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Minireview

Potassium transporters in plants – Involvement in K^+ acquisition, redistribution and homeostasis

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Abstract **Potassium is a major plant nutrient which has to be accumulated in great quantity by roots and distributed throughout the plant and within plant cells. Membrane transport of potassium can be mediated by potassium channels and secondary potassium transporters. Plant potassium transporters are present in three families of membrane proteins: the K^+ uptake permeases (KT/HAK/KUP), the K^+ transporter (Trk/HKT) family and the cation proton antiporters (CPA). This review will discuss the contribution of members of each family to potassium acquisition, redistribution and homeostasis.**

Keywords: Potassium uptake; Transporter; High-affinity; Low-affinity; Plant nutrition; Ion homeostasis

1. Introduction

Potassium is an essential plant nutrient and the most abundant cation in plants, constituting up to 10% of plant dry matter [1]. Plant tissue concentrations of potassium can vary within a wide range. Potassium content below 10 g/kg dry weight will lead to deficiency symptoms in most species, starting with chlorotic interveinal areas in the oldest leaves that become necrotic with progression of deficiency, and, under severe conditions, death of lateral and terminal meristems [2,3]. Potassium deficiency symptoms are visible evidence of the many essential functions that this element has in central metabolic processes like photosynthesis, protein biosynthesis, osmoregulation, turgor driven movements, and maintenance of the plasma membrane potential.

Plant subcellular compartments may vary considerably regarding their potassium levels, with the cytoplasmic concentration being tightly regulated and maintained at approximately 100 mM [2,4]. In contrast, vacuolar potassium concentrations are highly variable reflecting the potassium status of the plant – in times of sufficiency the vacuolar pool is stocked up, while under limiting conditions the vacuolar potassium storage is depleted to sustain a constant concentration in the cytoplasm. In its function as an osmoticum in the vacuole, potassium can be replaced by sodium, and vacuolar potassium can be exchanged against sodium to maintain physiologic potassium concentrations in the cytoplasm.

The high demand for potassium needs to be met through effective uptake from the soil solution by roots and further translocation into the aerial parts of the plant. Moreover, potassium needs to be distributed within cells into different compartments at the appropriate concentrations. These processes require K^+ to cross the plasma membrane when taken up from the soil or released into the xylem lumen, respectively the tonoplast when deposited into or retrieved from the vacuole. Membrane transport of potassium can be mediated either by potassium channels, utilizing the membrane potential to facilitate transport of potassium down its electrochemical gradient, or by secondary transporters. The importance and function of plant potassium channels is discussed in detail by H. Sentenac and coworkers elsewhere in this issue. Candidate genes for potassium transporters identified in the *Arabidopsis thaliana* genome revealed three major transporter families, the KT/HAK/KUP and TRK/HKT transporters and the CPA cation proton antiporter families [5], in addition to K^+ channels.

Proteins of the KT/HAK/KUP family are found in prokaryotes, fungi and plants where they mediate potassium transport probably through a K^+:H^+ symport mechanism as has been shown for HAK1 of *Neurospora crassa* [6]. In plants individual genes are involved in potassium acquisition from the external medium, in cell expansion, and possibly auxin distribution in roots. HKT transporters were initially studied regarding K^+ transport but subsequently received more attention due to their roles in Na^+ transport and salt susceptibility. An opposite trend is happening with the CPA families. Originally described as Na^+/H^+ exchangers involved in salt tolerance, several CPAs were recently shown to also transport K^+.

The mechanisms by which plants sense and adapt to a low potassium environment have been reviewed in detail recently [7,8]. Here we focus on potassium transporters from the three different families and their possible functions in plant cation homeostasis.

2. KT/HAK/KUP transporters

The KT/HAK/KUP family has been identified in plants by homology to K^+ uptake permeases (KUP) from bacteria [9] and high-affinity K^+ transporters (HAKs) from fungi [10].

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When the first members of this family were identified from plants, individual genes were assigned diverse acronyms. Depending on the authors they were called KT (for K^+ transporter) [11], HAK [12], or KUP [13,14] leading to the composite name of KT/HAK/KUP used for this family throughout this review.

Individual *KT/HAK/KUP* genes have been cloned from a variety of plant species including barley [12,15–17], pepper [18], ice plant [19], *Lotus japonicus* [20], grapevine [21], tomato [22,23] and seagrass [24]. Whole families became apparent from completely sequenced genomes [25–27]: *Arabidopsis* possesses 13 KT/HAK/KUPs [5], rice 25 (17 identified by Banuelos et al. [28] plus 8 predicted in the Aramemnon database [29]), and poplar 24 (plus 7 probable pseudogenes; Gierth and Mäser, unpublished). According to Aramemnon [29], all KT/HAK/KUP proteins from *Arabidopsis* and rice have between 10 to 14 transmembrane domains and, except for OsHAK3, a cytoplasmic N-terminus. The first *KT/HAK/KUP* genes in plants were described from *Arabidopsis* [11,13,14] and barley [12]. Initial studies established by complementation of yeast or *Escherichia coli* potassium transporter mutants and uptake studies that the identified KT/HAK/KUPs indeed mediate potassium transport.

Only AtKUP1 [14], AtKUP4 [30] and AtHAK5 [31] have been characterized in planta, either by overexpression in *Arabidopsis* suspension cells (AtKUP1) or analyses of T-DNA insertion mutants (AtKUP4, AtHAK5) and all mediated high-affinity $Rb^+(K^+)$ uptake. While results in planta and from expression in yeast are consistent for AtKUP4 and AtHAK5 [15,30,31], AtKUP1 appeared to be a dual affinity transporter when expressed in yeast cells [13] indicating that heterologous expression may yield kinetic results different from in planta function, for example due to differences in the membrane potential.

For HvHAK1 [12], OsHAK1 [28], AtHAK5 [15] and CaHAK1 [18] high-affinity potassium transport was demonstrated in heterologous expression systems. Interestingly, all of these proteins apparently clustered into a distinct group termed I A [15,18,28]. However, the majority of KT/HAK/KUPs belongs to other clusters which, as characterized so far, mediate low-affinity K^+ transport in heterologous systems [16,24].

An *Arabidopsis* T-DNA insertion mutant in *AtKUP4*, which does not belong to the high-affinity cluster, displayed strongly reduced root hair elongation and was called *tiny root hair* (*trh1*) [30]. *trh1* mutants also had a slightly reduced root $Rb^+(K^+)$ uptake, but the *trh1* phenotype was not restored to wild type by growing the plants in high $[K^+]$ media, indicating that potassium availability was not the reason for the observed phenotype. More recent research indicated that TRH1 is involved in the root-specific distribution of the phytohormone auxin, since auxin efflux from mutant roots was reduced compared to wild type roots and overexpression of TRH1 in yeast led to increased auxin efflux [32]. The physiological mechanism by which the potassium transporter TRH1 interacts with root auxin transport remains to be clarified.

A mutation in another KT/HAK/KUP transporter, AtKUP2, led to the *shy3-1* phenotype displaying a short hypocotyl in the dark, which also resulted from reduced cell expansion growth [33]. However, the present AtKUP2 point mutation did not diminish the potassium transport capacity, meaning that the *shy* phenotype was not necessarily caused by reduced K^+ transport. The results of both mutants, *trh1* and *shy3-1*, indicate that interactions of AtKUP proteins with yet to be identified cell components may be important for their function in plant cell expansion growth.

KT/HAK/KUP proteins may also be involved in cell expansion growth in other plant species. Two KT/HAK/KUPs from grape, VvKUP1 and VvKUP2, were highly expressed in berry skin at the beginning of grape development, suggesting that VvKUPs may function in potassium accumulation in grape berries [21]. Since increase in potassium concentration coincides with berry growth [21], this might be another example of KT/HAK/KUP involvement in potassium dependent cell expansion growth. LjKUP, a homolog identified from the legume *L. japonicus*, is strongly expressed in developing and growing root nodules [20]. However, strong expression of LjKUP persists in mature nodules implicating also other functions than growth. After all, function cannot be inferred from differential gene expression alone.

Because of its importance for plant nutrition, potassium uptake by plant roots from the soil has been in the focus of many studies. Pioneering work on potassium starved barley roots led to the description of a two component uptake system with mechanism I mediating K^+ (Rb^+) uptake from micromolar and mechanism II mediating uptake from millimolar external $K^+(Rb^+)$ concentrations [34]. Mechanism II is thought to consist mainly of potassium uptake channels. However, studies of an *Arabidopsis* T-DNA insertion mutant in the root K^+ channel gene *AtAKT1* indicated that AKT1 also contributes to high-affinity transport, mediating uptake from K^+ solutions as dilute as 10 μM [35,36]. Growth of the *akt1-1* mutant is impaired on low potassium media only in the presence of high ammonium concentrations [35], which block KT/HAK/KUP transporters [12], indicating that AKT1 may mainly be important for high-affinity potassium uptake into roots under conditions that inhibit other types of K^+ transporters, as predicted based on biophysical properties of K^+ channels [37].

Members of the KT/HAK/KUP family have been assumed to contribute to mechanism I since the identification of the high-affinity K^+ transporter HvHAK1 from barley, whose expression strongly increased in roots exposed to low $[K^+]$ media [12]. In *Arabidopsis*, *AtKUP3* transcripts increased in roots of K^+ starved seedlings [14]. *AtKUP3* expression in roots of mature plants was unaltered by potassium depletion [31,38]. Microarray experiments looking for *Arabidopsis* transcripts responsive to low potassium availability found increased expression of *AtHAK5* as the only member of the KT/HAK/KUP family induced under several conditions [31,39,40]. Interestingly, in other studies *AtHAK5* expression was found to be unaffected by potassium starvation [15,41] indicating that media composition, culture conditions, and experimental procedures may be critical. AtHAK5 clusters together with other potassium starvation induced KT/HAK/KUP proteins like HvHAK1, OsHAK1 or LeHAK5 [15,22,23,28].

T-DNA insertion mutants in *AtHAK5* displayed no apparent growth phenotype [31]. However, determination of $Rb^+(K^+)$ uptake kinetics in roots of potassium starved mutant and wild-type plants revealed a strongly impaired high-affinity $K^+(Rb^+)$ uptake in mutant roots [31]. Kinetic parameters for AtHAK5 estimated from the difference in $Rb^+(K^+)$ uptake between wild type and *athak5* roots yielded a K_m of 15–24 μM for AtHAK5, a value very similar to the K_m determined by overexpression of AtHAK5 in yeast [15]. $Rb^+(K^+)$ uptake kinetics

in *akt1-1* mutants compared to WS wild-type roots indicated a small contribution to potassium uptake at micromolar concentrations by AKT1 and an intermediate K_m of 0.88 mM [31]. Rubidium is commonly used as a potassium analog in physiological studies but also to reveal the structural basis for selectivity of potassium channels, where the authors found that "Rb^+ is nearly a perfect K^+ analog because its size and permeability characteristics are very similar to those of K^+" [42]. The use of Rb^+ as a tracer for K^+ to characterize uptake kinetics of potassium channels [34,43] has been brought into question, as well as the contribution of AKT1 to high-affinity potassium uptake [44]. It should be noted though that classical studies of K^+ uptake properties and kinetics used Rb^+ uptake as the benchmark (e.g. [34,43,45,46]) and therefore kinetics and K_m values are generally analyzed for Rb^+ as the reference in many studies, enabling comparison between classical and current research. Very few studies have used radiolabelled K^+ as a tracer for K^+ uptake confirming high-affinity kinetics with the expected lower K_ms for K^+ (e.g. [47]), but the $^{42}K^+$ tracer has a short half life and is more difficult to obtain and work with safely. Furthermore, at negative (hyperpolarized) membrane potentials even low Rb^+ permeabilities found for some K^+ channels will give rise to measurable Rb^+ fluxes, most likely due to the ability of proton pumps to hyperpolarize the membrane and compensate for a lower conductance. The Rb^+/K^+ selectivity of inward rectifying K^+ currents in *Arabidopsis* root cells has been determined as 0.65 [48] providing direct evidence for substantial Rb^+ permeability of plant K^+ channels in vivo.

To allow comparison to classical studies in barley [34], we plotted Rb^+ influx kinetics measured in *Arabidopsis* roots [31] using the "split scale" plot of Epstein and colleagues (Fig. 1). When comparing the Rb^+ uptake kinetics in wild-type roots and the data for AtHAK5 and AKT1 [31] it becomes apparent that AKT1 accounts for a large portion of the low-affinity and AtHAK5 accounts for a large portion of the high-affinity uptake (Fig. 1). Interestingly, the sum of AKT1 and AtHAK5 curves shows an apparent 84% of the low- and 78% of the high-affinity Rb^+ (K^+) uptake in wild type *Arabidopsis* roots (green curve in Fig. 1). This would leave roughly 20% of uptake in both mechanisms I and II to yet to be identified components. However, kinetic parameters for $Rb^+(K^+)$ uptake determined in knock-out mutants may reflect compensatory mechanisms in roots, e.g. loss of AtHAK5 protein activity could lead to plasma membrane hyperpolarization [36] thereby increasing high-affinity Rb^+ uptake activity through AKT1 and other transporters and thus underestimating AtHAK5 contribution to high-affinity uptake, with similar considerations for single *akt1* knock-outs. Moreover, $Rb^+(K^+)$ uptake activity in roots of the two mutant backgrounds, WS and Col-0, could be different, hence explaining the 20% discrepancy between Col-0 and additive AtHAK5 and AKT1 $Rb^+(K^+)$ uptake. Notwithstanding open questions, the analysis in Fig. 1 illustrates that AtHAK5 and AKT1 are major contributors to high- and low-affinity K^+ uptake in K^+-starved roots [31,35].

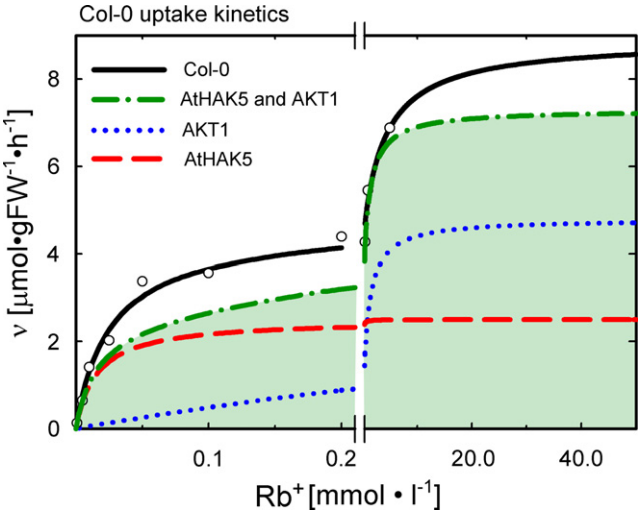

Fig. 1. AtHAK5 (dashed red line) and AKT1 (dotted blue line) Rb^+ uptake in *Arabidopsis* roots plotted individually and additive (dashed-dotted green line) compared to Rb^+ uptake in wild type Col-0 roots (solid black line). Curves were calculated from K_m and V_{max} determined from $^{86}Rb^+$ uptake kinetics in wild type Columbia, *athak5* and *akt1* roots [31] using one term (red and blue lines) or two term (green and black lines) Michaelis-Menten kinetics.

Candidate genes for contributing to the residual 20% of $Rb^+(K^+)$ uptake in roots are constitutively expressed KT/HAK/KUP proteins and potassium channels. Among the KT/HAK/KUP family *AtKUP4* through *ATKUP8* and *AtKUP10* are the most highly expressed genes in roots according to microarray analyses [31], the Genevestigator database [49] and RT-PCR studies [38]. While AtKUP4 (=TRH1) does not seem to be involved in potassium uptake as a primary function (see above), little is known about the other candidates and further research will need to clarify which KT/HAK/KUP proteins provide a basic, constitutive level of potassium uptake in roots.

3. HKT proteins

Plant HKTs (high-affinity K^+ transporters) belong to the Trk superfamily of cation transporters which are topologically related to K^+ channels [50,51]. Central to both are MPM building blocks of two transmembrane domains with a pore (P-) loop in between. While KcsA-type K^+ channels have one MPM block per subunit and function as tetramers, HKT/Trk proteins possess four MPM motifs in a single polypeptide (Fig. 2). HKT/Trk monomers are therefore thought to resemble K^+ channel tetramers in architecture [52]. There is an interesting analogy with the larger, voltage-gated cation channels: Shaker-type K^+ channels are tetramers of subunits with one MPM motif plus four additional transmembrane domains, while the cardiac Na^+ channel unites four MPM motifs and sixteen transmembrane domains in a single polypeptide (Fig. 2). Also the exon/intron boundaries of the transmembrane coding segments of NaV genes display a four-fold distribution pattern [53], indicating that voltage-gated Na^+ channels of mammals arose after sequential duplication of a eukaryotic (i.e. intron-containing) KV-type channel gene. *HKT/Trk* genes, in contrast, seem to have been selectively lost from the animal kingdom since they are only present in bacteria, fungi, and plants [54–65]. All known plant *HKT* genes contain two introns near the 3′ end. The distribution of the introns does not mirror the four-fold symmetry of the HKT proteins, consistent with the model that the ancestral *HKT/Trk* gene was prokaryotic and the introns were acquired in an ancestor plant before the split of monocots and dicots. The introns do,

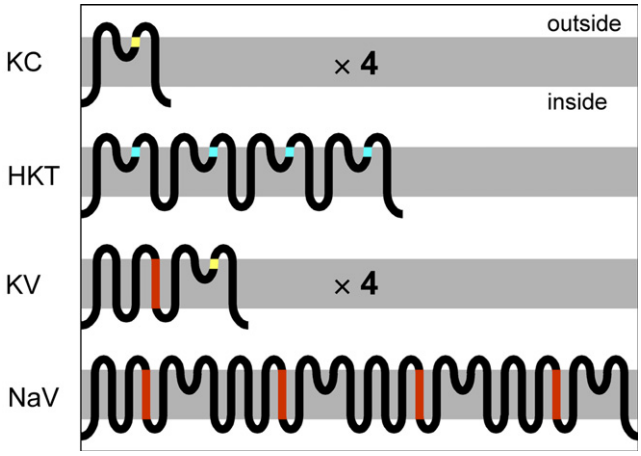

Fig. 2. Simplified topological models of a KcsA-type K^+ channel (KC), a HKT protein, a Shaker-type voltage-gated K^+ channel (KV), and a voltage-gated Na^+ channel (NaV). GYG K^+ filters are shown in yellow, single filter glycines in blue, and voltage sensory transmembrane domains in red. Extra- or intracellular domains are not shown.

however, reflect sequence similarities of the respective proteins: Plant HKT amino acid sequences group into two subfamilies, and the genes of subfamily one have longer introns than those of subfamily two [66]. Subfamily two exclusively contains monocot genes, subfamily one includes monocot genes and all known *HKTs* from dicots [66].

The first HKT/Trk gene identified from plants was wheat *TaHKT1*, which transported K^+ when expressed in *Xenopus laevis* oocytes and also showed large changes in outward currents in response to Cs+, Rb^+ and Na^+ [59]. Following up on these initial studies indicating a more complex cation transport mechanism than selective K^+ transport [59], detailed analyses in oocytes and yeast found that TaHKT1 functions as a high-affinity K^+, Na^+ co-transporter that switched to low-affinity Na^+ uniport at high $[Na^+]/[K^+]$ [47,67]. The *Arabidopsis* orthologue AtHKT1 transported only Na^+ in yeast or in oocytes [60]. Two genes identified from Eucalyptus, *EcHKT1* and *EcHKT2*, mediated Na^+-coupled K^+ transport in oocytes and rescued K^+ uptake deficient *E. coli* [61]. Of the several HKT/Trk paralogues in rice, OsHKT1 showed properties of a more Na^+ selective uniporter similar to AtHKT1, while OsHKT2 from the salt tolerant cultivar Pokkali was a K^+, Na^+ symporter similar to TaHKT1, again determined by expression in yeast or in *Xenopus* oocytes [62,64]. *OsHKT4* appeared to encode a low-affinity Na^+ transporter when expressed in yeast, while characterization of substrate specificity for OsHKT3, OsHKT6 and OsHKT9 has not yet been obtained [64]. Expression of *McHKT1* from ice plant (*Mesembryanthenum crystallinum*) suppressed K^+ uptake deficiency in yeast and predominantly caused Rb^+ and Cs+ currents in *Xenopus* oocytes [65].

As illuminated by the Nobel prize-winning work of MacKinnon et al., the potassium channel's P-loops serve to attract a substrate cation into the hydrophobic milieu of the plasma membrane. The alpha-helical P-loop ends in the so-called filter residues Gly-Tyr-Gly (GYG), which are devoid of secondary structure and bind the K^+ ion by means of their backbone carbonyl groups, mimicking coordination by water molecules [42]. Thus the kinetic barrier for stripping the potassium cation off its hydration layer is overcome without binding it too strongly for subsequent release [68], an obvious prerequisite for transport. The GYG triad is highly conserved among all types of K^+ channels. Given the topological resemblance to K^+ channels, it is reasonable to assume that HKT/Trk transporters engage backbone carbonyl groups in a similar way to bind their substrate cations. Indeed, the predicted P-loops of HKT/Trk proteins are followed by a highly conserved glycine, and it has been demonstrated for a bacterial transporter that these four glycines are critical for K^+ translocation [69]. Surprisingly, members of plant HKT subfamily one have a serine instead of the expected glycine at the first filter position [51]. Site-directed mutagenesis and functional expression studies in *Xenopus* oocytes or yeast mutants [51] showed that these proteins are more permeable to Na^+ compared to K^+ (with the possible exception of the HKTs from eucalyptus [70] and ice plant [65]). Null mutant plants may nevertheless exhibit potassium-related phenotypes, due to negative as well as positive effects of $[Na^+]$ on K^+ homeostasis.

Genetic disruption of the subfamily one member *AtHKT1* [60] in *Arabidopsis* decreased root Na^+ levels but increased the Na^+ content of the shoot, consistent with the finding that *athkt1* null plants were salt resistant in short-term root growth assays but salt hypersensitive when regarding long-term shoot growth [71]. A similar Na^+ distribution phenotype was observed for the *Arabidopsis* mutant *sas2* (sodium over accumulation in shoot), which mapped to *AtHKT1* and turned out to be a point mutation that strongly reduced the activity of the transporter [72]. Na^+ hyperaccumulation in the leaves as a consequence of *AtHKT1* disruption was also discovered by forward genetic screens [73,74]. Based on the activity of the AtHKT1 promoter in the phloem vasculature and the lowered phloem sap Na^+ content of *sas2* plants, AtHKT1 was proposed to mediate rootward Na^+ recirculation from the shoot by loading Na^+ into the phloem in leaves, respectively unloading it from the phloem in the root [72]. After immuno-localization of AtHKT1 to xylem parenchyma cells of *Arabidopsis* leaves, this model was refined to AtHKT1 unloading Na^+ from the xylem vessels into xylem parenchyma cells [75], from where Na^+ diffuses into the phloem via plasmodesmata and recirculates to the roots. This is in agreement with the higher xylem sap Na^+ levels of *athkt1* null mutant plants [75]. OsHKT8 (OsHKT1;5 according to the newly proposed nomenclature [66]), a subfamily one member from rice, also localized to the leaf xylem parenchyma based on a promoter:GUS reporter and controls the Na^+ content of the xylem sap [76]. Interplay between Na^+ and K^+ homeostasis was indicated by the finding that under salt stress, salt-sensitive *OsHKT1;5* Koshihikari-variant rice and *athkt1* null mutant *Arabidopsis* had a lower xylem sap K^+ concentration and a lower shoot K^+ content than rice nearly isogenic line *SKC1* or *Arabidopsis* wild-type plants, respectively [75,76].

Genetic disruption of *AtHKT1* suppressed the root growth phenotype at low $[K^+]$ of the root Na^+ hyperaccumulating mutant *sos3*, while overexpression of *AtHKT1* exacerbated the K^+-deficiency phenotype of *sos3* seedlings [77,78]. Disruption of *AtHKT1* suppressed the short-term root growth Na^+ hypersensitivity of *sos3* seedlings in the presence of high Ca^{2+} concentrations [77,79] and was reported to also suppress the salt hypersensitivity of mature *sos3* plants in long-term shoot growth experiments [78]. However, the latter effect was not generally reproducible [79] and *athkt1* mutants did not affect

Na^+ influx into roots [72,80]. The genetic recovery of root seedling growth in *sos3* mutants via *athkt1* mutation was explained in first order by a model in which the underaccumulation of Na^+ in *athkt1* roots (due to shoot Na^+ overaccumulation in *athkt1* [81]) can at least in part suppress the *sos3*-mediated overaccumulation of Na^+ in roots in *sos3* [79]. Additional genetic interactions of *sos* and *athkt1* mutants may also occur [79]. The role of AtHKT1 in Na^+ homeostasis and salt sensitivity is further discussed in this issue by Blumwald and Apse.

Focusing on K^+, candidate transporters are found in subfamily two of the HKTs which possess all four of the predicted filter glycines (except for rice OsHKT1, respectively OsHKT2;1 following the new nomenclature [66]). The glycine of the first P-loop was shown to be necessary for K^+ transport in OsHKT2 (OsHKT2;2) and wheat TaHKT1 (TaHKT2;1) [51]. The expression of *TaHKT1* as well as *OsHKT2* was induced in root tissue at low K^+ concentrations [59,62,82] and both proteins functioned as K^+/Na^+ symporters when expressed in *Xenopus* oocytes or in *Saccharomyces cerevisiae* [47,62], suggesting that Na^+ co-transport might facilitate root K^+ uptake from potassium-sparse soils. Na^+-coupled K^+ uptake has so far only been observed in bacteria, algae, and aquatic plants, not in roots of terrestrial plants [69,83], consistent with the finding that roots have several types of K^+ transporters as discussed above. TaHKT1 was shown to mediate low-affinity Na^+ uniport at high $[Na^+]/[K^+]$, leading to the model that HKT transporters have a Na^+ ion channel transport state [67]. Na^+ uniport was claimed to be the physiological function of subfamily two HKTs in barley roots [84]. However, no genetic *hkt* mutations were analyzed suggesting that the functions of more than one Na^+ influx transporter class may have been analyzed, and the conclusions were based on a time-course of Na^+ depletion from the medium over one hour, which does not allow to deduce the mechanism of transport [84]. Since the HKTs of subfamily two are confined to monocots (at present all known genes are from the grasses), they are genetically less amenable than subfamily one members. Genetic knock-down of *TaHKT1* in wheat reduced the Na^+ content of root exudate and the salt sensitivity in transgenic plants [85]. The possible role(s) of subfamily two HKTs in root K^+ and Na^+ uptake and distribution remain to be determined. Furthermore, compared to K^+ channels in animals and plants, in which biophysical ion transport properties have been analyzed under numerous conditions in many thousand studies [86], further biophysical analyses of HKT transporters would be useful to test hypotheses, including the proposed ion channel transport mechanism [87].

4. Monovalent cation:proton exchangers

Monovalent cation/proton antiporters of the two related families CPA1 and CPA2 are ubiquitous among plants, fungi, animals, and bacteria. As summarized in Fig. 3, both CPA1 and CPA2 can be subdivided further and some of the subfamilies are restricted to particular kingdoms, e.g. the two NhaP families to bacteria, the NHE-PM family to animals, and the SOS1 family to plants [88]. CPAs work electroneutrally, i.e. without affecting the electrical potential difference across the membrane. They tend to match the concentration gradients of protons (ΔpH) to that of their substrate cation(s). As such their function may be homeostasis of monovalent cations as well as regulation of the pH (pH-stat [89]). In the morning glory *Ipomoea* spp., for instance, tonoplast NHX proteins are critical for the alcalinization of the vacuole which makes the petals turn blue upon opening of the flower [90,91]. Whatever their physiological role, CPA proteins need to be tightly

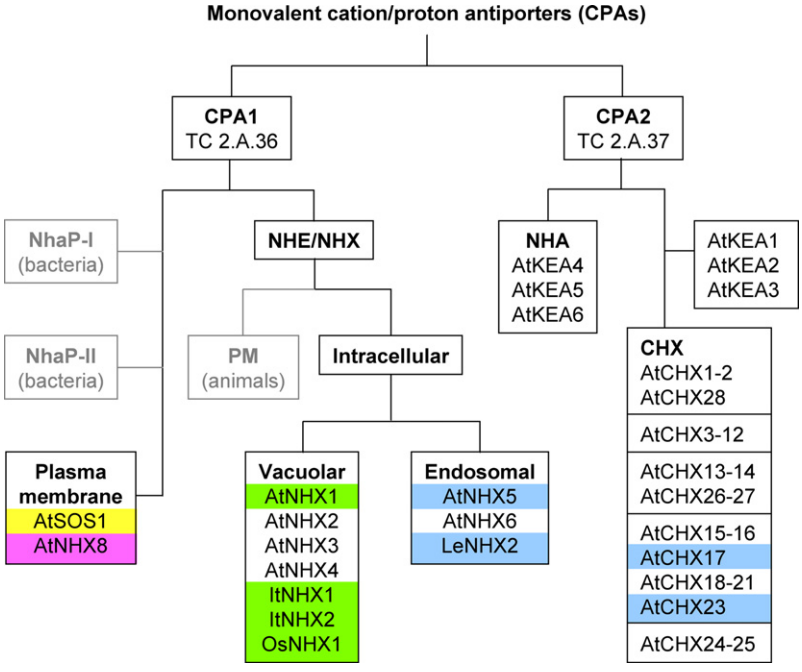

Fig. 3. The two CPA families and subfamilies therein (after [88]). Proteins for which experimental evidence about substrate specificity exists are colored (yellow, Na^+ preferred; blue, K^+ preferred; pink, Li^+ preferred; green, equal affinity to Na^+ and K^+). Subfamilies absent from plants are printed in grey. Abbreviations: PM, plasma membrane; At, *Arabidopsis thaliana*; It, *Ipomoea tricolor*; Os, *Oryza sativa*.

regulated – otherwise they would dissipate the potential energy stored in the concentration gradients of their substrates. The regulatory mechanisms are best understood for *Arabidopsis* AtSOS1. A Na^+/H^+ exchanger of the plasma membrane [92], AtSOS1 is an important determinant of salt sensitivity whose expression and activity are controlled by the protein kinase AtSOS2 and its Ca^{2+} sensor AtSOS3 [93]. AtSOS2 also enhances Na^+/H^+ exchange over the tonoplast [94]. The molecular mechanisms for regulation of other plant CPAs are not known, but might be the key to understanding the physiological roles of these proteins. A total of 24 SOS2-type kinases is encoded in the *Arabidopsis* genome [93], some of which might be involved in controlling additional CPAs.

Clustering of eukaryotic CPA1 proteins according to sequence similarity pleasingly reflects their subcellular localizations. The mammalian NHX proteins of the plasma membrane, the plant tonoplast NHX, and the endosomal ones fall into distinct subfamilies [88]. Thus it is possible to predict for a given exchanger its compartment of residence – but not necessarily its substrate cation. AtNHX8, the second *Arabidopsis* member of the SOS1 subfamily (Fig. 3), was shown to transport preferentially Li^+, not Na^+, when expressed in *S. cerevisiae*, and disruption of *AtNHX8* caused Li^+ hypersensitivity in *Arabidopsis* [95]. The tonoplast CPA1 protein AtNHX1 was initially identified as a Na^+/H^+ antiporter [96] and gained most attention because of its promising potential to enhance salt tolerance when overexpressed [97,98]. Subsequently, AtNHX1 was shown to accept also K^+ as a substrate [98]. *Arabidopsis atnhx1* null mutants exhibited lower shoot K^+ levels and impaired leaf expansion [99]. Purified AtNHX1 reconstituted in liposomes transported K^+ and Na^+ with equally low affinities, with K_m values of 42 mM for K^+ and 45 mM for Na^+ [100]. Interestingly, the C-terminal domain of AtNHX1 was implicated in K^+ selectivity [101], responding to a vacuolar calmodulin [102]. K^+/H^+ as well as Na^+/H^+ antiport was also observed for the vacuolar CPA1 proteins ItNHX1, ItNHX2 [91] and OsNHX1 [103]. The proposed function for CPAs of the tonoplast is that they make use of the pH gradient to indiscriminately load K^+ and Na^+ into the vacuole, for storage (in the case of K^+), detoxification (Na^+), and generation of turgor [104]. However, NHX1 being sensitive to amiloride, the molecular mechanism of amiloride resistant vacuolar K^+/H^+ antiport [105] remains to be identified.

Cation selectivity is probably more critical for the CPA1 proteins of the endomembrane system (Fig. 3), which are thought to be important for the regulation of endosomal pH. Excess loading of Na^+ into the Golgi or other compartments may be harmful to plant cells (conversely, acidification of endosomes might not be feasible by Na^+/H^+ antiport due to the luminal scarceness of sodium). Indeed, the few members of the endosomal CPA1 subfamily analyzed so far displayed a preference for K^+ over Na^+. Tomato LeNHX2 reconstituted in proteoliposomes transported K^+ with a low affinity but strong selectivity over other cations [106]. Based on functional expression in yeast, also AtNHX5 is thought to prefer K^+ to Na^+ [107].

The CPA2 family comprises several good candidates for K^+ transport. AtKEAs (K^+ exchange antiporters) resemble the bacterial K^+/H^+ antiporters KefB and KefC. However, as no experimental data are available on AtKEAs it remains to be shown whether they really deserve that name. Comprehensive data are available about tissue-specific expression of the CHX subfamily of CPA2 transporters in *Arabidopsis* [108]. Out of 28 genes, 18 are expressed specifically during microgametogenesis or in sporophytic tissue, suggesting that CHX proteins are involved in regulation of potassium homeostasis in the course of pollen development and germination [108]. Only two CHX proteins have been characterized in detail. AtCHX17 was reported to be preferentially expressed in roots under stress, i.e. high salt concentrations, low external pH, low external $[K^+]$, or abscisic acid treatment [109]. Moreover, analyses of *atchx17* T-DNA insertion mutants indicated a function in potassium homeostasis since mutant plants accumulated less potassium than wild type plants. When expressed in yeast, AtCHX17 co-localized with Golgi markers and complemented the pH sensitive phenotype of the yeast *kha1* mutant [110], suggesting a role for AtCHX17 in K^+ homeostasis and pH regulation under salt stress. A similar function and localization is suggested for the tomato CPA1 protein LeNHX2 [106], indicating functional similarities within the CPA1 and CPA2 families. The second CHX protein from *Arabidopsis* characterized so far, AtCHX23, localized to the chloroplast envelope [111]. Loss of function mutants displayed altered chloroplast ultrastructure along with strongly decreased, pH-dependent chlorophyll content in seedling leaves and an elevated cytosolic pH in guard cells. Growth of *atchx23* plants was improved by high $[K^+]$ but impaired by NaCl [111]. These data suggest that AtCHX23 is a $K^+(Na^+)/H^+$ antiporter of the chloroplast envelope involved in potassium homeostasis and stromal pH regulation.

5. Concluding remarks

While the in silico detection of K^+ channels is straightforward due to the highly conserved K^+ filter sequence, substrate prediction for plant HKT and CPA transporters requires experimental analyses, which have revealed many interesting functions of these transporters. Growth phenotypes of mutant plants do not necessarily provide conclusive evidence about substrate specificity – an *Arabidopsis* null mutant for a K^+ transporter may exhibit Na^+ related phenotypes and vice versa. The current model for the interconnection of K^+ and Na^+ homeostasis attributes such effects mainly to the double-edged role of Na^+, which in the cytosol competes with K^+ for its biochemical functions but in the vacuole substitutes for K^+ in its osmotic functions [44]. Indirect effects may also occur when transporters are expressed in K^+ uptake deficient or Na^+ hypersensitive yeast mutants and tested for the suppression of growth defects. It follows that live recording of cation translocation is the most reliable way to determine substrate specificity. However, of the 56 candidate K^+ transporters from *A. thaliana* covered in this chapter (13 HAK/KUPs, 1 HKT, 42 CPAs), the few that have been analyzed for biophysical transport exhibited unpredicted properties that reflected physiological function: AtHKT1 transported Na^+ and not K^+ when expressed in *Xenopus* oocytes [60], AtNHX1 transported Na^+ as well as K^+ when reconstituted in lipid vesicles [100]. We may expect further surprises as the remainder of the predicted K^+ transporters are examined more closely. Once all K^+ transporters are identified, overlays with their expression patterns in plants, expression profiles from microarray experiments [49],

and disruption phenotypes will reveal their roles in acquisition, redistribution and homeostasis of cations.

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