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High-affinity potassium and sodium transport systems in plants

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Abstract

All living cells have an absolute requirement for K^+, which must be taken up from the external medium. In contrast to marine organisms, which live in a medium with an inexhaustible supply of K^+, terrestrial life evolved in oligotrophic environments where the low supply of K^+ limited the growth of colonizing plants. In these limiting conditions Na^+ could substitute for K^+ in some cellular functions, but in others it is toxic. In the vacuole, Na^+ is not toxic and can undertake osmotic functions, reducing the total K^+ requirements and improving growth when the lack of K^+ is a limiting factor. Because of these physiological requirements, the terrestrial life of plants depends on high-affinity K^+ uptake systems and benefits from high-affinity Na^+ uptake systems. In plants, both systems have received extensive attention during recent years and a clear insight of their functions is emerging. Some plant HAK transporters mediate high-affinity K^+ uptake in yeast, mimicking K^+ uptake in roots, while other members of the same family may be K^+ transporters in the tonoplast. In parallel with the HAK transporters, some HKT transporters mediate high-affinity Na^+ uptake without cotransporting K^+. HKT transporters have two functions: (i) to take up Na^+ from the soil solution to reduce K^+ requirements when K^+ is a limiting factor, and (ii) to reduce Na^+ accumulation in leaves by both removing Na^+ from the xylem sap and loading Na^+ into the phloem sap.

Key words: Potassium transport, sodium transport.

Introduction

Among all the cations that were present in the sea, where the early evolution of life took place, K^+ was utilized by cells as the major cation of their internal environment, with the chief functions of maintaining electroneutrality and osmotic equilibrium. In this cellular K^+-rich environment further evolution of some biochemical processes made use of K^+ for regulatory purposes while, in others, there evolved protein activities that depended on K^+–protein interactions. Because these interactions were not mimicked by Na^+ or by any other cation, K^+ became absolutely necessary for living cells. This K^+ dependence did not confine the evolution of life to the sea, where K^+ has always been abundant. Quite the reverse, the emergence of the terrestrial plants in the Cambrian era (Heckman *et al*., 2001; Sanderson, 2003) and their evolution from Bryophytes to flowering plants (Qiu and Palmer, 1999; Nickrent *et al*., 2000) took place in oligotrophic environments where K^+ was at much lower concentrations than in sea waters. Despite this K^+ shortage terrestrial plants not only kept their K^+ dependence but also developed new functions for K^+, such as for hydraulic cell movements (Blatt, 2000).

Because, the K^+ requirement applies to every cell in multicellular organisms, after entering a plant, K^+ has to be transported to distant organs through the xylem. K^+ moves from the root symplast to the xylem sap and from this to the apoplastic space outside the bundle sheath, a process that involves many types of cells. Although, in most cells, the cytoplasmic K^+ concentrations are quite similar in non-stressing conditions, around 100 mM (Walker *et al*., 1996*a*; Cuin *et al*., 2003), the external K^+ concentrations and pH values to which root and internal cells are exposed are considerably variable (Leigh and Wyn Jones, 1984). Consequently, it is not surprising that plant genomes contain a high

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number of genes encoding K^+ transporters and channels (Mäser *et al*., 2001, 2002*b*; Véry and Sentenac, 2002, 2003). Some of these transporters have the specific capacity of taking up K^+ from very low K^+ concentrations and of keeping very high concentration ratios across the plasma membrane. This type of transporter may be important for many plant cells, but are crucial in root epidermal and cortical cells, where they determine the capacity of the plant to thrive in soils with very low K^+ concentrations.

The term 'high-affinity transporter' does not have a formal definition and it is used here to indicate that the transporter exhibits a K_m that is not much higher than that of the high-affinity 'Mechanism I' that was described in pioneering papers on K^+ transport (Epstein *et al*., 1963). Although under such low-K^+ conditions transport may involve a high energetic demand, the term high-affinity does not imply that the transporter necessarily mediates an active process (Rodríguez-Navarro, 2000). For example, the high-affinity K^+ transporter of *Neurospora crassa* is an active transporter because it mediates the accumulation of K^+ when the diffusion potential of K^+ is more negative than the membrane potential (Rodríguez-Navarro *et al*., 1986). By contrast, a channel may exhibit high-affinity for K^+ (Sentenac *et al*., 1992; Brüggemann *et al*., 1999), but cannot mediate active transport.

Although plants have an absolute requirement for K^+, and Na^+ is toxic for many biological reactions in the cytoplasm, this does not apply to vacuolar processes and the replacement of K^+ by Na^+ in the vacuole does not produce toxicity (Flowers and Läuchli, 1983; Subbarao *et al*., 2003). With an unrestricted supply of K^+, a significant proportion is in the vacuole; its replacement by Na^+ greatly reduces the total K^+ content of the plant and, consequently, its ability to take up Na^+ is an evolutionary advantage that plants have used. However, if Na^+ is scarce, the aforementioned difficulties for taking up K^+ apply to Na^+, which also requires a high-affinity transporter.

This paper aims to present an overview of the advances that have taken place in recent years on the functional understanding of high-affinity K^+ and Na^+ transport systems. There are references to a previous review by Rodríguez-Navarro (2000) for general concepts and this is complemented by more recent reviews on K^+ (Schachtman, 2000; Mäser *et al*., 2001, 2002*b*; Tester and Leigh, 2001; Shabala, 2003; Véry and Sentenac, 2003; Amtmann *et al*., 2004) and Na^+ (Blumwald *et al*., 2000; Tester and Davenport, 2003; Horie and Schroeder, 2004) transport, avoiding repetitions. Recent reviews on cation channels (Demidchik *et al*., 2002; Véry and Sentenac, 2002; Chérel, 2004) are also relevant to this paper.

K^+ transport

High-affinity K^+ uptake and HAK transporters

High-affinity K^+ uptake was firstly described by Epstein *et al.* (1963). It was shown that the roots of barley (*Hordeum vulgare* L.) seedlings that had been germinated in a diluted $CaSO_4$ solution developed a high-affinity K^+ influx with a K_m of 10–20 mM K^+, which did not discriminate between K^+ and Rb^+ and was unaffected by micromolar concentrations of Na^+. Interestingly, an additional characteristic was its low discrimination against Cs^+ (Zhu and Smolders, 2000; and references therein). This system was named high-affinity 'Mechanism I' (Epstein *et al*., 1963). Further studies on the regulation of this K^+ uptake system showed that it was rapidly up-regulated when the supply of exogenous K^+ was arrested (Glass, 1978; Glass and Dunlop, 1978). Similar research with other species proved that high-affinity K^+ uptake was a general capacity of plants and that the barley model applied to many species (Kochian and Lucas, 1988; Rodríguez-Navarro, 2000). During the last decade, several laboratories have been working on the identification of the plant genes that encode this uptake system, a system that shares all the aforementioned characteristics with fungal HAK transporters (Rodríguez-Navarro, 2000). However, the molecular and genetic characterization of transport systems starting from simple kinetic data is not an easy task. Fortunately, the use of yeast mutants, defective in K^+ uptake, proved an important tool that allowed rapid progress in the identification of plant cDNAs that encode K^+ transporters. A further combination of these findings with the genetic studies on the model plant *Arabidopsis thaliana* that are described below allowed a comprehensive model of high-affinity K^+ uptake in plants to be produced.

The first transporters that were identified in yeast mutants using cDNA libraries (Anderson *et al*., 1992; Sentenac *et al*., 1992) belonged to the class of inward-rectifier K^+ channels. From kinetic considerations, the identity of any of these channels with the high-affinity barley transporter was unlikely. The putative high-affinity barley transporter was eventually identified assuming sequence similarity to fungal HAK transporters (Santa-María *et al*., 1997). Later, BLAST searches and the systematic use of an RT- PCR approach led to the isolation of cDNAs from *Arabidopsis* (Rubio *et al*., 2000), rice (*Oryza sativa* L.) (Bañuelos *et al*., 2002), and pepper (*Capsicum annuum*. L) (Martínez-Cordero *et al*., 2004) that encoded transporters of the same family. Functional expression of these cDNAs (*AtHAK5*, *HvHAK1*, *OsHAK1*, and *CaHAK1*) in yeast mutants revealed that the encoded transporters exhibited the expected characteristics: a high capacity to deplete external K^+ to very low concentrations (<1 μM); very low K_ms for K^+, Rb^+, and Cs^+; NH_4^+ inhibited K^+ uptake, but was not transported; and Na^+ exhibited low-affinity for the transporters. Taken together, these lines of evidence strongly suggested that the identified plant HAK transporters were those that mediated the high-affinity K^+ uptake by roots or, at least, a major component of it. Additional evidence is that transcript expression is enhanced by K^+ starvation, paralleling the onset of the high-affinity K^+ uptake in roots.

The genetic model in Arabidopsis

The use of the model plant *Arabidopsis* provided the opportunity to add the use of powerful genetic approaches for the study of K^+ transporters. However, the use of this model plant presents some difficulties because uptake tests are difficult to perform and basic kinetic data of K^+ uptake (K_m and K_is of other alkali cations) are still incomplete. Nevertheless, using BLAST searches, 13 genes encoding proteins with sequence similarities to the above-mentioned HAK transporters have been identified in *Arabidopsis* (Mäser *et al*., 2001). This type of transporter has also been named KT and KUP (Quintero and Blatt, 1997; Fu and Luan, 1998; Kim *et al*., 1998). Out of the 13 putative K^+ transporters of this family, one of them, AtHAK5, is in the phylogentic cluster that is defined by the high-affinity HAK transporters of other plant species. As already mentioned, the kinetic characteristics of AtAHK5 expressed in yeast mutants are very similar to those taken as a model of high-affinity K^+ uptake in plants (Rubio *et al*., 2000) and *AtAHAK5* is induced in roots upon K^+ starvation (Ahn *et al*., 2004; Armengaud *et al*., 2004; Shin and Schachtman, 2004; Gierth *et al*., 2005). Interestingly, reactive oxygen species production is an early response of K^+ deficiency and externally added H_2O_2 was sufficient for the induction of the high-affinity component of Rb^+ uptake (Shin and Schachtman, 2004).

The characteristics described above for AtHAK5 suggested that it might be crucial for high-affinity K^+ uptake in *Arabidopsis*. However, the lack of detailed studies on the kinetics of root K^+ uptake hampers the establishment of the relevance of AtHAK5 because there are not enough plant kinetic data to which the functional expression in yeast can be compared. Significant progress in this line of research has been made with the recent characterization of two *Arabidopsis* lines with T-DNA insertions in *AtHAK5*, which has shed light on the role of this transporter in the plant (Gierth *et al*., 2005). *athak5* plants lack an inducible high-affinity transport system with a K_m for Rb^+/K^+ of approximately 15–24 μM, which agrees with the K_m shown by AtHAK5 expressed in yeast (Rubio *et al*., 2000).

The remaining system mediating K^+ uptake in the micromolar range of concentrations in *athak5* plants is the inward-rectifier K^+ channel AtAKT1. The involvement of AtAKT1 in high-affinity K^+ uptake goes against the paradigm proposing that channels mediate low-affinity K^+ uptake (Rodríguez-Navarro, 2000), but it is well documented by the characterization of a T-DNA insertion line in *AtAKT1*, which proved that the AtAKT1 channel mediates K^+ uptake from solutions that contained as little as 10 μM K^+ (Hirsch *et al*., 1998). By using membrane potential measurements in root cells, it was found that the AKT1 component of wild-type permeability was between 55% and 63% of total permeability when external K^+ was between 10 μM and 1000 μM and NH_4^+ was absent. The finding that NH_4^+ specifically inhibited the non-AtAKT1 component of K^+ uptake (Spalding *et al*., 1999) is consistent with the notion that this component involves a HAK transporter.

In contrast to the results obtained by membrane potential depolarization measurements, kinetic studies in wild-type and *atakt1* plants suggest a small contribution of AtAKT1 to K^+ uptake in the micromolar range of concentrations, because the K_m of the K^+ influx amounted to 0.9 mM $K^+(Rb^+)$ (Gierth *et al*., 2005). Although this conclusion might be taken as contradictory with that proposing that AtAKT1 mediated 55–63% of the K^+ uptake in the micromolar range of K^+ concentrations, both are consistent. The apparent contradictions can be explained by the use of Rb^+ as a tracer of K^+ in the study with the *atakt1* mutant (Gierth *et al*., 2005). In the case of the HAK component of uptake, the Rb^+ data apply directly to K^+ because HAK transporters do not discriminate between the two cations, but this is not necessarily true for a channel, which may be less permeable to Rb^+ than to K^+. Assuming a lower V_{max} and a higher K_m for Rb^+ versus K^+ influx when AtAKT1 is involved, the notion that this channel mediates a significant part of the K^+ uptake in *Arabidopsis* in the micromolar range of K^+ becomes apparent.

In general terms, the conclusion drawn from the experiments performed with the *Arabidopsis* mutants probably applies to many plants. However, for more quantitative conclusions two caveats must be considered: that the knockout of a gene may generate pleiotropic effects (see below) and that different growth conditions may change the proportion of the uptake that is mediated by the channel and the transporter. Regarding the growth conditions, in barley (Santa-María *et al*., 2000) and pepper (Martínez-Cordero *et al*., 2005) the contribution of the NH_4^+-sensitive (putatively HAK1-type transporters) versus the NH_4^+-insensitive (putatively AKT1-type channels) pathways of high-affinity K^+ uptake was lower in NH_4^+-grown plants than in plants grown in the absence of NH_4^+. Moreover, in pepper, the enhancement of the expression of *CaHAK1* transcripts by K^+ starvation was decreased by the presence of NH_4^+ in the nutrient solution.

All this suggests that, as a general rule for many plants suffering K^+ starvation, root high-affinity K^+ uptake can be mediated by an AKT1-type channel and an HAK1-type transporter, but that the former may be important only in plants grown in the presence of NH_4^+.

K^+ transport within the plant

After entry into the root symplast, K^+ has to be distributed to the rest of the plant cells, firstly by loading the xylem and later moving to the surrounding cells. In these movements K^+ crosses the plasma membranes of several types of cells, depending on plant species. Moreover, fluxes into and out of the vacuole are also involved in cell K^+ homeostasis. It is

clear that K^+ channels mediate many of these fluxes (Véry and Sentenac, 2003), even when K^+ is taken up from diluted solutions (Brüggemann *et al*., 1999), but other transporters are also involved. Kinetic characteristics and thermodynamic reasons (Smith and Epstein, 1964; Osmond and Laties, 1968; Reed and Bonner, 1974; Blatt, 1985; Bellando *et al*., 1995; see also Rodríguez-Navarro, 2000) strongly suggest that high-affinity HAK K^+ transporters are involved in some of these fluxes. Interestingly, at the xylem/bundle sheath interface in maize leaves, the permeability for Rb^+ is at least as high as that for K^+ (Keunecke *et al*., 2001), which resembles the most notorious characteristic of HAK transporters. All this suggests that the function of high-affinity HAK K^+ uptake transporters is not restricted to K^+ uptake from the soil solution in root epidermal and cortical cells. Consistent with this, AtHAK5 is expressed in shoots (Rubio *et al*., 2000; Ahn *et al*., 2004) and in stellar root cells (Gierth *et al*., 2005).

A more complex question about the KT/HAK/KUP transporters is the function (or existence) of the low-affinity members of this family (Senn *et al*., 2001; Bañuelos *et al*., 2002; Garciadeblas *et al*., 2002). In fact, most of the transporters of the KT/HAK/KUP family, 13 in *Arabidopsis* (Mäser *et al*., 2001) and 17 in rice (Bañuelos *et al*., 2002), do not belong to the cluster of the high-affinity transporters (Bañuelos *et al*., 2002), and in some cases it has been demonstrated that they are low-affinity K^+ transporters (Senn *et al*., 2001; Garciadeblas *et al*., 2002). A possible hypothesis is that the main characteristics of all the transporters of this family is to be K^+-H^+ symporters (Rodríguez-Navarro, 2000). At a first glance, it seems unlikely that an active mechanism is associated with a low-affinity K^+ transporter in the plasma membrane, because when K^+ is at millimolar concentrations in the external medium, the membrane potential as the unique driving force is sufficient to give rise to the observed transmembrane gradient. An alternative possibility is that some of these K^+-H^+ symporters locate to the tonoplast as shown for OsHAK10 (Bañuelos *et al*., 2002). Because the electrical potential across the tonoplast is less negative than that across the plasma membrane, active transport of K^+ from the vacuole to the cytoplasm may be necessary when the K^+ content of the vacuole is low. This occurs in root cells of barley plants under K^+-limiting conditions (Walker *et al*., 1996*a*). In Na^+-grown plants, the same flux is not active in leaves (Cuin *et al*., 2003), but has to be active in root cortical cells (Carden *et al*., 2003). What is interesting regarding this possibility is that some HAK transporters of *Mesembryanthemum crystallinum* L. that belong to group II are induced by high external Na^+ concentrations (Su *et al*., 2002).

Two *Arabidopsis* mutants that are defective in root hair (*attrh1*) (Rigas *et al*., 2001) and hypocotyl (*atshy3-1*) (Elumalai *et al*., 2002) growth were found to have mutations in genes of the KT/HAK/KUP family. The *attrh1* (*TRH1* is equivalent to *KT3* and *KUP4*) mutation reduces auxin efflux in isolated root segments and expression of the *atTRH1* cDNA in yeast cells accelerates auxin efflux (Vicente-Agullo *et al*., 2004). The AtTRH1 protein does not have sequence similarity to auxin transporters (Kramer, 2004, and references therein) and it is unlikely that it transports auxin. A possible explanation of this puzzle is that the *attrh1* mutation in plant cells and the expression of the *AtTRH1* cDNA in yeast cells affect the cellular pH, which might occur if AtTRH1 is a tonoplast K^+-H^+ symporter. Auxin is a weak acid (pK_a=4.75) and in its undissociated form is lipophilic (the acid but not the anion), and will readily cross the plasma membrane. This implies that the lower the cytoplasmic pH, the higher the passive efflux. This applies to yeast, which does not have auxin transporters, while the case of plant cells is obviously more complex.

Na^+ uptake

High-affinity Na^+ uptake and HKT transporters

High-affinity Na^+ uptake was described many years ago in the roots of K^+-starved barley seedlings (Rains and Epstein, 1967*a*, *b*). However, because the addition of low K^+ concentrations inhibited Na^+ uptake at the same time as it elicited a rapid K^+ uptake, it was concluded that the system that so strongly selected K^+ over Na^+ was the high-affinity 'Mechanism I' that transports K^+ (Epstein *et al*., 1963). Despite the interest of these results, high-affinity Na^+ uptake in plants received little attention for more than 30 years. Especially important is the repeated observation that Na^+ reduces K^+ requirements (Rodríguez-Navarro, 2000; Subbarao *et al*., 2003). It is assumed in explaining this observation that in the absence of external K^+, the uptake of some Na^+ is better than no monovalent cation at all. In the absence of external K^+, the cellular K^+ content decreases and, concomitantly, the cellular pH also decreases (Walker *et al*., 1996*a*, 1998), injuring the physiology of the cell. By contrast, if Na^+ is taken up and substitutes for K^+, a decrease of the cellular pH can be avoided (Carden *et al*., 2003). A low amount of Na^+ is unlikely to be toxic in the cytoplasm, and even high amounts of Na^+ are not toxic in the vacuole (Flowers and Läuchli, 1983; Blumwald *et al*., 2000; Subbarao *et al*., 2003).

Given the potential benefits of Na^+ uptake when K^+ limits the growth of a plant, it might be expected that high-affinity Na^+ uptake played a crucial role in the evolution of terrestrial plants as they conquered an oligotrophic medium in the Cambrian era. This prediction can be extended to fungi, which also colonized terrestrial environments (Blackwell, 2000; Heckman *et al*., 2001) and thrive in soils together with plant roots. The existence of a high-affinity Na^+ uptake in fungi (Benito *et al*., 2004) confirmed the suspected importance of Na^+ and high-affinity Na^+-uptake

systems for the physiology of organisms thriving in low-K^+ environments.

The first plant transporter that was reported to be involved in high-affinity Na^+ uptake was the wheat HKT1. Although HKT1 mediates Na^+-driven high-affinity K^+ uptake and low-affinity Na^+ uptake in yeast cells and *Xenopus* oocytes (Rubio *et al*., 1995) its involvement in the physiological function of Na^+ uptake was not proposed. Later it was found that the *Arabidopsis* AtHKT1 (Uozumi *et al*., 2000) and the rice OsHKT1 (Horie *et al*., 2001) mediated Na^+ uptake that was not coupled to K^+ uptake. Almost simultaneously, a study with transgenic lines of wheat that showed down-regulation of *HKT1* transcripts in certain conditions suggested that the transporter encoded by these transcripts mediated Na^+ uptake but not K^+ uptake (Laurie *et al*., 2002). In all these cases the uptake of Na^+ as a single ion was demonstrated only in the low-affinity range of concentrations.

After these reports, molecular studies in rice (Garciadeblás *et al*., 2003) have demonstrated that HKT transporters mediate the high-affinity Na^+ uptake that is carried out by K^+-starved plants and that this occurs without the cotransport of K^+. The basis of the proposal is that these transporters expressed in yeast cells mimic, almost exactly, high-affinity Na^+ uptake in the roots of rice, including the effects of several inhibitors. Consistent with the notion that high-affinity Na^+ uptake is only supplementary to K^+ uptake, it was found that high-affinity Na^+ uptake and the expression of the transcripts that encode the HKT1 transporters in barley, wheat (Wang *et al*., 1998; Haro *et al*., 2005), and rice (Garciadeblás *et al*., 2003) are greatly enhanced when plants are K^+-starved. Furthermore, Na^+ uptake is inhibited by K^+ in K^+-starved plants of rice, wheat, and barley (Garciadeblás *et al*., 2003). Based on these studies and those already described for HAK transporters, the pioneering proposal of Epstein should be modified in the sense that the high-affinity 'Mechanism I' of barley is made up of two systems, the HAK1 K^+ transporter and HKT1 Na^+ transporter, which operate in parallel (Fig. 1), when the plants are grown in the absence of NH_4^+.

An intriguing question that currently cannot be answered is whether all plant species can carry out root high-affinity Na^+ uptake. In fungi, in which Na^+ uptake has been extensively investigated and many complete genome sequences are available, it has been found that high-affinity Na^+ uptake does not exist in species that have adapted to media with high K^+ contents (Benito *et al*., 2004). The experiments needed to answer the question in plants are very simple because the high-affinity Na^+ uptake test is unmistakable. However, thus far, only some cereals and sunflower plants (*Helianthus annuus* L.) have been tested, and, although the results have been positive in these species (Garciadeblás *et al*., 2003), the existence of this transport system may not be a general rule. For example, it may be absent in *Arabidopsis*, because AtHKT1 seems to be a low-affinity Na^+ uptake transporter (Uozumi *et al*., 2000) and there is not another HKT transporter encoded in the *Arabidopsis* genome that could carry out the high-affinity uptake (Mäser *et al*., 2001). Moreover, Na^+ influx did not differ from the wild type in an *athkt1* insertional mutant (Essah *et al*., 2003). Although this is a convincing result, a definitive conclusion needs further research. The main caveat regarding the experiments with the *athkt1* mutant is to know whether, in the conditions of the experiments, AtHKT1 was functional in the wild-type plants and whether its activity is significant versus the activity of the channels that transport Na^+ (Tyerman and Skerrett, 1999; Davenport and Tester, 2000; Maathuis and Sanders, 2001; Demidchik *et al*., 2002; Essah *et al*., 2003) when tests are performed with the cation at millimolar concentrations.

Epstein's original proposal:

High-affinity mechanism I is a K^+ transporter that can transport Na^+ when K^+ is not present

Current formulation based on molecular findings:

High-affinity mechanism I is made up of two transporters, HAK1 and HKT1. HAK1 is a high-affinity K^+ transporter with low affinity for Na^+ and HKT1 is a high-affinity Na^+ transporter that is inhibited by K^+ although it does not transport K^+

Fig. 1. Modification of Epstein's pioneering proposal on high-affinity K^+ and Na^+ uptake in barley.

The properties of HKT transporters explain the controversy regarding their function

The function of HKT transporters as high-affinity Na^+ uptake systems conflicts with the description of the wheat HKT1 transporter and this conflict deserves further discussion. The wheat HKT1 transporter was originally characterized as the K^+-H^+ symporter that mediated the high-affinity K^+ uptake system of wheat roots (Schachtman and Schroeder, 1994), but it was later found that it cotransported Na^+-K^+ when expressed in yeast cells or *Xenopus* oocytes (Rubio *et al*., 1995). The latter finding has not completely eliminated the notion that considered HKT1 as a root high-affinity K^+ transporter (Horie and Schroeder, 2004). However, Na^+-stimulated K^+ uptake, resembling a Na^+-K^+ cotransport function, has never been shown to operate in the roots of any cereal (Maathuis *et al*., 1996; Walker *et al*., 1996*b*; Hayes *et al*., 2001; Garciadeblás *et al*., 2003). This negative observation does not rule out that HKT1 functions as a high-affinity K^+ transporter (Rubio *et al*., 1996), but it cannot be Epstein's high-affinity 'Mechanism I', which is kinetically different.

The apparent contradiction between the function of the rice HKT1 transporter, which is taken as a model of high-affinity Na^+ uptake, and the wheat HKT1 transporter lies in the complexity of the heterologous expressions of

HKT transporters. The same cDNA, either *HvHKT1* (barley) or *TaHKT1* (wheat), can be expressed as a Na^+-K^+ symport or Na^+ (or K^+) uniport in yeast, depending on the construct in which the cDNA is inserted. This can be explained by alternative initiations of translation, which give rise to different proteins with different kinetic properties (Haro *et al*., 2005). In barley, only the uniport function mimics the kinetics of Na^+ uptake in roots, which suggests that the Na^+-K^+ symport may either be an artefact or a function that is expressed in non-root epidermal and cortical cells. Interestingly, with the rice OsHKT1 transporter the problems show some similarities with those seen with the wheat transporter, because the expression of OsHKT1 in *Xenopus* oocytes has been reported to be either an Na^+ transporter (Horie *et al*., 2001) or a Na^+ or K^+ transporter that shows higher currents when Na^+ and K^+ are added together (Golldack *et al*., 2002). In yeast (perhaps in a particular construct), OsHKT1 behaves as a high-affinity Na^+ uptake system that is strongly inhibited by K^+ and to a lesser extent by Ba^{2+}, mimicking, as described above, the high-affinity Na^+ uptake exhibited by K^+-starved rice roots (Garciadeblás *et al*., 2003).

Now, the most likely explanation for the diverse results concerning HKT transporters is that, as described for the expression in yeast, some *HKT* mRNAs have alternative initiations of translations in the plant, which give rise to transporters with different kinetic properties that are adapted to different physiological conditions. If these alternative initiations of translations are not performed in heterologous systems exactly as they are in the plants, the properties of the expressed transporter could be variable and not physiological (Haro *et al*., 2005).

The versatility of functions of plant HKT transporters, which may be used by the plant in a mode that is not yet known, probably lies in their capacity for transporting two cations simultaneously. These transporters have a structure made up of four MPM (membrane-pore-membrane) motifs that also occurs in bacterial and fungal transporters of the same family (Rodríguez-Navarro, 2000; Kato *et al*., 2001; Zeng *et al*., 2004). Plant HKT and fungal TRK transporters are similar, and all the evidence suggests that they have two cation binding sites that must be occupied before the bound ions can cross the membrane (Rodríguez-Navarro, 2000; Haro and Rodríguez-Navarro, 2002, 2003; Garciadeblás *et al*., 2003). If the same alkali cation, K^+ or Na^+, can occupy the two sites, the result is a uniport of either K^+ or Na^+, as in the case of K^+ transport in *Saccharomyces cerevisiae* and Na^+ transport by OsHKT1, TaHKT1, HvHKT1, and AtHKT1. In the event that the same ion cannot bind the two sites (or can do so only with very low affinity), but that two different ions can and cross the membrane, the result is a Na^+-K^+ symport, as occurs with some constructs of TaHKT1 and HvHKT1. Finally, if Na^+ can bind the two sites with high affinity and be transported, and K^+ can bind one of the two sites with high affinity but cannot cross the membrane, the result is a Na^+ uniporter whose function is strongly inhibited in the presence of K^+, as described for OsHKT1 (Fig. 2). In other words, this transporter mediates Na^+ uptake, but only when K^+ is not available. All these descriptions apply when K^+ and Na^+ are bound at micromolar concentrations (AtHKT1 and OsHKT4 may be exceptions). When the cations are present at millimolar concentrations some of these transporters may uniport Na^+, K^+, or Rb^+, regardless of the function that they exhibit when the cations are at micromolar concentrations (Gassmann *et al*., 1996; Garciadeblás *et al*., 2003).

It is worth observing that the binding of two Na^+, as in OsHKT1 in Fig. 2, does not result in an active process (Rodríguez-Navarro, 2000). Although specific experiments aimed at thermodynamic calculation have not been reported, typical uptake experiments with plants or yeast cells expressing OsHKT1, in which external Na^+ can be depleted down to 1–2 mM (a slightly lower concentration can be reached during K^+ starvation of roots and yeast cells if the nutrient medium has an initial low amount of Na^+) (Garciadeblás *et al*., 2003; Haro *et al*., 2005), do not imply an active uptake. Roots with a membrane potential of −180 mV, which is a reasonable assumption (Spalding *et al*., 1999, and references therein), could reach a maximum cytoplasmic/external concentration ratio of Na^+ of 10^3, which means that the Na^+ concentration in the cytoplasm could not reach a value higher than 1–2 mM. It can be predicted that this value is not exceeded in the described experiments, because the amount of Na^+ that is taken up is

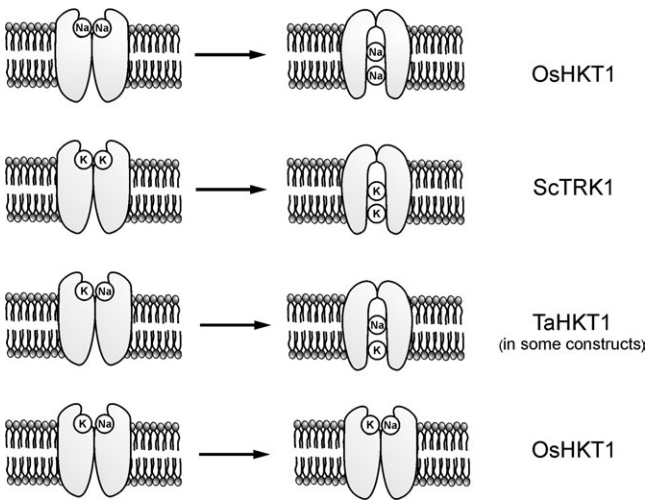

Fig. 2. Schematic representation of a hypothetical mechanistic model that explains the activity of fungal TRK and plant HKT transporters. The outer part of the pore has to bind two cations before these can move inside and cross to the other side of the membrane. The first two models are Na^+ or K^+ transporters, which bind two identical cations and allow them to move across the pore. The third model is a Na^+-K^+ symport, which accommodates one Na^+ and one K^+, but does not accommodate two Na^+ or two K^+. The fourth model is the Na^+ transporter presented in the first one but in a form that is inhibited by K^+, because K^+ binds the transporter but is not transported. Abbreviations: Os, *Oryza sativa*; Sc, *Saccharomyces cerevisiae*; Ta, *Triticum aestivum*.

low and a significant part of the Na^+ taken up is probably accumulated into the vacuole (Carden *et al*., 2003).

Making use of mutants (Diatloff *et al*., 1998; Rubio *et al*., 1999; Liu *et al*., 2000; Mäser *et al*., 2002*c*; see also Kato *et al*., 2001) and by comparing the sequences of the OsHKT1 and OsHKT2 transporters (Horie *et al*., 2001) the role of several amino acid residues in the Na^+/K^+ selectivity has been discussed. Although these data suggest functional domains, the fact that small changes in the protein change the function of these transporters (uniport or symport) suggests that more complete kinetic studies are necessary before a clear scheme of the structure–function relationships of these transporters can be made. It is especially important to determine the effect of the mutations on the affinity of the transporter in each one of the two binding sites and on the V_{max}. In addition, the kinetic response may be affected more by the aforementioned variability of the expressed protein than by the change of a particular amino acid residue. This applies to the Gly or Ser residue that is located at the end of the first P loop of the transporter. A Gly residue is probably necessary for K^+ transport in some species (Horie *et al*., 2001; Mäser *et al*., 2002*c*), but a Ser residue allows K^+ transport in others (Fairbairn *et al*., 2000; Liu *et al*., 2001; Golldack *et al*., 2002). Even AtHKT1, which is a model of a Na^+ transporter, transports K^+ when expressed in bacteria (Uozumi *et al*., 2000).

In addition to what has been described, high-affinity TRK-HKT transporters have other important characteristics whose physiological roles have not been sufficiently investigated so far. A striking characteristic is that some of them can function as Cl^- channels (Baev *et al*., 2004; Kuroda *et al*., 2004). Probably unrelated to this function or to that proper to transporting Na^+ or K^+, the expressions of some HKT transporters in yeast, or at least some constructs with the *HKT* cDNAs, are quite toxic. This is the case of OsHKT1 (Garciadeblás *et al*., 2003). In the case of the *Arabidopsis* transporter AtHKT1, which transports Na^+ (Uozumi *et al*., 2000; Berthomieu *et al*., 2003), some constructs cannot be studied in yeast because of their extreme toxicity (FJ Quintero and F Rubio, unpublished results).

Na^+ transport within the plant

As described for K^+, the Na^+ taken up by roots from the soil solution moves into the xylem from where it can be taken up by several types of cells in roots and shoots, and eventually returned to roots (Kramer *et al*., 1977; Johanson *et al*., 1983; Johanson and Cheeseman, 1983; Jeschke and Pate, 1991; Jeschke *et al*., 1992; Durand and Lacan, 1994; Lacan and Durand, 1995, 1996). Na^+ tolerance and differences in Na^+ tolerance in related plants may lie in the different capacities of different plants for carrying out these fluxes (Wolf *et al*., 1991; Blom-Zandstra *et al*., 1998; Watson *et al*., 2001; Davenport *et al*., 2005). It has already been mentioned that cellular Na^+ uptake can be mediated by channels (Tyerman and Skerrett, 1999; Davenport and Tester, 2000; Maathuis and Sanders, 2001; Demidchik *et al*., 2002; Essah *et al*., 2003). However, the most likely possibility is that HKT transporters and not channels mediate many of the internal Na^+ movements described in the aforementioned references. In the first place, because HKT transporters are the only known transporters that are specific for Na^+ and can carry out this uptake in the presence of K^+. In the second place, because it has been already shown that AtHKT1 mediates Na^+ distribution within the plant in *Arabidopsis* (Rus *et al*., 2001, 2004; Mäser *et al*., 2002*a*; Berthomieu *et al*., 2003; Gong *et al*., 2004). These considerations apply especially to the removal of Na^+ from the xylem sap, which may be a key process to limit the ascent of Na^+ to leaves.

Even assuming some uncertainty regarding the precision with which heterologous systems reproduce the physiological functions of HKT transporters, it is possible that some HKT transporters expressed within the plant exhibit low affinity for Na^+, AtHKT1 (Uozumi *et al*., 2000), McHKT1 (Su *et al*., 2003), and OsHKT4 (Garciadeblás *et al*., 2003). Taking into account the K^+ and Na^+ concentrations that can be expected in xylem and apoplast (Almeida and Huber, 1999; Grignon and Sentenac, 1991; Watson *et al*., 2001) the suitability of low-affinity HKT transporters for the function of removing Na^+ from the apoplast is clear. Less clear is the suitability of the high-affinity transporters to this function although TaHKT1 (Schachtman and Schroeder, 1994) and OsHKT1 (Garciadeblás *et al*., 2003) are expressed in shoots. However, as already mentioned, high-affinity HKT transporters may transport Na^+ in different modes and a comprehensive understanding of the functions of these transporters needs more extensive research.

HKT transporters across the species

The two plants whose genomes have been sequenced, *A. thaliana* and rice, are quite different regarding the number of existing *HKT* genes. In rice, there are eight HKT transporters that can be assigned either to the high-affinity or to the low-affinity groups, and the roots carry out a rapid, high-affinity Na^+ uptake (Garciadeblás *et al*., 2003). By contrast, in the genome of *A. thaliana* there is only one *HKT* gene and the encoded transporter exhibits low-affinity for Na^+ (Uozumi *et al*., 2000). Although information is still very limited, the comparison of rice and *Arabidopsis* suggests the existence of two plant models regarding Na^+ transport. In the two models, HKT transporters would be involved in Na^+ transport across the plasma membranes of different types of internal cells (xylem parenchyma, bundle sheath, companion, pith) but only in the rice model would high-affinity HKT transporters be expressed and mediate Na^+ uptake in root epidermal and cortical cells.

Regarding other plant species, *Eucalyptus camaldulensis* (Liu *et al*., 2001) and *M. crystallinum* (Su *et al*., 2003, and accession number AY231175) have at least two genes,

and the study of EST databases suggests that *HKT* genes may be present in most vascular plants. In rice, in which many ESTs have been identified, *HKT* ESTs are not abundant, which suggests that only in plants with a high number of sequenced ESTs the identification of *HKT* ESTs is probable. In dicotyledonous species most of the ESTs belong to a single gene, but *Medicago truncatula* L. has at least two *HKT* genes (EST accession numbers, BF632626 and CX528138).

High-affinity K^+ or Na^+ ATPases

In some fungi, high-affinity K^+ and Na^+ uptake is mediated by P-type ATPases. The K^+ and Na^+ K_ms of these ATPases are very low (perhaps less than 1 μM) and can mediate the uptake of these cations down to extremely low concentrations (Benito *et al*., 2004). The existence of this type of P-type ATPase has not been reported in plants and no genes encoding them exist in the *Arabidopsis* or rice genomes. Therefore, there is currently no reason to predict their existence in plants, but neither can their presence be ruled out. It is worth observing that the Na^+-ATPase described in *Physcomitrella patens* mediates Na^+ efflux, but not Na^+ uptake (Benito and Rodríguez-Navarro, 2003). In any case, the existence of K^+ or Na^+-uptake ATPases in fungi, which undertake the same function as other systems do in plants, suggests that the capacity to take up K^+ and Na^+ from the soil solution when these cations are at very low concentrations was of such selective advantage during evolution of terrestrial organisms that different systems evolved to fulfil to the same function.

Problems in the functional identification of K^+ and Na^+ transporters

In the case of K^+ and Na^+ transporters the power of genomics has been hampered by the difficulties underlying the functional studies of these genes. Unfortunately, the two obvious approaches, gene knockout and expression in heterologous systems, present problems that do not generally occur with other genes. In this review, several examples of these problems have been cited, and especially important are those that may be found in the use of yeast mutants for expressing K^+ transporters.

The problem of gene knockout is the pleiotropic effect caused by mutations that affect K^+ transporters. In fungi, it is known that the disruption of the *TRK* (Madrid *et al*., 1998; Mulet *et al*., 2004) but not *HAK* genes (Bañuelos *et al*., 2000) produces hyperpolarization and a consequent enhancement of K^+ uptake through non-K^+ transporters (Madrid *et al*., 1998). In addition, alteration of K^+ transport is related to multistability of the fungal growth pattern and can produce incomplete penetrance and variable expressivity of growth defects (Lalucque and Silar, 2004). In the case of transporters that are expressed in internal membranes (this may be the case of some KT-HAK-KUP transporters) and involved in the regulation of cellular pH, the disruption may affect vesicle trafficking (Brett *et al*., 2005) and also result in multiple responses. As far as is known, these problems have not been reported in plants, but possibly only because they have not been investigated.

The problems that have arisen with the use of heterologous expression systems have been previously described (Dreyer *et al*., 1999), and very little can be added except for the use of yeast mutants defective in K^+ uptake (*trk1 trk2* mutants). In addition to the problems that result from their highly hyperpolarized state, these mutants are quite unstable and revert to a condition requiring much less K^+ to grow. After cloning a cDNA it is possible to pick up a revertant and reach the conclusion that the cloned cDNA encodes a K^+ transporter. Unless the expressed transporter exhibits a very low K_m (1–100 μM K^+), the plasmid must be cured and afterwards it must be checked that the recipient clone is still K^+-dependent. The reason for this behaviour is not clear, but it is known that *trk1 trk2* mutants that originally keep a low-affinity K^+ transport lose it if additional mutations on the *PPZ1* or *PPZ2* genes are introduced (Ruiz *et al*., 2004).

Outlook

Over the last few years, the field of high-affinity K^+ uptake in plant roots has experienced significant progress and high-affinity Na^+ uptake has emerged as a new process that deserves further study, although its physiological importance is currently unknown. In addition to the models that this progress has originated and their importance for the emergence of new paradigms, the progress is also relevant because it has involved new techniques and approaches, which allow prediction of future rapid progress in the field. Good examples are some of the genes or plant mutants described in this review. The current model that is proposed here (Fig. 3) may not be entirely correct but is testable and a starting point for new working hypotheses. All this is well ahead of the knowledge of low-affinity K^+ and Na^+ transporters. *Arabidopsis* (Shin and Schachtman, 2004) and other plant species (Rodríguez-Navarro, 2000) grown under K^+-sufficient conditions exhibit low-affinity kinetics for Rb^+ or Na^+ influx, but thus far it cannot be said with certainty what protein mediates these influxes. Similarly, the proteins that mediate many of the processes of cellular Na^+ uptake that are well described in the references cited above under the heading 'Na^+ transport within the plant' are also unknown. The authors' proposal that HKT transporters are involved obviously needs further investigation. A technical problem is that the study of these transporters in yeast may be difficult because the yeast mutants that are available for expressing K^+ or Na^+ transporters have

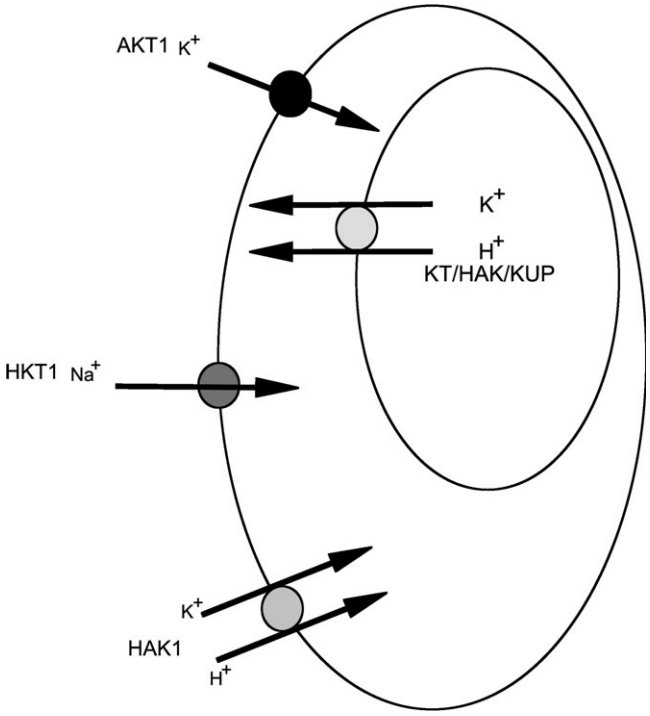

Fig. 3. Current hypothesis for the location of HAK, AKT, and HKT transporters in the plasma membrane and other transporters of the KT/HAK/KUP family in the tonoplast. The transporters in the plasma membrane are high-affinity K^+ or Na^+ transporters while the tonoplast transporters may exhibit low-affinity. HAK1 refers to transporters that are phylogenetically related to barley and rice HAK1 transporters, which include the *Arabidopsis* HAK5 transporter.

intrinsic low-affinity transporters (Madrid *et al*., 1998) that exhibit a high V_{max} and may dominate the uptake over the activity of the plant transporter (Santa-María *et al*., 1997; Rubio *et al*., 2000).

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