trends in plant science
reviews

Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants

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Potassium uptake is vital for plant growth but in saline soils sodium competes with potassium for uptake across the plasma membrane of plant cells. This can result in high Na^+:K^+ ratios that reduce plant growth and eventually become toxic. Our understanding of the molecular basis underlying the interaction between essential potassium and toxic sodium was limited until the recent cloning and electrophysiological characterization of several genes encoding different types of molecules that are involved in K^+ and Na^+ transport. These molecules, and their regulation, are important in determining the K^+:Na^+ homeostasis of plants in saline soils, although it is not yet known which is most critical in determining the K^+:Na^+ ratios in whole plants.

Potassium is a macronutrient that is required in high concentrations. It plays essential roles in the growth of all plants. In contrast to potassium, sodium is only essential for certain plant species[1] but can also be ‘beneficial’ to plant growth in relatively low concentrations[2] or toxic at high concentrations[3]. In all living cells, cytoplasmic K^+ concentrations are relatively high, whereas Na^+ concentrations are relatively low, which indicates the selective uptake of K^+ and, in some situations, the preferential exclusion of Na^+ from the cytoplasm. The low cytoplasmic Na^+ concentration in animal cells is maintained via the well-characterized Na^+:K^+ ATPases, and the inward gradient for Na^+ is used to energize the uptake of many solutes. Although cytoplasmic Na^+ concentrations are also low in plant cells, the energized uptake of solutes is primarily linked to the inwardly-directed proton gradient that is generated by proton pumps located in the plasma membrane.

In the soils where plants are rooted, potassium and sodium ions might compete for entry into plant root cells. This competition can have significant negative effects on plant growth in saline soils, where concentrations of sodium often exceed those of potassium. As approximately a third of the world’s irrigated soils and a large proportion of soils in dryland agricultural regions are saline, it is important to develop an understanding of how plant roots are capable of distinguishing between essential potassium and potentially toxic sodium ions. Distinguishing between these two ions is not trivial because they are similar in ionic radius and ion hydration energies, which are both factors in determining how these ions move through membrane proteins into cells and whole plants[4].

Sodium–potassium selectivity is an important factor in salt tolerance

In developing more salt-tolerant crop plants it is essential to understand what mechanism(s) make one plant more salt-tolerant than another. Halophytes, which are plants that grow naturally in saline environments, have developed specialized strategies for maintaining growth in spite of high tissue salt concentrations and for regulating Na^+ and Cl^- concentrations via specialized trichome structures, such as salt glands or bladders[5]. Non-halophytes evolved in non-saline environments and, within a particular species, the observed genetic variation for salt tolerance is often linked to a superior ability to exclude sodium[6] while maintaining potassium uptake. In woody perennials, such as citrus and grape, genetic variation for salt tolerance is also linked to an ability to exclude Cl^- ions (Ref. 7). In addressing the question of what mechanism(s) make one plant type more tolerant than another, many plant biologists have focused on the control of Na^+ uptake, which, in many cases, interacts with the uptake of K^+.

Genetic control of potassium and sodium uptake

At the whole plant level the genetic control of potassium and sodium selectivity has been studied in relation to salinity tolerance. A fundamental role of K^+ uptake, for growth under saline conditions, was recently demonstrated by the isolation of an *Arabidopsis* mutant that is hypersensitive to salinity and deficient in high-affinity K^+ uptake[8]. Progress in localizing a gene that is important in determining both K^+:Na^+ selectivity and salt tolerance has been made using *Triticum aestivum* as a model system. The hexaploid composition of the bread wheat genome has allowed cytogeneticists to construct aneuploid lines lacking chromosome arms. These aneuploid stocks were used to map a K^+:Na^+ discrimination trait to a single chromosome arm (4DL)[9]. This trait was subsequently mapped to a single locus (*KNA1*) that was later introgressed into durum (tetraploid) wheat[10], which is known to be more sensitive to salinity than bread (hexaploid) wheat. In studies on these durum wheat lines, the *KNA1* locus was shown to be important in salinity tolerance and K^+:Na^+ discrimination, but only at low external NaCl concentrations[10,11]. Therefore, modifier genes located in other regions of the D genome might be important in the K^+:Na^+ selectivity of bread wheat, or other genetically distinct mechanisms might be involved in determining the salt tolerance of bread wheat at higher salinity. The *KNA1* locus might have agronomic significance in sodic soils, where Na^+ concentrations are much lower than in saline soils. Thus, studies on the *KNA1* locus in wheat have shown that there is a genetic component to K^+:Na^+ selectivity, but have not elucidated the molecular mechanism that determines selectivity.

How do sodium and potassium interact in transport processes across the plant plasma membrane?

In most cases, charged ions do not move through the lipid bilayer, and so must cross the membrane via specialized proteins. Several

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Table 1. Guide to plant K^+ and Na^+ transport

Transport protein	Mode of transport	Membrane location	Tools and systems used for characterization	K^+:Na^+ Selectivity
KAT/AKT Inward K^+ channels	Passive diffusion	Plasma membrane	Molecular and electrophysiology *in planta* and in heterologous systems	Highly selective for K^+
HKT1 High-affinity K^+ transporter	Na^+-energized	Not known	Molecular and electrophysiology in heterologous systems	Transports both Na^+ and K^+
KUP or HAK High-affinity K^+ transporter	Not known	Not known	Molecular and radioisotopes in heterologous systems	Some Na^+ permeability
NSC Non-selective cation channels	Passive diffusion	Plasma membrane	Electrophysiology *in planta*	High Na^+ permeability
AtNXH1 Na^+–H^+ exchanger	H^+-energized	Vacuole and plasma membrane	Molecular and radioisotopes *in planta* and in heterologous systems	Not known
LCT1 Low-affinity cation transporter	Not known	Not known	Molecular and radioisotopes in heterologous systems	Transports both Na^+ and K^+

important tools have emerged that allow plant biologists to identify and characterize the function of the proteins that control the movement of potassium and sodium across plant cell membranes. In the 1980s, the patch clamp technique was successfully applied to the study of K^+ channels in higher plant cells[12,13] and, in the 1990s, molecular tools and model microbial systems revealed the identity of a large number of potassium channels and transporters (Table 1). When electrophysiological techniques and molecular methods are used together they provide a powerful method for dissecting the precise function of genes encoding transport proteins. These techniques have been used widely in neurobiology and are now routinely applied to understanding transport processes in plant cells.

Channels and their potassium–sodium selectivity

Under most conditions Na^+ would passively diffuse into the cell cytoplasm if Na^+-selective or other cation channels were present in the plasma membrane. In plants, highly Na^+-selective channels have not been found like those present in excitable cells of animals. Therefore, because of the thermodynamics and also the widely observed interaction between Na^+ and K^+ uptake, it is possible that Na^+ enters the cell cytoplasm through potassium channels.

A large number of highly selective plasma membrane K^+ channels have been identified in various plant cell types (for a review see Ref. 14). Potassium-selective uptake channels in plant root cells (often called ‘inward rectifiers’ because they mainly conduct current in one direction, i.e. into the cytoplasm) are thought to be mainly responsible for K^+ uptake by plant roots under relatively high extracellular K^+ concentrations. These same channels might also take up K^+ when extracellular concentrations are low and when high-affinity transporters are blocked[15]. Therefore, K^+-uptake channels have been suggested to function as a backup system for high-affinity K^+-uptake mechanisms[16]. The possible role of K^+-selective uptake channels in mediating Na^+ influx has been studied, and most inwardly rectifying channels appear to be highly selective for K^+ over Na^+ (Ref. 14). Therefore, it appears unlikely that K^+-uptake channels would mediate significant net Na^+ flux into plant cells.

Non-selective cation channels that are permeable to a range of monovalent cations and might play a role in mediating Na^+ uptake, have been identified in plant root cells[14,17]. In some studies these channels have been found to be more permeable to K^+, whereas, in other cases, non-selective channels have been shown to be more permeable to Na^+ (Ref. 14).

Recently, non-selective cation channels have been described using the patch-clamp technique. Na^+-dependent inward currents were identified in protoplasts of wheat root cortical cells[18], maize root cortical cells[19] and barley suspension-culture cells[20]. The current amplitude of these channels was dependent on the external Na^+ concentration as well as external Ca^{2+} concentration. At low Ca^{2+} concentrations (40–100 μM), large increases were observed in the amplitude of the Na^+-dependent inward currents. The calcium inhibition of Na^+ influx through non-selective cation channels correlates well with the reduction of Na^+ uptake by increased Ca^{2+} concentrations in soil[21]. Sixty to eighty percent of the inward current generated by some non-selective cation channels appears to be inhibited by levels of calcium commonly found in soil solutions ($>$0.5 mM). In a comparison of ion channel currents (patch clamp experiments) with whole root uptake of Na^+ (tracer flux analysis), calcium inhibited the Na^+ permeation through non-selective channels and whole root Na^+ fluxes to a similar extent ($K_i = 0.6$ mM Ca^{2+} activity for wheat)[22]. This analysis highlights the physiological relevance of the patch-clamp studies that are conducted on single cells. It also suggests that Ca^{2+}-insensitive Na^+-uptake pathways are probably present and involved in Na^+ and K^+ uptake by plant roots, because in most soils calcium levels are high enough to substantially inhibit Na^+ transport through non-selective channels.

Sodium-permeable channels coexist and function in concert with other channels in the plant cell membranes but, to date, there are no models that incorporate the full spectrum of channels and

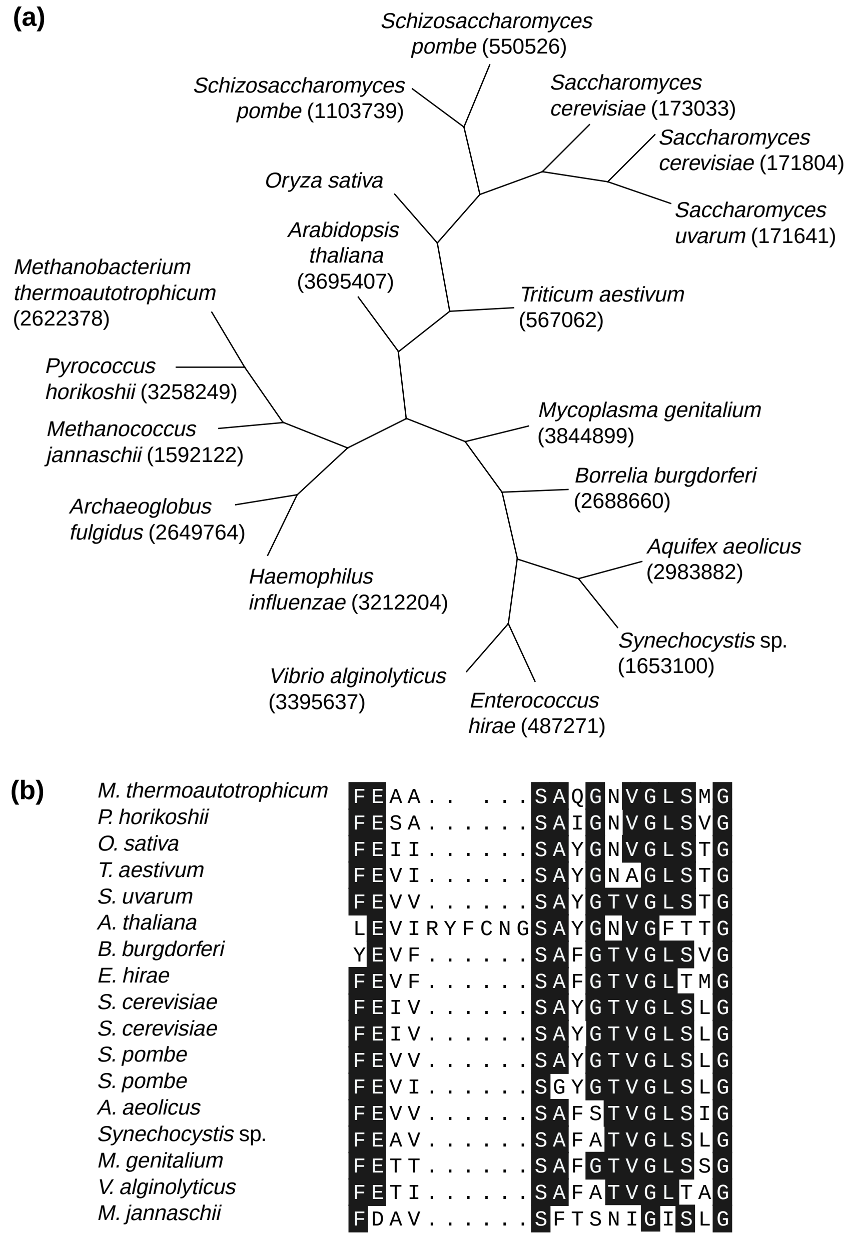

Fig. 1. Sequence comparisons of HKT1 and TRK homologues. (a) Phylogenetic tree of HKT1 homologues found in plant, bacterial and fungal species. Only full length proteins were used to construct the tree. The *Oryza sativa* sequence is unpublished (J. Bennett). Numbers under species names indicate the protein identification number. The tree was constructed using GCG version 8.0 on the Australian National Genomic Information Service (ANGIS). Protein sequences were aligned using 'Pileup', and the output file was then used for the construction of phylogenetic trees using the evolutionary analysis program 'Eprotpars'. (b) Sequence alignment of the most highly conserved region of the HKT1 homologues. The alignment was done using 'Pileup' and then shaded with 'Prettybox'.

transporters in any specific plant cell type. Such models will be vital to our understanding of the integration and regulation of the suite of channels present in plant cells. The existence of multiple channel types with differing K^+:Na^+ selectivities might reflect the need to coordinate the influx of these different cations. Sodium-permeable channels might provide certain cells with a greater degree of flexibility in Na^+:K^+ discrimination through cooperation with other inward cation channels at the plasma membrane[20]. In saline conditions, the reduced activity of these non-selective channels might increase tolerance owing to reduced Na^+ accumulation. It is not known how the activity of these non-selective channels is regulated, nor how the genes encoding non-selective channels respond to saline conditions.

Saccharomyces cerevisiae contains a similar type of non-selective cation channel. Whole cell patch clamp experiments revealed large inward K^+ currents in intact yeast cells when extracellular Ca^{2+} concentrations were low[23,24]. Whole cell inward currents resemble currents recorded in barley suspension-cultured cells. These channels mediate the flux of a range of monovalent cations into cells and, therefore, might represent a low-affinity Na^+-uptake pathway in yeast[24].

To date, the molecular identity of these non-selective cation channels is not known in plants or yeast. As most of these non-selective channels are more permeable to K^+ than Na^+, it might be possible to clone cDNAs encoding non-selective cation channels by complementation of K^+-uptake-deficient yeast mutants under appropriate conditions. It might also be possible to identify the non-selective channels studied in yeast[23,24] using a bioinformatics approach, and use the sequence information from yeast to construct a mutant for complementation of similar plant channels.

A cDNA encoding a low-affinity cation transporter (LCT1) was recently identified from a wheat root cDNA library. It appears to be relatively non-selective for cations and the uptake of rubidium ions (Rb^+) is blocked by mM concentrations of Ca^{2+} (Ref. 25), similar to the non-selective cation channels. LCT1 has also been shown to transport divalent cations, such as Ca^{2+} (Refs 25,26) and Cd^{2+} (Ref. 26). It is not known in which membrane LCT1 resides, or whether LCT1 is a channel or some other type of transporter. These details will be helpful for understanding the physiological function of this novel transporter.

High-affinity potassium uptake

The cDNA encoding a high-affinity K^+ transporter, *HKT1*, was isolated from a wheat root library and shown to be expressed in cortical cells[27]. Although HKT1 was originally described as a H^+-driven transporter[27], it was later shown to be energized by Na^+ (Ref. 28) and derepressed when roots were placed in a solution lacking K^+ (Ref. 29). The characterization of HKT1 provided the first molecular evidence for Na^+-energized uptake of solutes in higher plants, although earlier work showed that aquatic macrophytes exhibited Na^+-driven K^+-uptake mechanisms[30].

It is surprising that wheat does express a gene encoding a Na^+-energized transporter, as sodium is not an essential element and only in some situations is it beneficial for plant growth. The physiological

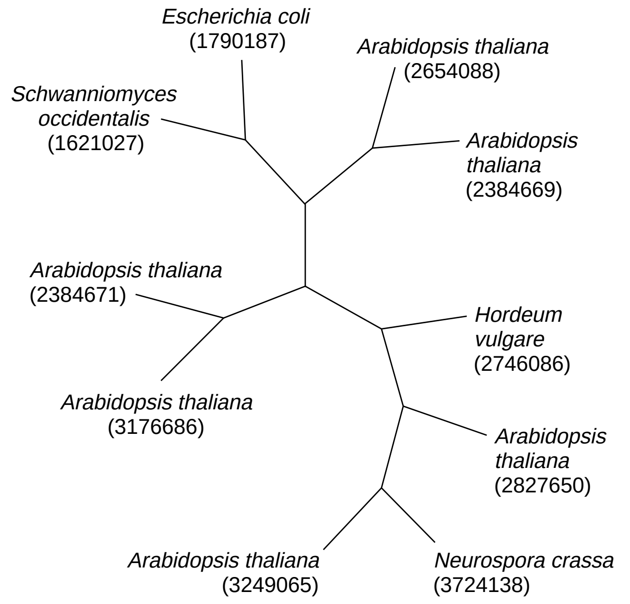

Fig. 2. Phylogenetic tree of KUP or HAK homologues found in plant, bacterial and fungal species. Numbers under species names indicate the protein identification number.

role of HKT1 is not clear. But, based on the fact that HKT1 is expressed in cortical cells and that Na$^+$ is not required for growth, HKT1 is probably not a primary uptake mechanism for K$^+$ and, therefore, might not be a major route for Na$^+$ entry into cells. However, evidence that HKT1 functions as a high-conductance pathway for Na$^+$ entry when expressed in oocytes[31] indicates that its role as a major route for Na$^+$ entry into the cell needs to be resolved. To gain a thorough understanding of HKT1's role in the K$^+$:Na$^+$-selectivity of plant cell membranes it is important to piece together a few more parts of the puzzle, including the precise subcellular localization and physiological function of HKT1.

One can easily envision that the different Na$^+$:K$^+$ ratios measured in whole plants might be determined by a single transporter, such as HKT1, because single amino acid changes can increase the K$^+$:Na$^+$-selectivity of this molecule when expressed in yeast cells[28,32]. However, it is known that HKT1 does not map to the same location as the *KNA1* locus, which is linked to K$^+$:Na$^+$ ratios at low salinity[10,27].

It is possible that HKT1 might not play a primary role in ion acquisition but is in some way involved in the ionic homeostasis of the cells in which it is expressed. The importance of HKT1 in maintaining ionic homeostasis is highlighted by the many bacterial, fungal and plant genomes that contain proteins that are orthologous to HKT1 (Fig. 1). HKT1 orthologs have been found in the most minimal genomes[33] and the most ancient genomes[34]. Interestingly, phylogenetic comparisons of bacterial, fungal and plant homologues show that plant and bacterial homologues are as closely related as plant and fungal homologues (Fig. 1). One region of these K$^+$-transport proteins is highly conserved (Fig. 1) and has been shown to be important in the binding of Na$^+$ to the transporter[35]. The precise transport mechanism of this widespread class of cation transporter is poorly described but emerging evidence suggests that these proteins function mainly as K$^+$-uptake mechanisms[27,36–38] and are energized by either Na$^+$ or H$^+$ (Refs 28,38). A model, based on an extensive series of experiments in oocytes and yeast, has been proposed for the function of HKT1. It suggests that the HKT1 protein has two binding sites; one that is relatively specific for Na$^+$ and the other which recognizes and transports both Na$^+$ and K$^+$ depending on the K$^+$:Na$^+$ ratios outside the cell[31]. In some bacteria and fungi, HKT1 orthologues are the primary uptake pathways for K$^+$ (Ref. 39); whereas in higher plants, multiple mechanisms for the acquisition of K$^+$ are present that allow for dynamic adaptation to changes in the soil environment, which varies over time and space. The origin of this widespread class of transporter, the different ions that are used to energize K$^+$ transport, and the structures involved in determining the functional characteristics, are the key pieces of the puzzle for understanding how Na$^+$ and K$^+$ compete for uptake across the plasma membrane via HKT1.

Another family of high-affinity transporters has recently emerged that might play a primary role in K$^+$ acquisition and might be important for the K$^+$:Na$^+$ selectivity of plant cells. The KUP or HAK1-like high-affinity K$^+$ transporters that have been described in *Arabidopsis* and barley, encode proteins that are similar to transporters identified in bacteria[40] and fungi[41]. KUP transporters represent a large [at least six genes have been identified in *Arabidopsis* (Fig. 2)] and potentially diverse family of K$^+$-uptake transporters. Na$^+$ has been shown to reduce K$^+$ transport in at least two members of this family[42,43]. Detailed characterization of HvHAK1 from barley, showed that Na$^+$ also permeates the transporter. The KUP-like transporters, similar to HKT1, are found in a range of organisms including bacteria, fungi and plants (Figs 1 and 2). When the distribution of *KUP* and *HKT1* is compared, it is interesting to note that HKT1 homologues are found more widely in bacteria (at least in those genomes available in public databases) whereas KUP homologues are found in only a few bacterial and fungal species. Because KUP appears to be a large gene family, its members might have different selectivities for K$^+$ compared with Na$^+$. Further functional characterization of these transporters, as well as details about the cellular localization of the family members are needed, to conclude whether KUP represents an important route for Na$^+$ uptake into plant cells.

Control of intracellular sodium concentrations

Little is known about the regulation of plant cell cytoplasmic Na$^+$ concentrations, which might be an important factor in determining K$^+$:Na$^+$ ratios in higher plant tissues. However, molecules that function as sodium and proton exchangers (or antiports) contribute towards regulating cytoplasmic concentrations by efflux of Na$^+$ from the cytoplasm into the vacuole or across the plasma membrane out of the cell (Fig. 3; Ref. 44,45). The activity or density of these Na$^+$-efflux mechanisms might be an important determinant of cytoplasmic K$^+$:Na$^+$ ratios of higher plant cells. Compartmentation of Na$^+$ into the vacuole is a particularly important mechanism for maintaining high K$^+$:Na$^+$ ratios in the cytoplasm. This compartmentation strategy is vital for the growth and survival of halophytes, which comprise the most salt-tolerant plant species, and of many non-halophytes, such as barley[46]. The capacity to compartmentalize Na$^+$ into the vacuole via antiports is dependent on the activity of the H$^+$-ATPase (Ref. 44) and perhaps the vacuolar H$^+$-pyrophosphatase[47], which establishes the H$^+$ gradient that energizes the transport of Na$^+$ against the electrochemical gradient. Therefore, any strategy for improved Na$^+$ compartmentation via overexpression of the Na$^+$–H$^+$ antiport might also need to increase the activity of molecules that contribute to the vacuole–cytoplasm proton gradient. This has been recently demonstrated in yeast through the expression of a plant vacuolar H$^+$-pyrophosphatase[47].

In addition to the Na$^+$–H$^+$ antiports that are found in yeasts[48,49], *S. cerevisiae* employs a plasma membrane Na$^+$-efflux mechanism ENA1, which hydrolyses ATP to pump Na$^+$ out of the cell[50] (Fig. 3). These Na$^+$ ATPases have only been identified in yeast[51] and it is unclear whether such a mechanism is important in higher plants. The yeast Na$^+$–H$^+$ antiport NHX1 (Ref. 48), which has been shown to be involved in the intracellular sequestration of sodium, has an *Arabidopsis* homologue (*AtNHX1*; Ref. 47). The expression of *AtNHX1* in *Arabidopsis* increases upon NaCl and KCl stress, which suggests a role in response to osmotic stress but does not fully complement the *nhx1* yeast mutant.

Regulation of potassium and sodium transport under conditions of high salinity

Salinity triggers several responses in plant cells that have not been fully characterized. However, evidence from yeast provides a glimpse of what we might expect to discover about plant cell response to salinity. When yeast cells are grown under highly saline conditions, uptake via TRK1 becomes more selective for K$^+$ over Na$^+$ (Ref. 52), leakage of K$^+$ is reduced[53] and Na$^+$-efflux increases because of the enhanced expression of *ENA1* (Ref. 54; Fig. 3). Calcium-signaling pathways under saline conditions appear to be vital for the regulation of K$^+$ and Na$^+$ uptake and efflux mechanisms under saline conditions. There are numerous reports describing calcineurin's pivotal role in gene expression changes that lead to salt tolerance (summarized in Ref. 55; Fig. 3). The link between calcium signaling pathways in yeast and plants during salinity stress was recently strengthened by the isolation of an *Arabidopsis* mutant that is hypersensitive to salt (Ref. 56). This mutant was shown to contain a deletion in the gene encoding a protein involved in calcium sensing[57] that might function to modulate a protein phosphatase or protein kinase. Further evidence illustrating the importance of calcium signaling comes from recent experiments with plants expressing an activated form of yeast calcineurin that show increased levels of salt tolerance[58]. Although the ionic composition of the salinized plants overexpressing an activated calcineurin was not reported, tolerance to salinity in these plants appears to involve specific root processes, which was demonstrated in a series of grafting experiments[58]. How the phosphorylation-state alters the function of membrane-transport mechanisms under saline conditions is poorly understood in higher plants. But, in yeast, the phosphorylation-state of certain molecules appears to increase the expression of genes encoding a Na$^+$ extrusion pump and leads to changes in the selectivity of the primary K$^+$-uptake mechanisms (Fig. 3).

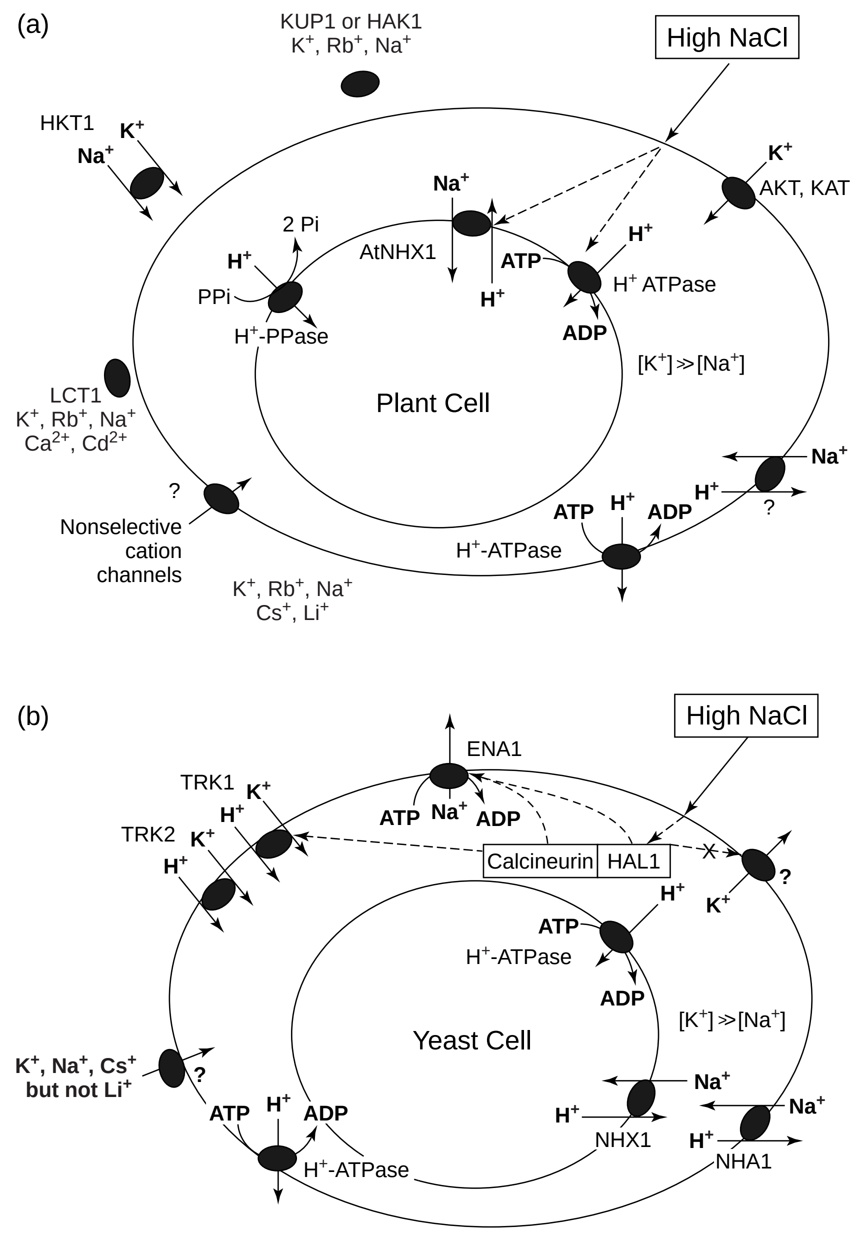

Fig. 3. Models showing the major transport processes that are important for maintaining K$^+$:Na$^+$ homeostasis in the cytoplasm of plants and yeast. (a) Transport mechanisms in a plant cell (see text for details). Some transport mechanisms lack arrows, which indicates that the mode of transport is unknown. Some transport mechanisms are not on a membrane because their membrane location is not known. Salt stress has been shown to increase the expression of *AtNHX1* and H$^+$-ATPase. (b) A yeast cell. TRK proteins are K$^+$ transporters, NHX1 and NHA1 are Na$^+$–H$^+$ antiports and ENA1 is a Na$^+$ pump. Two mechanisms that have not been described at the molecular level are highlighted with '?' and might be involved in Na$^+$ uptake and K$^+$ efflux. Two regulatory factors, calcineurin and *HAL1*, play a role in modulating K$^+$ and Na$^+$ influx and efflux, as indicated by the broken lines. Broken lines do not necessarily indicate a direct effect of the regulatory molecule on the transporter.

Outlook

Our understanding of the molecular basis of K$^+$ transport has led to new insights into how specific K$^+$-uptake mechanisms might be involved in K$^+$:Na$^+$ discrimination in higher plants grown under saline conditions. Yeast and *Arabidopsis* mutants have also provided important models of how plants and cells respond to and regulate specific transport processes in an attempt to control cytoplasmic K$^+$–Na$^+$ homeostasis when faced with an overabundance of extracellular sodium. We need to discover new pieces of the puzzle and to fully comprehend the existing pieces to gain a complete understanding of how and where the discrimination between K$^+$:Na$^+$ occurs in plants.

As our knowledge in this area increases it is probable that plant biologists will be able to engineer plants that can tolerate saline conditions better, by changing the functional characteristics of specific transporters or by modulating cellular response to high salinity. The creation of crop plants that are better adapted to saline, acid and nutrient-depleted soils should aid in sustaining yields in these degraded soils. Enhancing crop yields on marginal soils in both developed and developing countries is an important objective for maintaining the yield increases that are required to feed the rapidly expanding worldwide population.

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technical focus

GFP-based FRET microscopy in living plant cells

The fate and function of biomolecules in living plant cells is a challenging area of plant science. On the one hand, *in vitro* studies on isolated biomolecules are often difficult to extrapolate to *in vivo* function because of the complex organization and high degree of compartmentalization in living plant cells. On the other hand, the *in vivo* study of molecular function is technically demanding, as it requires the simultaneous capability of detecting molecules with high (subcellular) spatial resolution, high sensitivity, high specificity and yet with minimal perturbation of the cell state, in addition to obtaining information about the molecular state or molecular environment. The combination of green fluorescent protein (GFP) technology with fluorescence resonance energy transfer (FRET) microscopy offers these capabilities, and thereby creates new horizons for molecular plant cell biology, particularly in the field of signal transduction.

GFP and chromophore mutants

The GFP from the jellyfish *Aequorea victorea* is a 21 kDa apo-protein that spontaneously folds into a bright-green fluorescing structure. The gene encoding GFP can be expressed in many (non-jellyfish) cell types, enabling the use of GFP as a molecular marker for gene expression.

By fusing the gene encoding GFP with a gene encoding an endogenous protein, and the subsequent expression of the chimera, fluorescent fusion proteins can be produced and targeted to specific subcellular organelles. Consequently, the fate of these fusion proteins and the organelle dynamics can be monitored in living plants at the single cell level with

Box 1. Chromophore mutants of the green fluorescent protein (GFP)[a]

Essential amino acid substitutions, the resulting changed chemistry of the chromophore and the wavelengths for maximal absorbance and fluorescence are indicated.

- **Enhanced green fluorescent protein** (EGFP)
  Ser65 to Thr, Phe64 to Leu.
  Phenolate anion in chromophore.
  Absorbance/emission = 488/509 nm.
- **Yellow fluorescent protein** (YFP)
  Ser65 to Gly, Ser72 to Ala, Thr203 to Tyr.
  Phenolate anion in chromophore with stacked π-electron system (to Tyr203).
  Absorbance/emission = 514/527 nm.
  The addition of Val68 to Leu, Gln69 to Lys to YFP yields a more pH-insensitive (above pH 6.8) enhanced version of YFP with slightly red-shifted spectral properties:
  Absorbance/emission = 516/529 nm (Ref. 15).
- **Blue fluorescent protein** (BFP)
  Tyr66 to His, Tyr145 to Phe.
  Imidazole in chromophore.
  Absorbance/emission = 382/446 nm.
- **Cyan fluorescent protein** (CFP)
  Phe64 to Leu, Ser65 to Thr, Tyr66 to Trp, Asn146 to Ile, Met153 to Thr, Val163 to Ala, Asn212 to Lys.
  Indole in chromophore.
  Absorbance/emission = 434, 452/476, 505 nm.

[a] Some of the chromophore mutants of GFP are known under different names. Therefore, the abbreviations used here refer to chromophore mutant classes. For an overview of GFP chromophore mutants see Ref. 5.

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