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Review

Molecular insights into the structure and function of plant K^+ transport mechanisms

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Abstract

Our understanding of plant potassium transport has increased in the past decade through the application of molecular biological techniques. In this review, recent work on inward and outward rectifying K^+ channels as well as high affinity K^+ transporters is described. Through the work on inward rectifying K^+ channels, we now have precise details on how the structure of these proteins determines functional characteristics such as ion conduction, pH sensitivity, selectivity and voltage sensing. The physiological function of inward rectifying K^+ channels in plants has been clarified through the analysis of expression patterns and mutational analysis. Two classes of outward rectifying K^+ channels have now been cloned from plants and their initial characterisation is reviewed. The physiological role of one class of outward rectifying K^+ channel has been demonstrated to be involved in long distance transport of K^+ from roots to shoots. The molecular structure and function of two classes of energised K^+ transporters are also reviewed. The first class is energised by Na^+ and shares structural similarities with K^+ transport mechanisms in bacteria and fungi. Structure-function studies suggest that it should be possible to increase the K^+ and Na^+ selectivity of these transporters, which will enhance the salt tolerance of higher plants. The second class of K^+ transporter is comprised of a large gene family and appears to have a dual affinity for K^+. A suite of molecular techniques, including gene cloning, oocyte expression, RNA localisation and gene inactivation, is now being used to fully characterise the biophysical and physiological function of plants K^+ transport mechanisms.

Keywords: Plant; Potassium; Transport; Structure; Molecular biology

1. Introduction

The first studies relating to the molecular biology of plant potassium transport mechanisms were published about 8 years ago. A first potassium channel from plants was cloned from *Arabidopsis* by a French group [1], which was quickly followed by the cloning of another potassium channel using the same methods with a different cDNA library [2]. This early work on plant inward rectifying K^+ channels introduced new tools for studying plant membrane transport processes such as yeast complementation for cloning, yeast expression for studying the function of plant membrane transporter mechanisms [3] and the use of *Xenopus* oocytes for the electrophysiological characterisation of ion channel and transporters from plants [4]. Thus far, molecular studies have revealed at least three different types of K^+ selective channels and two other types of transport-

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Table 1
Key features of the different plant K^+ transport mechanisms that have been identified at the molecular level

Transporter	Gene name	Mode of transport	Tissue expression	Structural features	TMDs	Physiological function	Ref.
Inward rectifying K^+ channels	AKT, KAT	passive diffusion	roots, guard cells and probably other tissues	ankyrin domain nucleotide binding domain pore domain voltage sensor	6	uptake, osmoregulation	[1,2,4]
Two pore outward rectifying K^+ channels	KCO	passive diffusion	leaves, seedlings and flowers	EF hands pore domains voltage sensor	4	not demonstrated	[58]
Outward rectifying K^+ channels	SKOR	passive diffusion	roots, pericycle and xylem, parenchyma	pore domain voltage sensor	6	K^+ release to xylem	[65]
High affinity K^+ transporter	HKT	Na^+ energised, K^+ uptake	roots and leaves	highly conserved 16 amino acid motif	8	not demonstrated	[68,72]
High affinity K^+ transporter	KUP, HAK	not known	roots and leaves	none reported	12	not demonstrated	[57,86–88]

ers or carriers, one of which has been shown to be energised by Na^+ (Table 1). This review will focus on the structure, function and briefly describe what is known about the physiological role of these vital K^+ transport mechanisms.

2. Inward rectifying K^+ channels

Genes encoding inward rectifying K^+ channel α-subunits were amongst the first membrane transport systems to be identified in plants at the molecular level. It was surprising to discover that the inward rectifying K^+ uptake channels cloned from plants were similar in overall six transmembrane topology to Shaker channels from animals, which function as outward rectifiers [5,6]. One of the inward rectifying K^+ channels, KAT1, has experimentally been shown to contain six transmembrane domains further supporting the suggestion that these channels are similar to the Shaker superfamily [7]. KAT1 as well as other plant inward rectifying K^+ channels also contain a voltage sensing region (S4) and a pore (H5) which are features of channels from the Shaker superfamily [8]. KAT1 is also functionally similar to Shaker channels because it functions as a multi-ion pore [9,10]. Although the molecular mechanisms that determine rectification properties have not been fully elucidated, experiments have shown that the rectification of plant K^+ uptake channels is due to an 'intrinsic' gating process [11,12] rather than by Mg^{2+} blockade as has been shown for inward rectifiers from animal systems [13]. In the Shaker channel, three mutations have been shown to convert the channel from an outward rectifier to voltage-gated inward rectifier [14]. The change in function of this channel (i.e. activation at more hyperpolarised potentials) was shown to be due to a recovery from inactivation. Chimeras between the KAT1 channel and Xsha2, which is an outward rectifier K^+ channel, showed that inward rectification of KAT1 was due to the amino terminus and first four transmembrane segments [15]. The involvement of the N-terminus in rectification properties of plant inward rectifying K^+ channels [15] is in contrast to inward rectifying K^+ channels in animals. In animal systems the C-terminus and a single charged amino acid in the last transmembrane domain play a major role in de-

termining the rectification properties of the channel [16–19].

2.1. Expression patterns and tissue specificity

Inward rectifying K^+ channels form a family of genes expressed in different tissues, but our understanding of channel gene tissue specific expression is not complete. The GUS reporter system was been used to localise the expression of at least two different K^+ channels. The *Arabidopsis KAT1*, as well as its potato homologue *KST1* appear to be expressed primarily in guard cells [20,21]. The expression and localisation of another *Arabidopsis* K^+ uptake channel *AKT1* was studied in *Brassica napus* and in *Arabidopsis*. In those studies of the *AKT1* promoter, activity was not affected by K^+ nutrition and was restricted primarily to roots [22]. The *AKT1* promoter activity was not affected by K^+ nutrition and was restricted primarily to the roots. Some promoter activity was detected in leaves, in specialised hydathode cells that are involved in the process of guttation. In roots most of the promoter activity was detected in cells that have access to the soil solution including the epidermis, cortex and endodermis. In contrast to *AKT1*, another K^+ uptake channel called *AKT2* appears to be primarily expressed in the leaves of *Arabidopsis* [23,24].

2.2. Subunit composition

As has been demonstrated for animal K^+ channel α-subunits [25,26], plant K^+ uptake channel α-subunits also form tetramers. These studies demonstrating the composition of plant inward rectifying K^+ channel α-subunits in vivo were performed using size exclusion chromatography and anti-AKT1 antibodies [27]. Studies using the yeast two-hybrid system have revealed specific details about how channel α-subunits might interact. In the case of AKT1, specific sequences in the cyclic nucleotide-binding site and in a region between the last 81 amino acids and a region between the hydrophobic core and the cyclic nucleotide-binding site were suggested to play a role in the tetramerisation process. The yeast two-hybrid system was also used to screen for proteins that interact with the K^+ uptake channels KST1 [28]. In that screening, additional K^+ channels, rather than additional subunit types, were identified by using the C-terminal regions of KST1 as bait. Further analysis showed that a domain called K_{HA} (hydrophobic and acidic amino acid residues) which is located in the C-terminus was important for the clustering of K^+ channels as visualised using a green fluorescent protein tag. The physiological significance of clustering is uncertain because deletion of the K_{HA} domain reduced clustering, but did not abolish or change the function of the channels [28].

Plant K^+ channel α-subunits physically assemble indiscriminately. This was clearly demonstrated using the yeast two-hybrid system [28]. These physical interactions have also been shown to lead to functional changes in channel properties [29,30]. A series of experiments showed that when cRNAs encoding the channels *KAT1*, *KST1*, *AKT1*, *SKT1* and *AtKC1* were co-injected into *Xenopus* oocytes, the proteins assembled to form heteromultimers as judged by changes in caesium and calcium sensitivity. A very surprising result was that two previously studied channels (AKT1 and SKT1) that had been shown to be electrically silent in oocytes, were found to mediate electrical currents when they were co-injected into oocytes [30]. Experimental evidence does not explain this result, nor is it understood why these channels (AKT1 and SKT1) mediate electrical currents in insect cells [31,32], but not *Xenopus* oocytes.

A dominant negative point mutation in KAT1 was used in *Xenopus* oocytes to suppress the activity of wild type KAT1 channels [29]. These results further support the conclusion that subunits of plant K^+ uptake channels form multimeric complexes and it was suggested that this 'dominant negative' system could be used to reduce the activity of channels in vivo [29]. Tissue specific expression of genes encoding K^+ uptake channels, rather than specific amino acid motifs in the proteins appear to determine ion channel heteromeric assembly *in planta*.

In addition to α-subunits, plant inward rectifying K^+ channels may also have β-subunits. Putative β-subunits have been identified based on sequence similarity to β-subunit polypeptides from other organisms [33]. Although a physical association between KAB1 was shown to occur with KAT1 [34], this association does not lead to changes in channel function [34] as has been shown for some K^+ channels in non-plant systems [35]. Database searches of the

Arabidopsis genome suggest that KAB1 belongs to a small gene family or may even be a single gene. Expression patterns indicate this β-subunit is present in flowers, roots and leaves. The expression of a rice β-subunit was shown to be reduced in older leaves of K^+ starved plants and the protein levels appeared to be developmentally regulated with very high levels of KOB1 protein being found in the youngest leaves. At this time the physiological significance of these putative K^+ channel subunits is uncertain. The possibility exists that this β-subunit may associate with other channel types, such as outward rectifiers (KCO or SKOR, see Table 1). Although a physical association was demonstrated in vitro one could speculate that such results are an artefact of the system used, because there is no evidence showing an in vivo association between KAT1 and KAB1.

2.3. Ankyrin domain and nucleotide binding site

A well known structural feature found in some plant K^+ inward rectifying channels is the ankyrin domain [1]. This region is found in the C-terminus of some K^+ channel proteins that have been grouped into the *AKT* family. This ankyrin domain may facilitate the binding of channel proteins to the cytoskeleton, but is probably not involved in the interactions between K^+ channel subunits [27]. Recent work with *KAT1* suggests that a microtubule-interaction site (MIS) may be present in the C-terminus of this protein, but studies in which the MIS was deleted have failed to establish a functional role for this MIS [36]. The importance of cytoskeleton attachment for channel function was also tested in oocytes by adding cytoskeleton-disrupting agents such as colchicine [36]. A 21 h colchicine treatment reduced KAT1 currents as expressed in *Xenopus* oocytes by about 50%, whereas the deletion of the MIS had no effect on channel properties. It is uncertain whether there were non-specific effects of the colchicine because the expression of other channels was not compared. The role of the MIS in KAT1 as well as the ankyrin domain in AKT channels remains to be fully determined.

Located near the ankyrin domain is a putative cyclic nucleotide binding site [1,27]. The function of this site has not been determined. However, there are a number of studies showing the importance of the nucleotide ATP in the function of K^+ channels in plants [37,38]. In excised membrane patches ATP was shown to increase the open probability of inward rectifying K^+ channels [38]. In heterologous expression systems the absence of ATP was shown to be involved in the run down of KAT1 and KST1 ion channel currents in excised patches [11,20]. The importance of the cyclic nucleotide binding site in the regulation of channel activity is unclear but this domain is important for the overall function of KAT1 because deletion of regions of the C-terminus that contain the cyclic nucleotide binding site abolished the function of KAT1, whereas deletions distal to the cyclic nucleotide binding site resulted in functional channels as expressed in *Xenopus* oocytes [36].

2.4. The pore loop

The region between transmembrane domains S5 and S6 contains the pore helix (H5) of K^+ selective channels [39]. This pore helix contains highly conserved amino acid residues (GYG) that in part determine the selectivity of K^+ channels [40] and have been termed the K^+ channel signature sequence. These highly conserved amino acids are found in channel proteins from bacteria, plants and animals. A K^+ channel protein from *Streptomyces lividans* has now been crystallised and studied using X-ray analysis to 3.2 Å [41]. Although this bacterial channel only contains two transmembrane domains there is a high degree of structural and amino acid homology between the H5 and S6 regions in the six transmembrane channels and the *S. lividans* channel. Therefore, based on the structural data obtained from this model system and by analogy, the H5 region in plant channels should be cradled inside the pore of the channel forming the selectivity filter. The selectivity filter in *S. lividans* is the narrowest region of the pore estimated to be 12 Å long with the rest of the pore being wider. Using the same *S. lividans* channel, gating was studied and shown to involve the movement of transmembrane helix two (TM2) [42]. TM2 corresponds to the S6 region of the six transmembrane channels such as KAT1 and Shaker which has also been implicated in gating of these channels [43].

Numerous studies have been completed to elucidate how the amino acid configuration of the plant

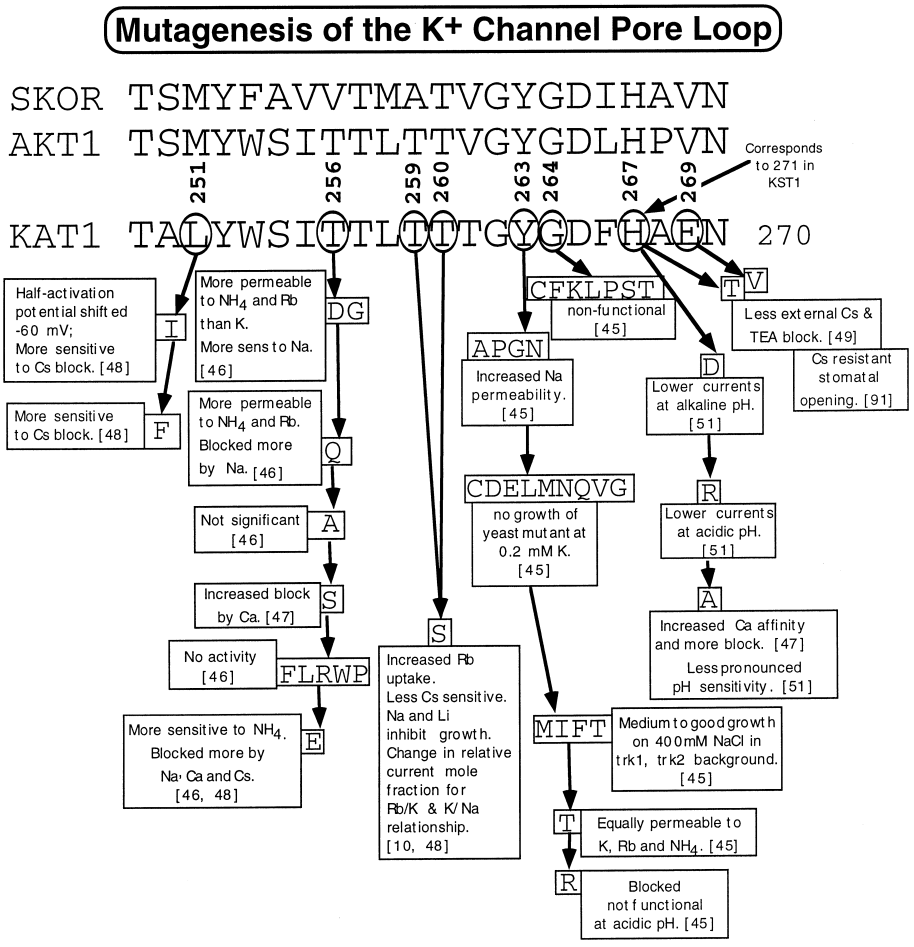

Fig. 1. Sequence alignment of the pore loops of three K^+ channels from plants – SKOR1, AKT1 and KAT1. The results of extensive mutagenesis experiments are shown. Arrows point to amino acids that have been substituted for circled amino acids in KAT1. Boxes next to or below substituted amino acids describe the functional changes that were measured. In the case of H267 the experiments were done on the corresponding amino acid in KST1 [51].

inward rectifying K^+ channel pore loop specifies function. Most of those studies have used KAT1, which can be easily expressed in yeast [2] and in *Xenopus* oocytes [4,44]. Site directed and random techniques have been used for mutagenesis of the pore region of plant inward rectifying K^+ channels. The use of random mutagenesis has been possible because the yeast heterologous expression system can be employed for selection of interesting mutants. In Fig. 1 I have summarised where specific mutations have been created and how those mutations change channel functional characteristics.

The pore of an ion channel is an extremely important structure in determining cellular homeostasis, because to a large extent the selectivity of the pore determines which ions move into or out of cells. Several specific amino acid residues have been identified that are involved in determining selectivity [45,46]. Two of the conserved amino acid residues (Gly and Tyr/Phe) found in the pore region of all selective K^+ channels were extensively mutagenised using a random mutagenesis protocol [45]. This work revealed that the Gly^{264} residue could not be changed unless there was a corresponding change in the neighbouring Tyr^{263} residue. For example functional channels were only recovered when double mutations were created between Y^{263} and G^{264} such as GA, LA DS, QA [45]. One mutation, DS, studied in detail using oocytes and electrophysiological techniques, was no longer able to select between the monovalent

cations K^+, NH_4^+, Na^+ or Rb^+. This study also showed conclusively that a tyrosine or phenylalanine in position 263 is essential for full selectivity of KAT1.

One amino acid residue, Thr^{256}, has been studied in detail (Fig. 1), because it is thought to be located in the narrowest region of the pore and involved in selectivity [46–48]. Changes in Thr^{256} resulted in very large increases in NH_4^+ and Rb^+ permeability as well as blockade by Na^+. Other amino acids have also been identified as being important in blockade by ions such as Cs^+ and Ca^{2+} [9,47–49,91]. It is well known that protons activate guard cell inward rectifying K^+ channels [50]. This activation was shown to be partially due to a specific histidine residue located in the pore [51] as well as a histidine residue located in a linker between transmembrane domains S3 and S4. The results of these studies, which are summarised in Fig. 1, confirm that this highly conserved region, found in all selective K^+ channels, functions as a pore helix in plant inward rectifying K^+ channels.

2.5. Voltage sensor and N-terminus

Plant inward rectifying K^+ channels contain a region of positively charged amino acids that are separated by hydrophobic and polar amino acids. A similar more highly charged region (S4 domain), with charged residues separated by two hydrophobic amino acids is found in Shaker channels and has been shown to be involved in the voltage activation of these outward rectifying K^+ channels. A few studies have tested whether this S4 like region in plant inward rectifying K^+ channels is involved in voltage sensing and activation of these channels. Two single charged amino acid residues R176 and R177 were changed to either serine (R176S) [52], leucine (R176L) [53] or glutamine R177Q [53]. The R176S substitution was shown to affect voltage dependent gating in such a way as to shift the half-activation voltage of the channel more positive ($V_{1/2}$ of KAT1 = −119 mV and $V_{1/2}$ of R176S = −80 mV). In addition to this mutation, several N-terminal deletions were also shown to affect the $V_{1/2}$ of steady state activation, but in the opposite direction. One deletion in amino acids Δ20–34 shifted the $V_{1/2}$ of activation from −104 mV to −169 mV. Several other deletions such as Δ2–34 and Δ16–34 appeared to abolish the function of KAT1. However, the investigators [54] reasoned that the apparently non-functional mutations might have shifted the voltage dependence to a negative activation potential which was outside of the range of the electrophysiological analysis. Therefore they tested this hypothesis by introducing the R176S mutation into deletions Δ2–34 and Δ16–34, which shifted the activation potential of these mutant channels back into the range where they could be recorded. The experiments that neutralised positive charges or introduced additional positive charges in the S4 domain of KAT1 [30,51–53] suggest that this region is involved in voltage sensing and activation. In plant inward rectifying K^+ channels the N-terminus of the polypeptide is also important to voltage sensing and activation. It has been proposed that this region of KAT1 forms a cytoplasmic part of the vestibule that the S4 region traverses [52].

2.6. Physiological roles

The physiological role of these K^+ uptake channels is clearly established in certain cell types. For example in guard cells inward rectifying K^+ channels are involved in the net influx of K^+ and stomatal opening [8,55], whereas the channels expressed in the root have been shown to be involved in high and low affinity K^+ uptake [56].

The physiological role of *AKT1* was recently demonstrated using plants in which the *AKT1* gene was inactivated by the insertion of a T-DNA tag [56]. The absence of a inward rectifying K^+ channel in this mutant (*akt1*) was demonstrated at the cellular level using high-resolution patch clamp techniques. A phenotype of lower growth at 100 μM K^+ was only observed when *akt1* knockouts were grown with high concentrations of ammonium in the medium. That result suggests ammonium inhibits one of the parallel K^+ uptake pathways such as the KUP/HAK transporters [57].

Based on studies with the *akt1* mutant it appears that this channel is involved in high affinity uptake which was somewhat surprising because of previous theoretical arguments that suggested there should be a distinct external K^+ concentration where high or low affinity uptake systems would dominate. The in-

volvement of channels in high affinity uptake is based on measurements of root membrane potential as low as −230 mV and growth experiments with the *AKT1* knockout mutant at high ammonium concentrations in the medium [56]. It is now fairly certain that channels and other energised K^+ uptake mechanisms contribute to *high* affinity K^+ uptake. It is still not certain whether there are mechanisms other than K^+ selective channels that contribute to *low* affinity K^+ uptake. These studies on the expression of K^+ channels and knockout mutants highlight the importance of K^+ uptake channels in plant K^+ nutrition and stomatal function. Genomics research projects should help to provide a complete inventory of all the genes encoding K^+ uptake channels, which could then be used to complete the picture of where all the different genes encoding K^+ uptake channels are localised and how their expression is modulated by varying environmental conditions.

3. Outward rectifying K^+ channels

3.1. Two pore loops

Electrophysiological techniques have shown that outward rectifying K^+ channels are present in most plant cell types that have been studied. The frequency with which these channels have been seen in patch clamp studies, suggested that the genes encoding these channels should be abundantly expressed and relatively easy to clone. Only recently has the molecular identity of this class of channel been revealed in plants. In both cases of where genes encoding these channels have been cloned, the database of EST sequences has revealed their identity.

The first gene encoding an outward rectifying K^+ channel in plants was cloned by searching databases using the highly conserved P-domain motif TXGYGD [58]. Searches with this motif led to the discovery of a unique EST from *Arabidopsis* and the subsequent isolation of a full-length cDNA clone called *KCO1*. Several genes encoding this type of channel can be found in the protein database from potato, *Arabidopsis* (KCO1) and *Samanea saman* (*SPOCK1*). The structure of the protein encoded by KCO1 is similar to others from yeast and humans [59,60] because there appear to be 'two pores' (Fig. 2). Overall the sequence homology to the previously cloned TOK1 and TWIK-1 is low (approx. 20%), but in the case of TWIK-1 the topology (four transmembrane domains) is the same. In contrast the overall topology of TOK1 (eight transmembrane domains) is different from KCO1, but the function (outward rectifying K^+ channel) is similar. KCO1 also has two EF hand domains, which is a structural feature unique to this type of K^+ channel, as compared to other K^+ channels from plants. This EF hand motif appears to play an important role in regulating channel activity in response to calcium. KCO1 was expressed in insect cells and in that system its activity is modulated by internal Ca^{2+} with maximal current observed at about 300 nM internal Ca^{2+} [58]. At lower levels of calcium, below 100 nM, currents were completely abolished. Single channel conductance of KCO1 was high – about 64 pS.

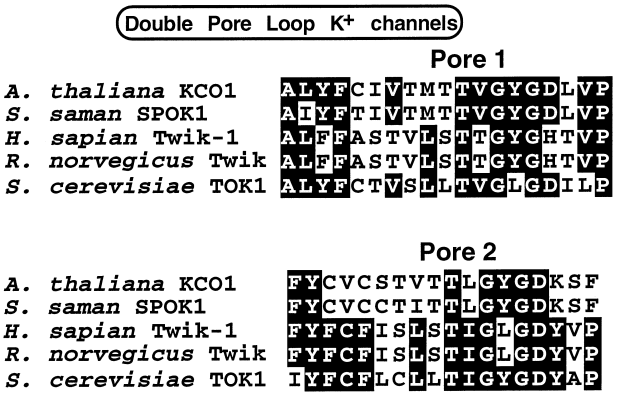

Fig. 2. Sequence alignments of the two pores found in plant, animal and fungal outward rectifying K^+ channels. Accession numbers are *Arabidopsis thaliana* (2181186), *S. saman* (4323298), *Homo sapian* (1086491), *Rattus norvegicus* (4103372), *S. cerevisiae* (995913).

The tandem pores of plant outward rectifying K^+ channels KCO1 and SPOCK1 are slightly different in amino acid sequences from TWIK and TOK1. KCO1 and SPOCK1 contain two GYG sequences whereas the other channels have only one GYG motif (Fig. 2). In TOK1 the two different pores were mutated at equivalent positions and results showed qualitatively similar but quantitatively different effects [61]. The significance of these two highly conserved K^+ channel signature sequences in the cloned plant channels may have implications for their selectivity as compared to other channels that contain the two pore motifs.

A unique feature of these outward rectifying K^+ channels in both plant and animal systems is their ability to sense extracellular K^+ concentrations [62–64]. Modulation by extracellular K^+ [60] was recently demonstrated to be an intrinsic feature of the yeast outward rectifying K^+ channel [61].

3.2. Shaker-like outward rectifier

A second class of outward rectifying K^+ channels has recently emerged that is involved in the release of K^+ into the xylem sap [65]. In plants, the main pathway for ion movement from roots to leaves is via the xylem which forms part of the vascular system. The release of ions into the xylem sap is an important process for providing leaves with nutrients that are essential for photosynthesis and other physiological and biochemical processes. Very little was known about the molecular basis of how the xylem sap K^+ content is regulated, except for the fact that stelar cells which surround the xylem were shown to have K^+ outward rectifying channels [66,67]. The cloning of SKOR revealed that this protein has six predicted transmembrane domains similar in the topology to Shaker outward rectifying K^+ channels and KAT/AKT inward rectifying channels. SKOR contains an S4 sequence and a P domain that are both similar to those found in other K^+ channels [65].

Characterisation of *SKOR* using molecular and biophysical techniques clearly showed that it encodes for an outward rectifying K^+ channel involved in the release of K^+ to the xylem. Electrophysiological studies in *Xenopus* oocytes showed that SKOR functions as on outward rectifying K^+ channel and expression analysis showed that *SKOR* is expressed in the root pericycle and parenchyma cells. To confirm the physiological role of this channel, studies were completed on an *Arabidopsis* knockout mutant (*skor-1*). This mutant had reduced concentrations of K^+ in shoots and xylem exudate. Complementation of the *skor-1* mutant by the *SKOR* gene restored wild type K^+ content to the shoots of these complemented *Arabidopsis* plants. This work identified the first K^+ channel in the plant kingdom that has the same six transmembrane topology as the Shaker superfamily and is also a K^+ selective *outward* rectifying channel. This work illustrates how molecular techniques (cloning, oocyte expression, GUS and Northern localisation and gene inactivation) can now be used in plants to fully characterise the biophysical and physiological function of ion transport mechanisms [65].

4. High affinity K^+ transporters

4.1. HKT1

Yeast complementation also revealed the existence of a high affinity K^+ transporter (HKT1) [68] with significant sequence similarity (approx. 20%) to the two major K^+ uptake mechanisms in *Saccharomyces cerevisiae* [69]. This transporter may not have been isolated in previous screenings [1,2] because the cDNA library used for complementation was from wheat roots that had been starved of K^+. Homologues of HKT1 are found widely in eubacteria, archeabacterial, fungal as well as plant genomes [70].

When HKT1 was cloned and characterised it was thought to encode a major proton energised high affinity mechanism for K^+ acquisition [68]. Subsequent work has been unable to verify the role of HKT1 as a major mechanism for K^+ acquisition but has clearly demonstrated that the transporter is energised by Na^+ when expressed in yeast and *Xenopus* oocytes [71,72]. Part of the problem of verifying the physiological role for HKT1 is that no one has been able to show Na^+ energised K^+ uptake *in planta* [73].

In determining whether HKT1 is a major mechanism involved in K^+ acquisition from the soils it is important to assess where it is expressed and how expression is regulated. Rapid derepression of HKT1 gene expression occurs when K^+ is completely withdrawn from the growth medium [74]. The physiological significance of these studies is uncertain because plants may only be exposed to soil solutions completely lacking K^+ after a strong rain that leaches the K^+ away from the roots. The generally accepted dogma is that ion uptake mechanisms should be located in epidermal cells of the root [75]. Several transporters have now been localised to the epidermis (e.g. nitrate and phosphate) [76,77], but *HKT1* was shown to be expressed in the root cortex and in cells bordering the vascular tissue in leaves [68]. The localisation of *HKT1* gene expression may suggest

some other physiological role for HKT1, perhaps in the control of xylem unloading or in the scavenging of K^+ that may leak out of cortical cells [78]. In the root cortex the scavenging role of HKT1 is supported by the findings that cortical cells are better able to maintain cytoplasmic K^+ concentrations when external concentrations are low [79]. Cells bordering the xylem have been called xylem contact cells and have specialised functions [80]. In these cells HKT1 may be involved in controlling the delivery of solutes to mesophyll and epidermal cells. Although the physiological role of HKT1 has not yet been clearly defined, the presence of HKT1 homologues in the genomes of a wide range of organisms and its regulated expression in response to K^+ deprivation suggest that it plays a role in the coordinated response to K^+ deprivation in the external medium.

The structure and function work on HKT1 has been very innovative because of the novel structure of this transport protein and the fact that there were no previously established structural models, as was the case for inward rectifying K^+ channels. Durell et al. [81] have recently proposed that the HKT1 family of symporters are related to K^+ channels. They used the relationship between the bacterial KtrAB [82] and 2TM (transmembrane) K^+ channel protein families to link more distantly related K^+ symporter proteins to K^+ channels [81]. They have proposed a structural model for the symporters such as

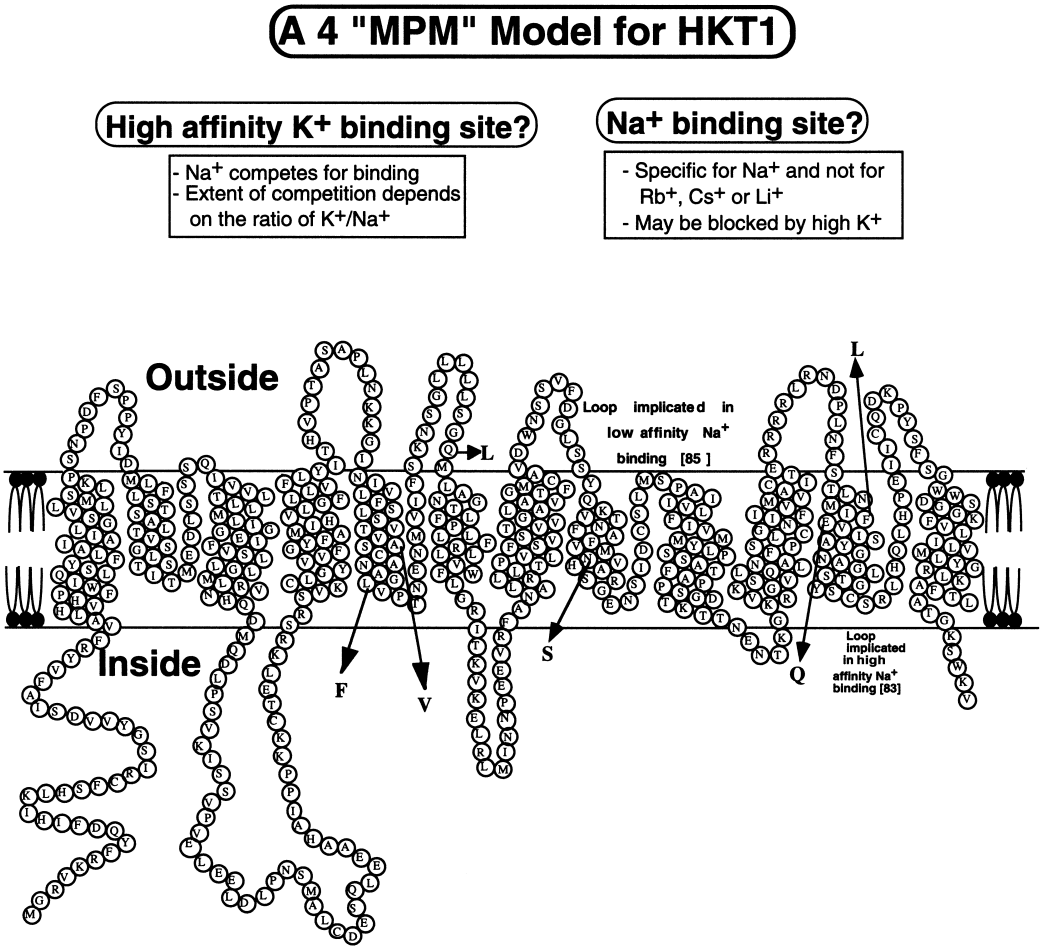

Fig. 3. A topological model of HKT1 based on the consensus of five membrane transporter predication programs and a recently proposed model [81]. The functional features that were proposed by [71] and the mutations that alter functional characteristics are highlighted [72,83,85].

HKT1, that suggests there are four sequential MPM motifs (transmembrane helix-pore-transmembrane helix). In Fig. 3, I have attempted to fit HKT1 to the four MPM model proposed by Durell et al. [81]. This model will need to be experimentally verified using molecular biological techniques, until it becomes possible to use X-ray diffraction for the determination of protein structure.

The HKT1 system has also been ideal for structure and function studies because of the ability to express this transporter to high levels in yeast and *Xenopus* oocytes. Two approaches have been used to dissect how the structure of this protein determines its function. One approach has been random mutagenesis and selection in yeast, while the other has used site directed mutagenesis of highly conserved residues. Since HKT1 is a Na^+ energised K^+ uptake mechanism and in high concentrations Na^+ is toxic to yeast, random mutations have been identified by selecting for cells with mutagenised HKT1 containing plasmids that confer growth on high concentrations of NaCl. Two different strains of yeast have been used for these screenings. One strain, called CY162, is deficient in two K^+ transporters [69] and the other strain, 9.3, is deficient in the same two K^+ transporters and a Na^+ efflux pump which makes the cells more sensitive to Na^+. In a landmark paper, Rubio et al. [72] used the CY162 strain to isolate mutants of HKT1 that lead to changes in the protein structure, which increased the salt tolerance of the yeast cells in which they were expressed (Fig. 3). Cells expressing the mutant form of HKT1 (A240V and L247F) took up less Na^+ and were more salt tolerant. This work was of great significance because it verified the concept that transporters could be engineered to increase the stress tolerance of an organism. Further proof of concept will require the replacement of HKT1 by the salt tolerant transporter *in planta* and subsequent testing for salt tolerance.

A functional model for the structure of HKT1 has been proposed based on a set of experiments carried out in yeast and *Xenopus* oocytes [71]. The model proposes that HKT1 has two independent binding sites for ions but does not suggest the location of the sites or how transport is coupled between these 'independent' sites [71]. In Fig. 3 a four MPM model is presented which is based on the consensus of several membrane prediction programs as well as the recently published analysis by Durell et al. [81]. According to a proposed model [71] one site is for high affinity K^+ binding and the other for high affinity Na^+ binding (Fig. 3). The high affinity Na^+ binding site is presumably involved in the energisation of transport and has been shown to be highly specific for sodium ions and not other alkali metal ions such as Rb^+, Li^+ or Cs^+. The other site is selective for K^+ but is also susceptible to binding Na^+ as was well as other alkali ions such as Rb^+ at a 1000 times lower affinity. When Na^+ alone is present in the solution, HKT1 translocates significant amounts of this ion as measured in *Xenopus* oocytes which suggests that HKT1 could also function as a Na^+ transporter. The interaction between the ions and the two binding sites depends on the ratio and concentration of potassium and sodium ions in solution.

One important question is where specific structures are located on the HKT1 protein. Highly conserved regions of orthologous proteins may point to areas that are essential to various functions, as has been the case for the pore loop of K^+ channels that plays an important role in determining selectivity [8]. In the HKT1 protein, a region of 16 amino acid residues was identified and modelled to be in a loop region at the C-terminal end of the protein [83]. This loop region is now thought to be located in the fourth MPM region of the symporter in the helical region that forms the pore [81,84]. Mutations in this region that is conserved between HKT1 homologues in different phyla resulted in changes to protein function that reduced the affinity of a putative high affinity Na^+ binding site (Fig. 3). More experiments will be necessary to prove this region of the protein is essential to high affinity Na^+ binding [83], but it is interesting that this highly conserved region has now been located in the fourth pore of the symporter [81]. Additional random mutagenesis has identified a single mutation N365S (Fig. 3) that confers high salt tolerance to 9.3 yeast cells [85]. This mutation (N365S) along with the previously described A240V and Q270L mutations increase the salt tolerance of yeast cells by reducing the Na^+ inhibition of Rb^+ uptake and lowering the rate of Na^+ transport as well as the affinity of the low affinity binding constant for Na^+. Electrophysiology experiments also clearly show that the shift in the reversal potential of the current voltage curves in response to

changes in external Na^+ is clearly different in HKT1 and the N365S and A240V mutants [85]. These differences in reversal potential show that HKT1 and the mutants differ in ionic selectivity. While the random mutagenesis results on HKT1 are interesting and have physiological relevance to engineering salt tolerance in higher plants, it is not clear how mutations that are 100 amino acids apart can have similar effects on transporter function [72,85]. These results may suggest that the two binding sites are not completely independent and highlight the co-transporter function of HKT1. In the future it will be important to have models that show where the sites of high affinity K^+ and Na^+ binding are located on the protein, if these are indeed independent binding sites.

4.2. HAK1/KUP1

Recently the genes encoding a third class of K^+ transport proteins (KUP or HAK) have been identified. These transporter genes appear widespread in plant species including monocots and dicots [57,86–88] and are structurally similar to the *Escherichia coli* KUP1 K^+ uptake mechanism [89] and a high affinity K^+ transporter HAK1 from the fungus *Schwanniomyces occidentalis* [90]. The *HAK1* cDNA from *S. occidentalis* was expressed in an uptake deficient *S. cerevisiae* mutant and was shown to be more efficient at depleting the external medium of K^+ than the yeast TRK1 mechanism. As in the case of HKT, this family of transporters is also found in bacteria (such as *Rhizobium*, *Lactococcus*, and *Sinorhizobium*) and fungi, but is not as widespread as HKT1 homologues [70]. A human homologue reported by [57,86] is no longer found in the databases. This plant K^+ transport protein has been modelled to contain 12 transmembrane domains [87] and in *Arabidopsis* at least six isoforms can be identified in the gene databases [70].

cDNAs from barley and *Arabidopsis* encoding these transporters have been cloned and characterised in yeast [57,86,88] and in plant cells [87]. In studies on the barley clone as expressed in a yeast mutant, medium containing 76 mM ammonium was shown to inhibit the growth of cells dependent on HvHAK1 when concentrations of K^+ were at or below 0.5 mM K^+. These results suggest that KUP1 is the parallel K^+ pathway that is being blocked in the studies where Hirsch et al. [56] show that the *AKT1* knockout only has a phenotype under conditions of high ammonium. *HvHAK1* which is expressed mainly in roots has a high affinity for Rb^+ (18 μM) and its expression is enhanced by K^+ starvation. Na^+ inhibits Rb^+ uptake ($K_i = 15 \pm 2$ mM) and is also taken up by HvHAK1.

Two groups published a comprehensive functional characterisation of the transporter gene encoding AtKUP1 at the same time [87,88]. Both groups showed that the transporter has a dual affinity for K^+ of 22–44 μM and 11 mM. The results of these two studies are yet to be fully resolved. For example in one study the AtKUP1 complements a K^+ uptake deficient yeast mutant whereas in the other study the researchers had to employ a novel *E. coli* expression system (triple deletion Trk, Kup, Kdp) to demonstrate complementation because attempts in yeast failed. In the case of the *Arabidopsis* gene family, a complex and somewhat confusing expression pattern has emerged with transcripts found in all tissues and expression of certain genes increased and decreased by both high and low concentrations of K^+. The large *KUP/HAK* gene family and their expression within most plant tissues suggests that this transporter is vital for K^+ nutrition of plants. The multiplicity of genes also suggests that these transporters will have slightly different functional characteristics as well as physiological roles.

5. Conclusions

The molecular biology of plant K^+ transport has already been greatly aided by research into plant genomics (e.g. [33,58,65]). The full *Arabidopsis* genome sequence will provide a complete inventory of the genes encoding K^+ transport mechanisms that will need to be localised and expression studies will need to be carried out in varying environmental conditions. Although much has been learned about the molecular biology of specific plant K^+ transport mechanisms, it is certain that the next 5–10 years of research will lead to a more complete appreciation of the complexity of K^+ transport mechanisms including those that function in the different plant tissues and intracellular membranes.

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