in heterologous expression systems (Leng *et al*., 1999, 2002). *Arabidopsis* mutants lacking individual AtCNGCs have either higher or lower $Cs^+$ accumulation in shoots than the wild type (Hampton *et al*., 2005). It has been suggested that the absence of individual AtCNGCs might alter the expression of genes encoding other $Cs^+$ transporters, which contributes to the higher $Cs^+$ accumulation in some mutants (Hampton *et al*., 2005).

When plants are grown in media containing low $K^+$, members of high affinity $K^+$ transporter family *KUP*/*HAK*/*KT* (Maser *et al*., 2001) have been suggested to function as $Cs^+$ transporters in *Arabidopsis* (White and Broadley, 2000). AtHAK5 (At4g13420) appears to be a promising candidate for a $Cs^+$ transporter (Rubio *et al*., 2000), and the gene encoding AtHAK5 is the only member of this family whose expression is consistently up-regulated in $K^+$-starved *Arabidopsis* (Ahn *et al*., 2004; Shin and Schachtman, 2004; Gierth *et al*., 2005). Furthermore, both high affinity $K^+$ and $Cs^+$ uptake in *Arabidopsis* are induced by $K^+$ starvation and inhibited by $NH_4^+$ (Cao *et al*., 1993; Hampton *et al*., 2004; Shin and Schachtman, 2004). Although AtHAK5 has been shown to be a high affinity $K^+$ transporter in kinetic studies, and KUP transporters are known to be inhibited by $NH_4^+$, a physiological role for AtHAK5 has not been demonstrated *in planta*. In this study, several questions were asked related to the function of AtHAK5. First, it was asked whether AtHAK5 is localized to the plasma membrane and whether it is required for root elongation under low $K^+$ conditions. Then the question of whether AtHAK5 contributes to high affinity $Cs^+$ uptake and accumulation in *Arabidopsis* was explored. It is also reported that *AtHAK5* is up-regulated in an *akt1* mutant that shows increased $Cs^+$-sensitive root elongation. Our data provide the basis for a proposed model regarding the role of AtHAK5 and AKT1 in plant sensitivity to environmental $Cs^+$.

## Materials and methods

### *Plant materials and growth conditions*

The *athak5* T-DNA insertion lines used in this study were obtained from the SALK collection and homozygous lines were identified as outlined on the SIGNAL website at http://signal.salk.edu/tdnaprimers.2.html (Alonso *et al*., 2003). *Arabidopsis* line SALK_005604 was designated as *athak5-2* to be consistent with the previous study (Gierth *et al*., 2005). Our analysis indicated that *athak5-2* was a null allele. *Arabidopsis* line SALK_130604 was designated as *athak5-3*. The T-DNA insertion was located in exon 6 in *athak5-2* and in exon 4 in *athak5-3*.

*Arabidopsis* seeds of Wassilewskija (Ws2; N1601), Columbia-0 (Col-0; N1092), and *atakt1-1* (Hirsch *et al*., 1998) were used in this study. For *in vitro* plate assays *Arabidopsis* seeds were sterilized with 75% ethanol and 18–20 seeds were sown on the surface of

0.8% agarose (SeaKem LE Agarose, Cambrex Bio Science). Seeds were directly sown and germinated on the different media used in the low potassium and high caesium experiments. After 2–3 d at 4 °C, plates were placed in a growth chamber with a 16/8 h day/night cycle and maintained at 22 °C. Plates were oriented vertically for measuring root elongation and for GUS staining or horizontally for examining cotyledon development, total chlorophyll and $Cs^+$ content. The growth medium contained 0.5 mM $H_3PO_4$, 2 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 74 μM Fe-EDTA, 5 μM $H_3BO_3$, 1 μM $MnCl_2$, 2 μM $ZnSO_4$, 0.1 μM $CuSO_4$, 0.075 μM $NH_4Mo_7O_{24}$ and 1% sucrose. $K^+$ and $Cs^+$ concentrations were adjusted with KCl or CsCl. pH was adjusted to 5.8 with $Ca(OH)_2$.

### *Construction of plasmids and transformed plants*

Transgenic plants expressing *AtHAK5* under the control of the FMV promoter and *AtHAK5* native promoter were generated. *AtHAK5* cDNA was amplified without stop codon using *AtHAK5* forward primer with *Pst*I (CTGCAGATGGATGGTGAGGAACATCAAA-TAG) and *AtHAK5* reverse primer with *Nco*I (GGTACCTAACT-CATAGGTCATGCCAAC). The product was fused with three HA tags and cloned into pCAMBIA 1380 vector (CAMBIA, Canberra, Australia). The *AtHAK5* promoter was amplified using a forward primer with *Xho*I (CTCGAGGTAACAATACAATTGGTGAG-GAGC) and a reverse primer with *Hin*dIII (AAGCTTTTTTT-TTTTTTTTTTTTTGTGTTGTG). The FMV promoter (Sanger *et al*., 1990) and *AtHAK5* promoter were then cloned into the AtHAK5 with HA tags in pCABMIA1380. For the localization assay, the –1365 bp to 0 AtHAK5 promoter was cloned into the binary vector with a GUS reporter gene as described in Collier *et al*. (2005). The binary vector constructs were introduced into the *Agrobacterium tumefaciens* strain GV3101. *Arabidopsis* plants were transformed by the *Agrobacterium*-mediated floral dip method (Clough and Bent, 1998).

### *GUS staining*

To localize GUS activity, plants 5 d after germination were stained with 1 mM X-Gluc solution (Vicente-Agullo *et al*., 2004) and were destained as described by Malamy and Benfey (1997). The pictures were taken using a Nikon SMZ1500 dissecting microscope.

### *Membrane isolation and purification*

To determine the subcellular localization of AtHAK5 protein, a construct expressing a fusion of the AtHAK5 protein and an N-terminal haemagglutinin (HA) epitope tag driven by *AtHAK5* promoter was created. The construct was then transformed into *Arabidopsis* wild type (Col-0). Microsomal fractions were isolated from transgenic plants which were grown in liquid culture on a shaker for 3 weeks and subsequently starved of $K^+$ for 2 d at 22 °C with 16 h daylight at 200 μmol $m^{-2}$ $s^{-1}$. Microsomes were layered onto a continuous sucrose gradient (5% to 50%) and centrifuged at 35 000 rpm for 24 h at 4 °C using a Beckman SW 41 swinging bucket rotor. Thirty-two 1 ml fractions were collected from the gradient according to the method of Barkla *et al*. (1999). The linearity of the sucrose gradients was measured with a refractometer. Protein (100 μg) from each fraction was precipitated in 1:1 (v/v) ethanol:acetone by incubation overnight at –20 °C and then centrifuged at 10 000 *g* for 20 min at 4 °C. Pellets were resuspended with SDS sample buffer and 20 μg of protein was electrophoresed on 5%/10% acrylamide SDS-PAGE gels. SDS-PAGE-separated proteins were transferred onto hydrophobic polyvinylidene difluoride (PVDF) membrane (Amersham Bioscience, Piscataway, NJ, USA). Western blot analyses were performed as previously described (Vera-Estrella *et al*., 2004). An antibody was used against the HA tag (COVANCE, Berkeley, USA) for detecting AtHAK5, a calreticulin antibody (Nelson *et al*., 1997) for ER localization, a SOS1 antibody (Qiu *et al*., 2003) for plasma membrane localization and a PPase antibody (Kim *et al*., 1994) for tonoplast localization.

### *Total chlorophyll and tissue $Cs^+$ content measurement*

Total chlorophyll was extracted using ice-cold 80% acetone (Arnon, 1949) and the absorbance at 645 nm ($A_{645}$) and 663 nm ($A_{663}$) were measured. Total chlorophyll per fresh weight tissue (μg $mg^{-1}$) was calculated using the equation: $20.2(A_{645})+8.02(A_{663})$ (Arnon, 1949).

For tissue $Cs^+$ content measurements, whole seedlings were collected and washed with water and dried at 60 °C for 3 d. After weighing, the dried tissue was extracted with 0.5 M HCl at 37 °C for 2 d (Hunt, 1982). $Cs^+$ content was measured using an EDL (Electrodeless Discharge Lamp) with a Perkin Elmer Analyst 300 atomic absorption spectrometer.

### *$Cs^+$ uptake in seedlings*

Older plants than those used for plate assays were used for $Cs^+$ influx experiments, *Arabidopsis* seeds were sown on perforated polycarbonate discs over agar containing the complete MS basal salt mix. Seven days after sowing, plants were transferred to a hydroponics system situated in a Saxcil growth cabinet (Hampton *et al*., 2004). In the hydroponics system, plants were supported on polycarbonate discs over 450 ml of aerated nutrient solution containing complete MS basal salt mix for 7 d. Plants were then transferred for a further 7 d to either MS solution with 2 mM $K^+$ to produce $K^+$-replete plants or an MS solution containing 0.5 μM $K^+$ to produce $K^+$-starved plants. Caesium influx experiments were performed on intact plants as described by Hampton *et al*. (2004). Briefly, polycarbonate discs supporting *Arabidopsis* were placed over 455 ml of an aerated, 'single salt' solution containing 50 μM CsCl radiolabelled with 104 kBq $l^{-1}$ $^{134}CsCl$. After 20 min, plants were transferred to 450 ml of a solution containing 50 μM CsCl plus 1 mM $CaCl_2$ for 2 min to remove $^{134}Cs$ from the root apoplast. Plant roots were blotted with tissue paper, and roots of individual plants were harvested, weighed, and their Cs contents estimated from $^{134}Cs$ γ-emissions determined using a gamma counter. Since the rate of $Cs^+$ accumulation by plants was constant over a 20 min period (CR Hampton and PJ White, unpublished data), these measurements approximate unidirectional influxes.

### *Analysis of shoot $Cs^+$ concentration*

For the analysis of shoot $Cs^+$ concentration in Ws2 and *atakt1-1*, seeds of Ws2, and *atakt1-1* were grown on 0.8% agar in polycarbonate boxes in a growth room at 24 °C as described by Hampton *et al*. (2004). Agar contained 1% (w/v) sucrose and a basal salt mix at 10% of the full-strength Murashige and Skoog formulation. The effect of $Cs^+$ concentration in the agar ($[Cs]_{agar}$) on $[Cs]_{shoot}$ was determined in the presence of 2 mM $K^+$. The $[Cs]_{agar}$ was raised to 0.3, 1, 10, 100, 178, 300, 562, 794, 1000, 1778, 3162, and 10 000 μM using CsCl. The radioisotope $^{134}Cs$ (Radioisotope Centre Polatom, Świerk, Poland) was used to quantify $[Cs]_{shoot}$ and the agar spiked with $^{134}Cs$. Boxes were placed in a growth room set to 24 °C with 16 h light per day. Illumination was provided by a bank of 100 W 84 fluorescent tubes (Philips, Eindhoven, Netherlands) giving a photon flux density of 45 μmol photons $m^{-2}$ $s^{-1}$ at plant height. Shoots were harvested 21 d after sowing for the analysis of $[Cs]_{shoot}$. Six shoots of each

accession were bulked and their $^{134}Cs$ content was determined by counting γ-emissions for 900s per sample on a well-type automatic gamma counter (Wallac 1480 Wizard, Perkin-Elmer Life Sciences, Turku, Finland).

### *Yeast $Cs^+$ uptake*

A previous study (Rubio *et al*., 2000) demonstrated that changing the leucine to histidine at position 776 of AtHAK5 was required for sufficient activity of AtHAK5 to complement a yeast mutant deficient in $K^+$ uptake. To introduce the mutation in the AtHAK5 protein, a corresponding point mutation was introduced in the *AtHAK5* cDNA with a PCR-based oligonucleotide-directed method with mutagenic primers 5′-TCGAAGCATCTCAAGGTTGG-CATG-3′ and 5′-CCTAGGAATCGCAAGTGCTTTGTC-3′. The sequence of the amplified DNA was verified and then the cDNA with the point mutation was cloned into the pYES2 vector at the *Bam*HI and *Xba*I sites. Also a C-terminal 3× HA tagged version of HAK5 L776H was tested for complementation. The pYES2-*AtHAK5* containing the base change was transformed into the $K^+$ uptake-deficient yeast cells CY162 (Δ*trk1*, Δ*trk2*) (Ko and Gaber, 1991).

For $Cs^+$ uptake assays, yeast cells were grown overnight at 30 °C in arginine phosphate media (AP) supplemented with 100 mM $K^+$, 2% sucrose and 2% galactose. Yeast cells with density $OD_{600}$ 1.0∼1.2 were pelleted, washed twice with Milli-Q $H_2O$ and resuspended in AP media without $K^+$ and grown at 30 °C for 6 h. The yeast cells were then resuspended in uptake buffer containing 1% sucrose, 1% galactose, and 10 mM MES at pH 6.0 with $Ca(OH)_2$. Aliquots of yeast cells (5 ml) were taken in 10 min intervals for a 30 min period after CsCl was added. The yeast cells were immediately filtered through 0.8 μm nitrocellulose membranes and washed with 5 ml of cold 20 mM $CaCl_2$ solution. The filters were incubated in a 0.5 M HCl solution at 37 °C for 36 h, and $Cs^+$ was determined by atomic absorption spectrometry (Perkin Elmer AAnalyst 3000). For studying the effect of $NH_4^+$ on the $Cs^+$ uptake, the yeast cells were pretreated with $NH_4^+$ for 30 min before $Cs^+$ was added to the uptake buffer.

### *Transcriptional profiling and quantitative PCR*

Seeds of Ws2 and *atakt1-1* were sown on perforated polycarbonate discs and placed over agar containing the complete MS basal salt mix. After 14 d the polycarbonate discs were transferred to a hydroponics system situated in a Saxcil growth cabinet (Hampton *et al*., 2004) where they were placed over 450 ml of aerated nutrient solution containing 8.0 mM $NO_3^-$, 4.0 mM $Ca^{2+}$, 0.76 mM $SO_4^{2-}$, 0.75 mM $K^+$, 0.75 mM $Mg^{2+}$, 0.25 mM $H_2PO_4^{2-}$, 0.1 mM FeNaEDTA, 0.05 mM $Cl^-$, 0.03 mM $H_2BO_3$, 0.01 mM $Mn^{2+}$, 0.001 mM $Na^+$, 0.001 mM $Zn^{2+}$, 0.003 mM $Cu^{2+}$, and 0.0005 mM $MoO_4^{2-}$. Total RNA was extracted from root tissue after a further 7 d (21 d after sowing) as described by Hampton *et al*. (2004). Samples of total RNA were then either sent to NASC (Nottingham, UK) for labelling and hybridization to Affymetrix *Arabidopsis* ATH1 GeneChips (Affymetrix Inc., Santa Clara, USA) or used to assay AtHAK5 gene expression by quantitative PCR. All experiments were repeated three times. The analysis of microarray data was performed as described by Hampton *et al*. (2004).

To confirm differences in the expression of *AtHAK5* between Ws2 and *atakt1-1* plants, quantitative PCR was performed as described by Hammond *et al*. (2003). Primers for quantitative PCR were designed to the cDNA sequence of *AtHAK5* genes and 18s rRNA control using the Primer3 primer design tool (Rozen and Skaletsky, 2000). The primers for *AtHAK5* were 5′-CGTTTTCATTGTTCTTCAGG-3′, 5′-ATCTTCTGGTTCTTGGTTTG-3′ and the primers for 18s rRNA are 5′-CATAAACGATGCCGACCAG-3′, 5′-AGCCTTGCGAC-CATACTCC-3′.

## Results

### AtHAK5 *localizes to the plasma membrane and is required for root elongation under low $K^+$ conditions*

*AtHAK5* is expressed in root cells under low $K^+$ conditions and contributes to high affinity $K^+$ uptake (Ahn *et al*., 2004; Shin and Schachtman, 2004; Gierth *et al*., 2005). To test the hypothesis that AtHAK5 is localized in the plasma membrane, transgenic plants containing native promoter-driven *AtHAK5* cDNA with a 3× haemagglutinin epitope tag (HA) were created. After growing these plants in a nutrient-replete medium for 3 weeks, they were starved of $K^+$ for 1 d or transferred to nutrient-replete medium as a control. Membranes from the roots of these plants were separated on a continuous sucrose gradient and probed with various antibodies. Calreticulin was used as the ER marker, SOS1 as a plasma membrane marker, and the PPase as the tonoplast marker. AtHAK5 was detected in membrane fractions corresponding to the endoplasmic reticulum (ER), plasma membrane (PM), and vacuolar membrane (Fig. 1). Under potassium-replete conditions, the AtHAK5 protein was not found in the plasma membrane (Fig. 1A) whereas under potassium-deficient conditions a fraction of the total protein was in the plasma membrane fraction (Fig. 1B). Protein also remained in the ER and may have been localized to the vacuolar membrane which may be due to the presence of the HA tag at the *c*-terminus.

To determine the physiological significance of AtHAK5 in $K^+$ nutrition, root elongation in wild-type plants and in two *athak5* mutants possessing null alleles of *AtHAK5* was measured. The SeaKem agarose used in these studies was previously determined to contain trace amounts of $K^+$, which was estimated to be 1–3 μM. After 6 d of growth, both *athak5* mutants had significantly shorter roots than those of the wild type when grown in the absence of added $K^+$ or with very low levels (1 μM $K^+$) of $K^+$ (Fig. 2). However, with higher concentrations of $K^+$ (10 μM) in the growth medium, there were no differences in root length between wild-type plants and the *athak5* mutants (Fig. 2).

### *$Cs^+$ sensitivity of* athak5

In previous work, a yeast strain expressing *AtHAK5* was shown to possess the ability to take up $Cs^+$ (Rubio *et al*., 2000). Therefore, it was determined whether *athak5* mutants differed from wild-type plants in their response to rhizosphere $Cs^+$. The root elongation of wild-type plants and two mutants lacking *AtHAK5* was examined on vertical plates containing media with low (100 μM) and high (1.75 mM) $K^+$ concentrations and a range of CsCl concentrations. In the presence of 100 μM $K^+$, roots of both *athak5* mutants were significantly shorter than those of wild-type plants when the $Cs^+$ concentration in the

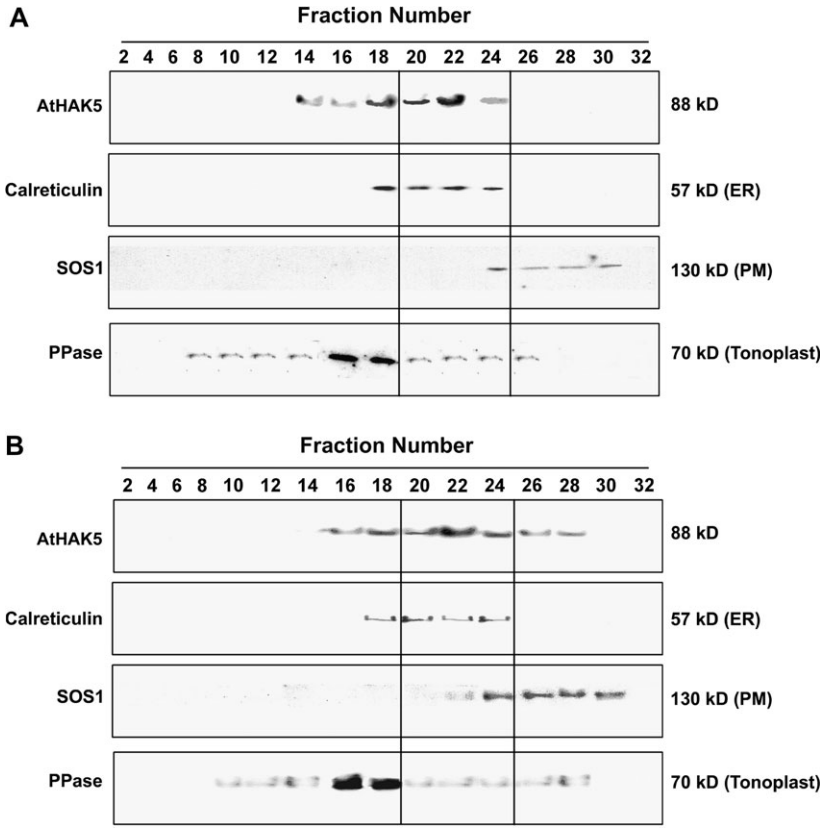


**Fig. 1.** AtHAK5 localizes to the plasma membrane under conditions of $K^+$ deprivation. Membrane fractions containing 20 μg of protein from promoter::AtHAK5::HA transgenic plants were separated on 10% SDS-PAGE gels and transferred to PVDF membrane. Antibodies were used to probe protein fractions, from top to bottom: HA antibody for AtHAK5::HA, calreticulum antibody for endoplasmic reticulum (ER), SOS1 antibody for plasma membrane (PM), and PPase (vacuolar $H^+$-pyrophosphatase) antibody for tonoplast. Molecular masses of the bands are indicated. (A) Protein from plants grown in full nutrients and (B) from plants grown without potassium.

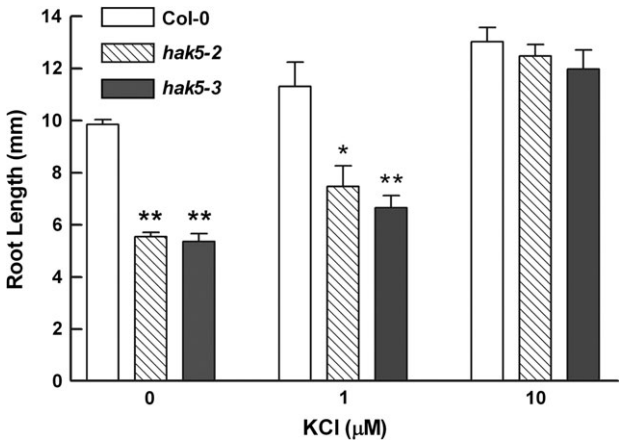


**Fig. 2.** $K^+$-dependent root length of 6-d-old *athak5* knockout and wild-type seedlings as measured in agarose plate assays. Data represent means ±SD ($n$=4, each replicate was comprised of 18–20 plants). Significant differences are indicated with asterisks (*$P$ < 0.05 and **$P$ < 0.01).

medium was greater than or equal to 200 μM (Fig. 3A). Thus, the expression of *AtHAK5* enhanced the $Cs^+$ tolerance of *Arabidopsis* grown at low $K^+$ concentrations. When grown in media containing 1.75 mM $K^+$, there were no differences in root elongation between *Arabidopsis* lacking AtHAK5 and wild-type plants (Fig. 3B).

During the measurement of root elongation, it was noticed that the cotyledon of the knockout lines remained green whereas those of the wild type were bleached. To examine cotyledon development, seeds of Col-0 and *athak5-3* were germinated on the same media used for the root elongation assay except that the plates were horizontally placed. When seeds of Col-0 and the *athak5-3* mutant were germinated on plates containing 100 μM KCl, both lines had a similar appearance. However, when plants were grown on 300 μM $Cs^+$, the cotyledons of wild-type plants bleached after 6 d and appeared to contain very little chlorophyll (Fig. 4A), whereas the cotyledons of *athak5-3* remained green for 12 d (not shown). Total chlorophyll content of wild-type plants and two *athak5* mutants was measured 6 d after germination. When grown in the absence of $Cs^+$, chlorophyll content did not differ among the lines (Fig. 4B). However, the chlorophyll content of both wild-type and *athak5* plants was significantly reduced in the presence of 300 μM $Cs^+$, although the mutants contained significantly more chlorophyll than wild-type plants. Analysis of the plants grown

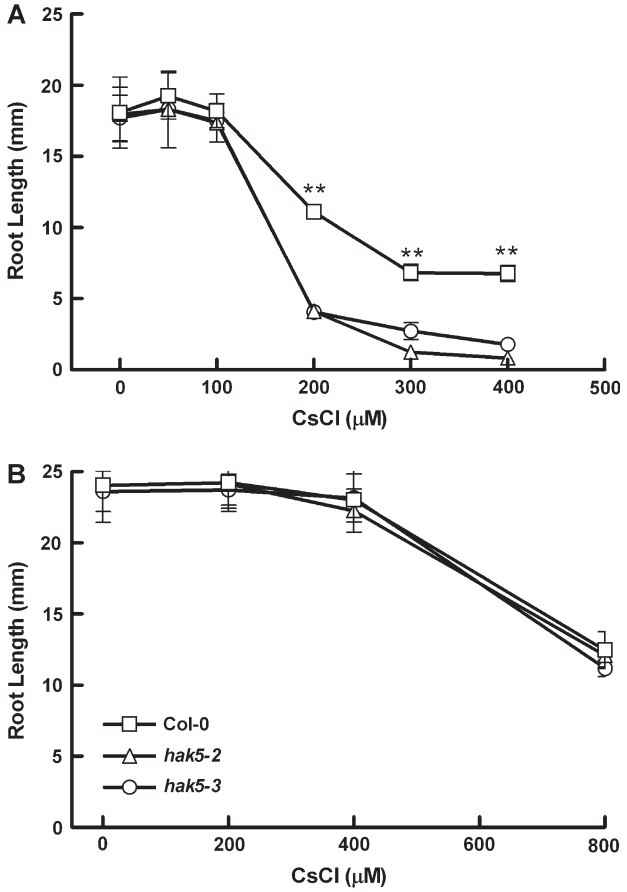


**Fig. 3.** Root elongation in response to increasing $Cs^+$ concentrations of 6-d-old *athak5* knockout and Col-0 plants in the presence of (a) 100 μM KCl or (B) 1.75 mM KCl. Data represent means ±SE ($n$=4). Significant differences are indicated with asterisks (*$P < 0.05$ and **$P < 0.01$) as measured in agarose plate assays.

in the presence of 300 μM $Cs^+$ showed that the *athak5* mutants contained significantly less $Cs^+$ than wild-type plants (Fig. 4C). Since the uptake of $Cs^+$ has been associated with a decrease in chlorophyll concentration in *Arabidopsis* leaves (Le Lay *et al*., 2006), both these data also suggest that AtHAK5 mediates the uptake of $Cs^+$.

### *Effect of $NH_4^+$ on AtHAK5-mediated $Cs^+$ uptake in yeast and $K^+$ starvation-induced* AtHAK5 *expression* in planta

$Cs^+$ influx is inhibited by $NH_4^+$ in $K^+$-starved *Arabidopsis* (Hampton *et al*., 2004). To test the hypothesis that AtHAK5 mediates $NH_4^+$-inhibited $Cs^+$ influx, the cDNA of a modified version of *AtHAK5,* with enhanced transport activity (Rubio *et al*., 2000), was transformed into a mutant yeast strain (CY162) defective in $K^+$ uptake and unable to grow on media containing low $K^+$ concentrations. The yeast system is useful in these types of studies because the expression of the transporter is less subject to other control factors that may be imposed by the plant

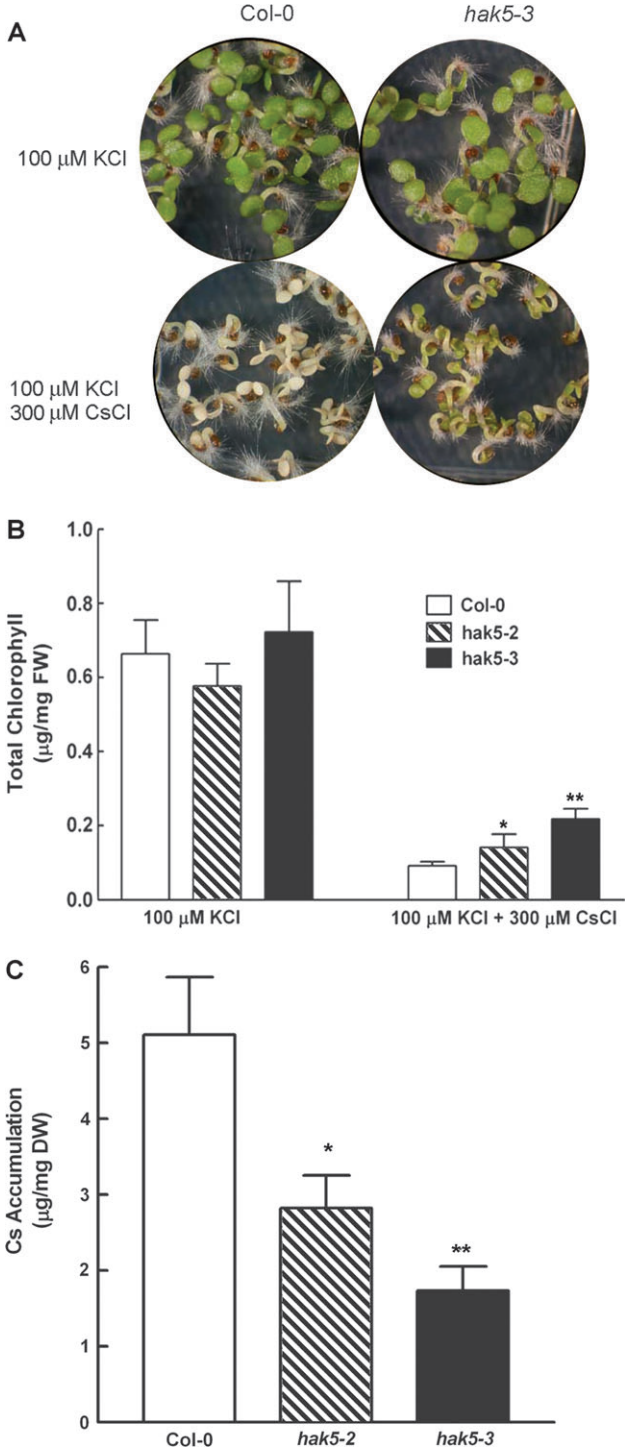


**Fig. 4.** Chlorophyll content and $Cs^+$ accumulation in 6-d-old *athak5* knockout and Col–0 plants under low potassium and 300 μM $Cs^+$. (A) Images of 6-d-old seedlings showing the differences in leaf coloration reflecting chlorophyll levels between an *athak5* knockout line and the wild type (Col-0) grown in 100 μM KCl on horizontally oriented plates in the presence (bottom) or absence (top) of 300 μM $Cs^+$. (B) Total chlorophyll content measured in plants treated as in (A). FW, fresh weight. (C) $Cs^+$ accumulation in seedlings grown in the presence of 300 μM $Cs^+$. DW, dry weight. Values in (B) and (C) are means ±SD ($n$=4). Significant differences are indicated with asterisks (*$P < 0.05$ and **$P < 0.01$) as measured in agarose plate assays.

because gene expression is induced by galactose. Expression of the modified *AtHAK5* cDNA enabled this yeast mutant to grow on media containing low $K^+$ concentrations, as previously reported (Rubio *et al*., 2000). Consistent with the observations of Rubio *et al*. (2000), yeast expressing *AtHAK5* also displayed concentration-dependent $Cs^+$ uptake (data not shown). Yeast containing the empty vector was also tested and the levels of $Cs^+$ uptake were undetectable with or without added ammonium in the short-term (data not shown). $Cs^+$ influx was examined at a series of $NH_4$ concentrations. At 100 μM $NH_4^+$, $Cs^+$ influx was significantly higher than without added $NH_4^+$, whereas concentrations of $NH_4^+$ at or above 2 mM inhibited $Cs^+$ influx (Fig. 5A).

These results (Fig. 5A), in combination with previous studies (Ashley *et al*., 2006), show that millimolar concentrations of $NH_4^+$ reduce $Cs^+$ and $K^+$ uptake through *AtHAK5*. *In planta* the reductions in uptake through *AtHAK5* by $NH_4^+$ may be due to a direct blockade of the transporter or they could also be due to the reduced expression of the transporter in the presence of high $NH_4^+$. To test whether $NH_4^+$ alters *AtHAK5* expression, the effect of $NH_4^+$ was examined at a series of concentrations on $K^+$ deprivation-induced *AtHAK5* GUS (β-glucuronidase) expression. The seedlings grown on medium without $K^+$ showed strong GUS staining (Fig. 5B) which was not detectable in plants grown on $K^+$-replete medium (data not shown). The addition of 100 μM $NH_4^+$ was sufficient to almost completely suppress the $K^+$ deprivation-induced *AtHAK5*::GUS staining. At higher $NH_4^+$ concentrations, no *AtHAK5*::GUS staining was detected (Fig. 5B) even under

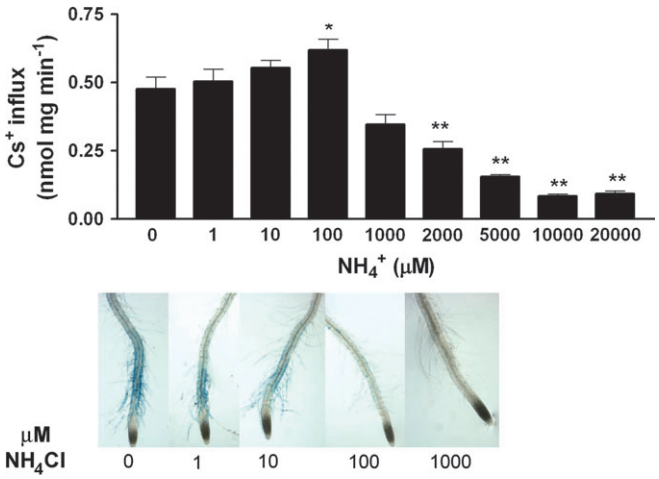


**Fig. 5.** Effects of $NH_4^+$ on AtHAK5-mediated $Cs^+$ uptake in yeast and $K^+$ starvation-induced *AtHAK5* expression in *Arabidopsis*. (A) $Cs^+$ influx into yeast as a function of increasing $NH_4^+$ concentrations at 300 μM $Cs^+$. Significant differences compared to 0 $NH_4^+$ are indicated with asterisks (*$P < 0.05$ and **$P < 0.01$). (B) Representative images for *AtHAK5*::GUS expressing lines. Five-day-old seedlings grown on medium without KCl were transferred to plates with the indicated concentration of $NH_4Cl$ for 1 d followed by GUS staining. Each of the images is representative of at least five samples.

$K^+$-deprived conditions. The response of *athak5* to $NH_4^+$ was also examined on media with low (100 μM) and high (1.75 mM) $K^+$ concentrations containing a range of $NH_4^+$ concentrations. No differences were found between the *athak5* insertions lines and the wild type in terms of root elongation, fresh weight, and growth rate (data not shown).

### *$Cs^+$ influx to* Arabidopsis *wild-type and* athak5 *plants*

The plate assays above demonstrated that AtHAK5 confers $K^+$ uptake at a very low concentration range and that the AtHAK5-mediated $Cs^+$ uptake is not the dominating $Cs^+$ influx pathway since the *athak5* mutant could not survive on media with high $Cs^+$ concentration (300 μM) longer than 12 d. To determine the extent that AtHAK5 contributes to $Cs^+$ uptake by more mature *Arabidopsis* plants *in vivo*, $Cs^+$ influx was compared in wild type and *athak5* knock-out lines grown in a hydroponics system.

$Cs^+$ influx was determined with either $K^+$-replete intact plants (previously grown in the presence of 2 mM $K^+$) or with plants that had been starved of $K^+$ (previously grown in the presence of 0.5 μM $K^+$). When plants had been grown in a solution containing 2 mM $K^+$, no significant difference in $Cs^+$ influx in wild-type plants and *athak5* mutants was observed (Table 1). Potassium deprivation increased $Cs^+$ influx to wild type by 35% and, interestingly, also increased $Cs^+$ influx to the *athak5* mutants, but by a lesser degree (between 18–21%) (Table 1). However, there were no significant differences in $Cs^+$ influx in wild-type plants and *athak5* mutants under the $K^+$-deprivation condition (0.5 μM).

### AtHAK5 *is up-regulated in* atakt1*, which shows $Cs^+$-sensitive root elongation*

AtAKT1 is the dominant $K^+$ uptake channel in the root cells of *Arabidopsis* (Hirsch *et al*., 1998; Spalding *et al*., 1999). To test if AtAKT1 is the main $Cs^+$ entry pathway in the plant, $Cs^+$ influx was determined with either $K^+$-replete intact plants (previously grown in the presence of 2 mM $K^+$) or with plants that had been starved of $K^+$

**Table 1.** *$Cs^+$ influx into roots from a hydroponic solution containing 50 μM CsCl in* Arabidopsis *accessions Ws2, Col-0,* akt1-1, athak5-2, *and* athak5-3

Plants were initially grown in hydroponic nutrient solutions containing either 2 mM or 0.5 μM $K^+$. All data are expressed as mean ±SE ($n$=3).

| Accession | Cs influx (μmol $g^{-1}$ FW root $h^{-1}$) | |
|---|---|---|
| | [K]=2 mM | [K] = 0.5 μM |
| Ws2 | 0.251±0.004 | 0.313±0.010 |
| *akt1* | 0.275±0.032 | 0.343±0.006 |
| Col-0 | 0.215±0.013 | 0.291±0.009 |
| *athak5-2* | 0.205±0.004 | 0.243±0.015 |
| *athak5-3* | 0.223±0.009 | 0.270±0.002 |

(previously grown in the presence of 0.5 μM $K^+$). No significant difference in $Cs^+$ influx in the roots of wild-type plants (Ws2) and *atakt1* mutant was observed (Table 1), although $Cs^+$ influx of *atakt1* under both conditions was 10% higher than those of wild type. This demonstrates that AtAKT1 is not a main $Cs^+$ uptake pathway. To confirm this conclusion further, the $Cs^+$ content was measured in the shoots of wild type (Ws2) and the *atakt1* mutant grown on agar containing various $Cs^+$ concentrations. Parallel regression analysis indicated that although $Cs^+$ concentration in shoots of *atakt1* tended to be higher than in shoots of wild-type plants (Fig. 6), this was not significant at $P < 0.05$.

To test the $Cs^+$ sensitivity of *atakt1*, the growth of *atakt1* roots was examined on agar containing either 100 μM (Fig. 7A) or 1.75 mM KCl (Fig. 7B) and a range of $Cs^+$ concentrations. The root length of *atakt1* was less than the wild type at 100 μM $K^+$ at all $Cs^+$ concentrations tested (Fig. 7A). With higher concentrations of $K^+$ the root elongation of *akt1* was inhibited more by $Cs^+$ than in the wild type between 400–800 μM $Cs^+$ (Fig. 7B).

To investigate the mechanism of increased sensitivity of *atakt1* to $Cs^+$, the expression of the whole genome was compared between the roots of *atakt1* and wild-type (Ws2) plants using the *Arabidopsis* ATH1 Genome Array, which represents approximately 24 000 genes. The complete set of the microarray data is available from the microarray database of Nottingham *Arabidopsis* Stock Centre (NASC) with experimental reference number 76 (http://affymetrix.arabidopsis.info/narrays/experiment-browse.pl). After excluding those genes with signal values lower than 50, a comparison showed that only four genes were up-regulated and four genes down-regulated at 2-fold or higher levels (Table 2). Surprisingly, the most up-regulated gene in *atatk1* was *AtHAK5*, with a 2.86-fold increase and a *P*-value of 0.0178. This result was verified by quantitative PCR which indicated that *AtHAK5* exhibited a 25-fold greater expression in roots of *atakt1-1* compared with the wild-type plants. However, biological variation in AtHAK5 expression in both *atakt1-1* and Ws2 was large, which led to a non-statistically significance difference (*t* test, $P=0.073$).

## Discussion

There are 13 genes encoding KT/KUP/HAK transporters in *Arabidopsis*. The KT/KUP/HAK family of transporters was originally identified in bacteria (Schleyer and Bakker, 1993) and later in the soil-borne fungus *Schwanniomyces occidentalis* (Bañuelos *et al*., 1995). In fungi, the KT/HAK/KUP transporters mediate high affinity $K^+$ uptake, whereas in *E. coli* they mediate low affinity $K^+$ uptake (Uozumi, 2001). Some of the plant KT/HAK/KUPs have been shown to have both a high affinity and a low affinity

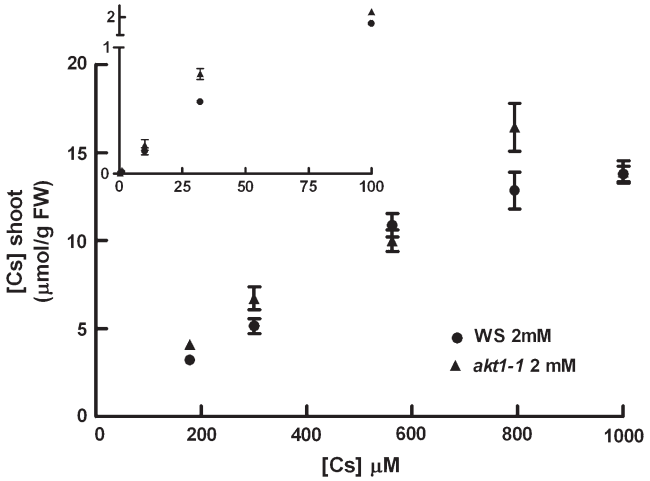


**Fig. 6.** The relationship between the shoot $Cs^+$ concentration ($[Cs^+]_{shoot}$) versus the $Cs^+$ concentration in the agar ($[Cs^+]$) of wild type (Ws2) and *atakt1* plants grown for 21 d on mineral-replete agar containing different concentrations of $Cs^+$ concentrations and 2 mM KCl. All data represent means ±SE ($n$=3). Insert shows more detail at the lower concentrations.

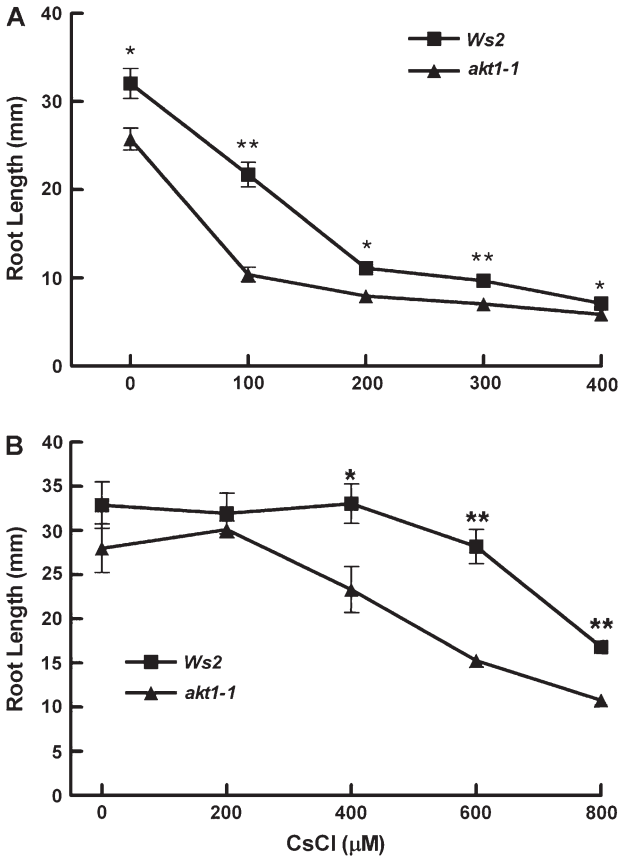


**Fig. 7.** Effects of CsCl on root elongation of 8-d-old Ws2 and *akt1* seedlings on medium containing (A) 100 μM KCl or (B) 1.75 mM KCl. All data represent means ±SE ($n$=4). Significant differences are indicated with asterisks (*$P < 0.05$ and **$P < 0.01$) as measured in agarose plate assays.

**Table 2.** *The most differentially expressed genes in wild type (Ws2) as compared to* atakt1-1 *plants based on results from microarray experiments*

| Gene | Fold change[a] | Name |
|---|---|---|
| At4g13420 | 3.0 | AtHAK5 |
| At5g26130 | 2.5 | Putative pathogenesis-related protein |
| At5g10040 | 2.5 | Unknown |
| At3g29970 | 2.2 | Germination protein-related |
| At3g27950 | 0.5 | Putative early nodule-specific protein |
| At1g34310 | 0.5 | ARF12, AUXIN RESPONSE FACTOR 12 |
| At3g10720 | 0.5 | Putative pectin esterase |
| At5g43360 | 0.5 | Phosphate transporter PHT3 |

[a] The fold change is the transcript abundance in *atakt1*, relative to the wild type.

for $K^+$ (Santa-Maria *et al*., 1997; Fu and Luan, 1998; Kim *et al*., 1998; Rubio *et al*., 2000; Senn *et al*., 2001). Characterization of two *Arabidopsis* mutants with defects in *AtKT*/*HAK*/*KUP* genes revealed that the AtKT/HAK/KUPs are involved in cell expansion (Foreman and Dolan, 2001; Rigas *et al*., 2001; Elumalai *et al*., 2002). At least one AtKT/HAK/KUP transporter, AtHAK5, has been shown to mediate high affinity $K^+$ uptake (Rubio *et al*., 2000; Gierth *et al*., 2005). The role of this transporter *in planta* is unclear, except for data showing that the gene encoding this transporter is up-regulated in response to potassium deprivation (Ahn *et al*., 2004; Armengaud *et al*., 2004; Gierth *et al*., 2005) and that the $K^+$ content of a *athak5* knockout was slightly decreased compared to the wild type when grown with 40 μM $K^+$ (Gierth *et al*., 2005).

Two important unresolved questions were identified about the function of *AtHAK5* in *Arabidopsis* that were addressed in this manuscript. In our study, biochemical methods were used to determine in which membrane AtHAK5 resides and the root elongation on low $K^+$ concentrations was characterized to determine whether AtHAK5 contributes to plant growth. It has been proposed that the members of the KT/HAK/KUP family function in different subcellular locations (Senn *et al*., 2001). The only available data on the membrane localization of KT/HAK/KUP transporters are from a rice homologue, OsHAK10. Based on visualization of an OsHAK10-GFP fusion protein, it was concluded that OsHAK10/KUP10 was localized to the vacuolar membrane (Bañuelos *et al*., 2002). In this report it is shown that an AtHAK5-HA fusion protein is located in the plasma membrane which supports a role for AtHAK5 in high affinity $K^+$ uptake (Gierth *et al*., 2005). To determine why much of AtHAK5 remained in the endoplasmic reticulum fraction, we checked if the HA tagged AtHAK5 protein was functional in yeast. The yeast strain CY162 that is defective in $K^+$ uptake (Schachtman and Schroeder, 1994) was transformed with the c-terminal HA tagged cDNA containing the L776H substitution. The tagged AtHAK5 cDNA did not complement the yeast mutant on low $K^+$ media (not shown). In yeast, the tagged protein was either not targeted to the plasma membrane or was unable to function because it was misfolded. However, it appears that some of the tagged protein *in planta* was induced by $K^+$ deprivation to relocate from ER to the plasma membrane. A slight increase in uptake in the lines overexpressing the tagged protein (not shown) could also be measured. The reason for the relocation of the tagged protein under potassium-deprived conditions is unknown at this time and could be due to the increased abundance of AtHAK5 protein triggered by the expression of the endogenous gene or due to some regulatory mechanism associated with protein trafficking. The cycling or trafficking of transport proteins between membranes under conditions of stress has been shown recently for plant aquaporins (Vera-Estrella *et al*., 2004) and an *Arabidopsis* boron transporter AtBOR1 (Takano *et al*., 2005). Similar to what has been observed, AtBOR1 accumulates at the plasma membrane under conditions of boron deficiency but is relocalized to internal membranes when boron is supplied.

Measurement of root elongation showed that this high affinity transporter plays a significant role in the growth of *Arabidopsis* at very low potassium concentrations. Root growth was less in *athak5* mutants than in wild-type plants when 1 μM $K^+$ or less was added in the growth medium. This is consistent with studies on a HAK transporter in pepper that was shown to function below concentrations of 10 μM $K^+$ (Martinez-Cordero *et al*., 2005). These low $K^+$ levels might be required to reveal a phenotype in part because of the contaminating amounts of $K^+$ in agarose. SeaKem agarose was used which was found to have very low concentrations of $K^+$, approximately 1–3 μM. From these results it appears that multiple mechanisms contribute to potassium uptake at low concentrations and that AtHAK5 is one mechanism of primary importance at very low potassium concentrations.

The increased expression of *AtHAK5* during $K^+$ starvation and the demonstration that AtHAK5 transports $Cs^+$ indicate that AtHAK5 may contribute to $Cs^+$ influx under $K^+$-deficient conditions (Rubio *et al*., 2000; White and Broadley, 2000; Hampton *et al*., 2004). $Cs^+$, however, does not affect the expression of *AtHAK5*, as revealed by genome-wide expression analyses (Hampton *et al*., 2004; Sahr *et al*., 2005). To determine the role AtHAK5 plays in $Cs^+$ transport, the growth and $Cs^+$ uptake of two null mutants compared to the wild type was studied. At low $K^+$ concentrations in the growth medium with added $Cs^+$, it was shown that wild-type plants expressing *AtHAK5*

have greater root elongation than plants lacking AtHAK5. Although AtHAK5 is a plasma membrane $Cs^+$ transporter in roots, knocking out *AtHAK5* was not sufficient to alleviate the detrimental effect of $Cs^+$ on root elongation, but it did slow the loss of chlorophyll as compared to wild type. The increased sensitivity to $Cs^+$ of the plants lacking AtHAK5, as determined by root growth, may be due to the blockade by $Cs^+$ of other $K^+$ uptake pathways such as AKT1 (Bertl *et al*., 1997; Schachtman, 2000). Under these conditions AtHAK5 may remain an important pathway for $K^+$ uptake at low concentrations when other pathways are blocked. At higher concentrations of $K^+$ there were no differences between the wild type and the *athak5* knock-outs with added $Cs^+$. Under these high $K^+$ conditions other low affinity $K^+$ uptake pathways could provide plants with $K^+$ (Very and Sentenac, 2003). Based on our short-term uptake measurements there may be other $Cs^+$ influx pathways since we only measured a 20% decrease in $Cs^+$ influx in the *athak5* knock-out which was not statistically different from the wild type (Table 1). However, in longer term experiments (Fig. 4), about half of the $Cs^+$ accumulation could be attributed to AtHAK5 suggesting it may be a major pathway under certain conditions.

Ammonium inhibition of $Cs^+$ influx was previously demonstrated for *Arabidopsis* grown under $K^+$-deficient conditions (Hampton *et al*., 2004). In pepper, $NH_4^+$ competitively inhibits CaHAK1 and also reduces the expression of this transporter in roots (Martinez-Cordero *et al*., 2005). In *Arabidopsis* the inhibitory effects of $NH_4^+$ on AtHAK5 have not been characterized and could be due to the inhibition of $K^+$ transporter function or a $NH_4^+$ induced repression of *AtHAK5* expression. Therefore, we tested how the expression of AtHAK5 responds to $NH_4^+$ *in planta* and whether this transporter is blocked by $NH_4^+$ in yeast cells expressing *AtHAK5*. In yeast, it was found that 2 mM $NH_4^+$ strongly inhibited the AtHAK5-mediated $Cs^+$ uptake and the expression of AtHAK5 decreased *in planta* when ammonium was added to the growth medium. In addition to the inhibition of AtHAK5-mediated $Cs^+$ uptake at high ammonium concentrations, it was also observed that low concentrations of $NH_4^+$ (100 μM $NH_4^+$) stimulated $K^+$ uptake. The effects of low concentrations of $NH_4^+$ on $Cs^+$ uptake were similar to the increased uptake of $K^+$ at low $Na^+$ concentrations (Spalding *et al*., 1999). Both $NH_4^+$ and $Na^+$ permeate $K^+$ transporters slowly (Bertl *et al*., 1997) and might bind and modulate the function of these transport proteins to facilitate $Cs^+$ or $K^+$ influx at low concentration. However, the effects of 100 μM $NH_4^+$ and higher concentrations on AtHAK5 activity may be of limited significance, since soils rarely contain higher than 100 μM $NH_4^+$.

Both AtHAK5 and AtAKT1 contribute to $K^+$ uptake under low $K^+$ conditions (Hirsch *et al*., 1998; Spalding *et al*., 1999; Gierth *et al*., 2005, Xu *et al*, 2006). Plants lacking one of these transporters will have a greater dependence on the remaining transporter. Plants lacking *AtAKT1* are more sensitive to $NH_4^+$ (Spalding *et al*., 1999), $Na^+$ (Qi and Spalding, 2004), and $Cs^+$ (Fig. 2A) which could be due to the sensitivity of AtHAK5 to $NH_4^+$, $Na^+$, and $Cs^+$. Similarly, the shorter root phenotype of *athak5* under $Cs^+$ stress could be explained by the sensitivity of AtAKT1 to $Cs^+$. As support for this hypothesis, AKT1-mediated $K^+$ currents in yeast cells are strongly inhibited by $Cs^+$ but much less by $NH_4^+$ (Bertl *et al*., 1997), and AtHAK5-mediated $Cs^+$ uptake in yeast is inhibited by $NH_4^+$ (Fig. 5B) which is consistent with previous findings that $Cs^+$ influx of *Arabidopsis* under $K^+$-deficient condition was inhibited by $NH_4^+$ (Hampton *et al*., 2004).

### *Effect of Cs on the* akt1 *knock-out*

Microarray analysis showed that one of the major changes in gene expression in the *akt1-1* null mutant is an up-regulation of *AtHAK5*. The consistent increase in $Cs^+$ influx and accumulation in *akt1* may therefore be due to the up-regulated expression of *AtHAK5*. The increased expression of *AtHAK5* in *akt1-1* may also confer the $Cs^+$ -sensitive growth phenotype of this mutant. However, the increased uptake of $Cs^+$ in *akt1-1* was only 10% (not significantly different from the wild type) and, therefore, it seems unlikely that this increased $Cs^+$ influx is the sole cause of the $Cs^+$-sensitive phenotype of *akt1-1*. We speculate that it is more likely that the $Cs^+$-sensitive growth phenotype of *akt1* is due to the impaired $K^+$ nutritional status of plants lacking the $K^+$ transporter AKT1.

The extraction of radiocaesium from contaminated soils using plants has been proposed as a phytoremediation technology. However, since it is likely that there are no specific $Cs^+$ transporters in plants, $K^+$ uptake pathways must be targeted to accelerate $Cs^+$ accumulation. $Cs^+$ in soil could be extracted with plants overexpressing certain $Cs^+/K^+$ transporters. However, although AtHAK5 contributes significantly to $Cs^+$ uptake by roots of $K^+$-deficient plants, it is not a promising target for phytoremediation purposes because plants overexpressing *AtHAK5* had only a marginally greater rate of $Cs^+$ influx and accumulation than wild-type plants (data not shown). Another strategy, to utilize agricultural soils contaminated by radiocaesium, is to grow crops that accumulate less radiocaesium by preventing $Cs^+$ from entering plant root cells. To do so, the selectivity of the complement of $Cs^+/K^+$ transporters must be modified such that $K^+$ can be accumulated but not $Cs^+$. When grown in media containing low $K^+$ concentrations, *Arabidopsis* lacking *AtHAK5* show reduced rates of Cs influx (Table 1) and accumulation (Fig. 4C), but they also show reduced growth rates (Fig. 2) presumably because they lack sufficient $K^+$. Similarly, *Arabidopsis* plants lacking certain AtCNGCs have reduced radiocaesium

accumulation rates, but these mutants exhibit other detrimental phenotypes including reduced growth (Hampton *et al*., 2005). It may, therefore, be necessary to manipulate the cationic selectivity of individual transporters. Proof of concept has been demonstarted for the KAT1 $K^+$-channel protein (Anderson *et al*., 1992; Schachtman *et al*., 1992), which is expressed predominantly in guard cells (Nakamura *et al*., 1995). Mutagenesis of the channel reduced $Cs^+$ uptake which increased resistance to $Cs^+$ toxicity (Ichida and Schroeder, 1996; Ichida *et al*., 1997, 1999).

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