Ion Channels and Plant Stress: Past, Present, and Future

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Abstract Perhaps the most significant change in plant electrophysiological studies that began some 25 years ago was a shift in focus from more basic electrical and biophysical properties of plant membranes to pursuing the understanding of the plant physiological and cell biological functions of individual plant ion channel types. In the 1990s, ion channels were characterized as targets of upstream signal transduction mechanisms, and in the later 1990s powerful combined molecular genetics, patch clamp, and plant physiological response analyses further manifested the importance of ion channels for many biological and stress responses of plants. Essential metals and ions in the intracellular and intraorganellar spaces of plant cells contribute to the activities of regulatory proteins, signal transduction, and to the maintenance of turgor pressure, osmoregulation, toxic metal chelation, and membrane potential control. A large number of studies on mineral nutrition have sustained the profitable cultivation of plant growth and development, and provided important knowledge on plant physiological mechanism of absorption of minerals from soils. Abiotic stress and biotic stresses are a global problem for plant growth in agricultural and noncultivated lands. Ion channels in plant cells play crucial functions in adapting to and overcoming abiotic and biotic stresses. Plant membrane transport systems play an important role not only in the uptake of nutrients from the soil but also in the adaptation to stress and environmental change.

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A revolution has taken place in the understanding of cell physiological, biophysical, molecular, and interaction network properties of plant ion channels and transporters as summarized here. However, many exciting and stimulating questions remain open to discovery, promising that research on plant ion channels will continue to be a vibrant area of research for many years to come.

1 Introduction

Among plant nutrients, potassium or K^+, is the most abundant cellular cation controlling cellular homeostasis, plant movements, cell expansion, guard cell turgor, membrane potentials, and many other processes. Potassium ions also counteract toxic effects of cations such as sodium (Na^+). Potassium transport properties have served as a classical model for understanding mechanisms of plant ion transport (Epstein et al. 1963). Studies indeed show that principles learned from K^+ transport and K^+ channel analyses can be applied to other transport systems.

Characterization of ion channel functions in plant stress responses led to the formulation of models of how multiple ion channels and transporters can function together in mediating a response. Studies in guard cells led to an early model for the interplay of a network of ion channels and proton ATPases in mediating stomatal opening and closing (Shimazaki et al. 1986; Schroeder and Hagiwara 1989; Schroeder and Hedrich 1989; Thiel et al. 1992; Lemtiri-Chlieh and MacRobbie 1994; Ward and Schroeder 1994; Davies and Sanders 1995; Blatt et al. 1999). Remarkably, studies of rapid changes in plant pathogen responses and other rapid stimulus-responses show ion transport behaviors that, at least in general terms, show similarity to those mediating stomatal closing (Nurnberger et al. 1994; Jabs et al. 1997). In guard cells, cytosolic Ca^{2+} activates anion efflux channels and inhibits K^+ uptake channels (Schroeder and Hagiwara 1989), which together with Ca^{2+} inhibition of plasma membrane proton pumps (Kinoshita et al. 1995) causes anion and K^+ efflux and depolarization of the plasma membrane to reduce the turgor pressure of guard cells.

Calcium (Ca^{2+}) concentrations are tightly controlled at low submicromolar concentrations in the cytosol. Increases in Ca^{2+} concentrations and stimulus-induced enhancement in Ca^{2+} sensitivity (Young et al. 2006) function as an effective signal which modulates calcium-binding proteins thus transmitting signals in signal transduction pathways. Ion channels that mediate Ca^{2+} influx into the cytosol from the extracellular space and from organelles have been characterized in electrophysiological studies (Miller et al. 1990). However, the genes encoding these ion channels still remain mostly uncharacterized in plant cells, probably due to the presence of large gene families with overlapping functions (Shimazaki et al. 1986; Blatt 2000; Ward et al. 2009). Anion channels in the plasma membrane have also emerged as major mechanisms regulating signal transduction and ion transport. Two types of

anion channel currents (slow (S)-type and rapid (R)-type) have been characterized extensively in guard cells and in hypocotyl cells (Keller et al. 1989; Schroeder and Hagiwara 1989; Marten et al. 1992; Colcombet et al. 2001), and genes encoding the anion conducting subunits of slow-type anion channels have been identified using *Arabidopsis* mutants (Negi et al. 2008; Vahisalu et al. 2008). Recent genetic approaches have led to identification of two additional gene families that encode anion conducting channel subunits that play major roles in aluminum resistance (Sasaki et al. 2004; Furukawa et al. 2007; Magalhaes et al. 2007). Yet another class of proteins exists, which shows similarity to mammalian chloride channels, AtCLCs. Functional characterization of the AtCLCa membrane protein showed that it encodes a nitrate-proton exchanger in the vacuolar membrane, rather than an anion channel (De Angeli et al. 2006) and additional CLC proteins are targeted to other organelle membranes (Marmagne et al. 2007).

In this chapter, we will provide an overview of the classes of different ion channels that have been characterized and their underlying gene families. In several cases, we discuss examples of their physiological functions in guard cells as well as in other cell and tissue types. The relevance of these channels in stress responses in many plant cell types is discussed throughout this book. Figures 1 and 2 summarize progress over the past 25 years in the identification of plant ion channel classes, technical advances, and major genes encoding plant membrane transport systems. Figure 1 exemplifies the accelerating pace of discovery in this thriving field.

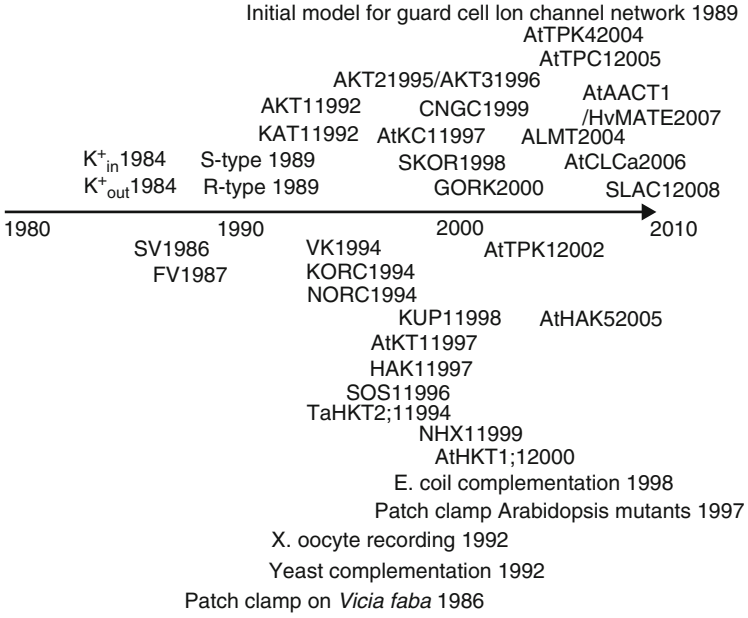

Fig. 1 Time-line of progress on the identification of individual plant ion channel classes, the genes encoding these plant ion transporters, and introduction of new techniques

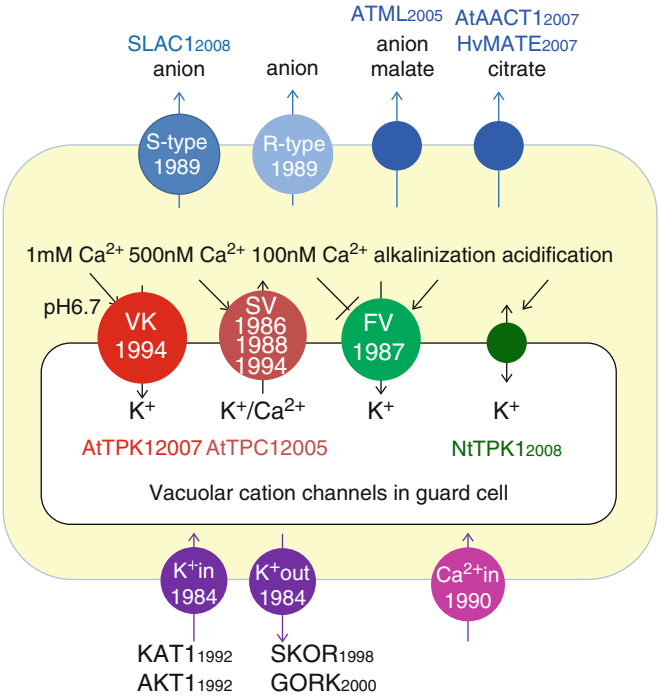

Fig. 2 Simplified scheme of several of the cation and anion channels in the plasma membrane and in the vacuolar membrane of plant cells, which were identified and characterized in patch clamp studies. Genes encoding some of these ion channels have been cloned and characterized (see text)

2 Plasma Membrane K^+ Channels in Guard Cells

The first characterizations of single plant ion channels were reported in 1984 in the analyses of leaf cells (Moran et al. 1984) and guard cells (Schroeder et al. 1984). These successful applications of patch clamp techniques for the measurement of plant ion channels opened the door to electrophysiological characterizations of ion channels in plant membranes of land plant cells, which are usually orders of magnitude smaller than the classically analyzed giant algae cells (Curtis and Cole 1938; Tazawa 1968, 1972). They also reported the measurement of sodium, potassium, and chloride ions in protoplasm of algal cells, which may be more difficult to measure than plant cells. Two major classes of voltage-dependent K^+ channels were characterized in guard cells; hyperpolarization-activated "inward-rectifying" K^+ channels and depolarization-activated "outward-rectifying" K^+ channels (Schroeder et al. 1984, 1987; Blatt 1988). Inward-rectifying K^+ channels are activated by hyperpolarization via electrogenic proton pumps controlled by blue light signals (Assmann et al. 1985; Shimazaki et al. 1986). The opening of stomatal pores is regulated by the accumulation of K^+ in guard cells. Both inward- and

outward-rectifying K^+ channels were proposed to contribute to the physiological transport of K^+ into and out of guard cells during stomatal movements (Schroeder et al. 1987). Subsequent studies in many different plant cell types including coleoptiles, root hair cells, aleurone, root cortex, and xylem parenchyma cells showed that these types of K^+ channels are widely distributed and were proposed to have important functions in K^+ transport and membrane potential control (Bush et al. 1988; Kourie and Goldsmith 1992; Gassmann and Schroeder 1994; Wegner and Raschke 1994; Maathuis et al. 1997; de Boer and Volkov 2003).

2.1 Characterization of K^+ Channel and Transporter cDNAs

In 1992, two distinct K^+ channel genes, KAT1 and AKT1, were isolated from *Arabidopsis thaliana* by complementation of K^+ uptake deficient yeast mutants (Anderson et al. 1992; Sentenac et al. 1992). Both genes encode six putative transmembrane regions and a predicted voltage sensor domain, and resemble Shaker K^+ channels in *Drosophila* neurons. For the isolation of these genes, both groups used yeast mutants which are unable to grow at low concentrations of K^+ in the medium. The use of yeast expression systems provides a powerful method for isolation of channel and transporter cDNAs and for structure-function analyses of these transporters (Frommer and Ninnemann 1995; Uozumi et al. 1995; Hoth et al. 1997; Nakamura et al. 1997).

Electrophysiological characterization of the KAT1-encoded protein in *Xenopus* oocytes showed that KAT1 functions as a heperpolarization-activated K^+ channel (Schachtman et al. 1992). Thus these studies led to the first isolation and characterization of eukaryotic inward-rectifying K^+ channel genes (Anderson et al. 1992; Schachtman et al. 1992; Sentenac et al. 1992), as hyperpolarization-activated K^+ channels genes had not yet been identified in animal genomes (Kubo et al. 1993; Ward et al. 2009).

AKT1 expression in *Xenopus* oocytes failed to show ion channel activities, but insect cells (Sf9 cell line) expressing AKT1 displayed an inwardly rectifying K^+ conductance (Gaymard et al. 1996). Other types of *Arabidopsis* K^+ channel genes have been isolated after this; a weakly inward-rectifying K^+ channel, AKT2 (Cao et al. 1995; Ketchum and Slayman 1996), depolarization-activated K^+ channels, SKOR and GORK (Gaymard et al. 1998; Ache et al. 2000), and a silent channel, AtKC1 which is likely to modulate other K^+ channels (Dreyer et al. 1997; Reintanz et al. 2002). The role of the silent regulatory subunit has been confirmed for the carrot AtKC1 homolog, KDC1 (Bregante et al. 2008). The cytosolic regulatory components, calcineurin B-like proteins (CBLs), and CBL-interacting protein kinases (CIPKs) are closely associated with several ion channels and transporters that function in adaptation to salinity or ion stress in plant cells. The complex of CBL1/CIPK23 directly controls AKT1-mediated K^+ uptake in roots and enhances K^+ uptake when ambient K^+ becomes deficient (Li et al. 2006; Xu et al. 2006).

Interestingly, *Escherichia coli* was shown to be another heterologous expression system suitable for functional expression of both plasma membrane-located and

organelle membrane-located plant channels/transporters (Uozumi 2001). Using this system, K^+ uptake activities of KAT1, AKT2, HKT-type transporters, and KUP-type transporters were measured (Kim et al. 1998; Uozumi et al. 2000; Uozumi 2001). Moreover, the transmembrane topologies of the *Shaker*-type K^+ channel KAT1 and the Na^+/K^+ transporter, HKT1 (TaHKT2;1) were determined by means of a bacterial alkaline phosphatase fusion approarch (Kim et al. 1998; Uozumi et al. 1998, 2000; Kato et al. 2001; Uozumi 2001).

KUP/HAK/KT genes encode a separate class of important plant K^+ uptake transport proteins and were isolated after earlier genomic EST sequencing showed plant isoforms with homology to *E. coli* Kup and yeast HAK transporters (Quintero and Blatt 1997; Santa-Maria et al. 1997; Fu and Luan 1998; Kim et al. 1998). The *Arabidopsis* genome sequence shows the presence of 13 genes KUP/HAK/KT genes in the *Arabidopsis thaliana* genome (Mäser et al. 2001; Ahn et al. 2004), and the physiological role of AtKUP4 and AtHAK5 has been reported (Rigas et al. 2001; Gierth et al. 2005). AKT1 and AtHAK5 likely together mediate K^+ uptake from soil. The transport mechanism by which these KUP/HAK/KTs mediate K^+ uptake into plants cells remains unknown (Maathuis and Sanders 1994). An important question for future research will be the characterization of the interplay of several different K^+ transporter/channel classes in mediating K^+ transport.

3 Critical Roles of Plasma Membrane Anion Channels in Plant Stress Responses

Stomatal closing is mediated by the release of ions and organic solutes from guard cells. Electrophysiological studies led to a model for the mechanisms that can drive K^+ release from guard cells. Electrophysiological research on outward-rectifying K^+ channels indicated that inhibition of proton pumps would not suffice for depolarization-activation of K^+ channels (Schroeder et al. 1987; Schroeder 1988). Elevation of the cytosolic Ca^{2+} concentration in guard cells led to the activation of a novel class of plant ion channels – S-type anion channels (Schroeder and Hagiwara 1989). Due to the electrochemical gradient of anions across the plasma membrane of guard cells, activation of anion channels causes anion efflux leading to depolarization. Anion channels were therefore proposed as drivers of ion efflux, thus controlling stomatal closing (Schroeder and Hagiwara 1989). Further research revealed additional types of anion channel in guard cells with properties different from those of S-type anion channels (Keller et al. 1989). These so-called R-type anion channels can also mediate anion efflux leading to stomatal closing.

Anion channels in guard cells are permeable to chloride, nitrate, sulfate, and malate (Keller et al. 1989; Schroeder and Hagiwara 1989; Schmidt and Schroeder 1994). Patch clamp analyses of the plasma membrane of *Vicia faba* guard cells revealed that these two types of anion channel conductances coexist in the membrane (Schroeder and Keller 1992). R-type anion channels are characterized as rapidly activating with kinetics that are time- and voltage-dependent and that show

inactivation (Keller et al. 1989; Hedrich et al. 1990). The other class of depolarization activated anion channels exhibits extremely slow voltage dependent activation and deactivation properties – the S-type anion channels (Schroeder and Hagiwara 1989; Schroeder and Keller 1992). It has been proposed that R-type and S-type anion channels may be encoded by the same channel protein (Linder and Raschke 1992), despite their relatively significant differences in some biophysical and regulatory properties. The plant hormone abscisic acid, which is induced in response to drought stress, activates both S-type and R-type anion channels (Grabov et al. 1997; Pei et al. 1997, 1998; Raschke 2003; Raschke et al. 2003; Roelfsema et al. 2004). S-type and R-type anion channels have also been characterized in hypocotyl cells of *Arabidopsis* and were also shown to co-exist in the same cells (Colcombet et al. 2005). Studies in the *Arabidopsis* hypocotyls also suggested that these two anion channels can be clearly distinguished in these cells (Colcombet et al. 2005). Nevertheless, it is possible that these two very different anion currents share molecular components (Raschke 2003).

4 Roles of Anion Channels in Stress Responses and Identification of Anion Channel Gene Families

SLAC1 (slow anion channel-associated 1) encodes a homologue of bacterial dicarboxylate/malic acid (C4-dicarboxylate) transport proteins and was identified as an S-type slow anion channel (Vahisalu et al. 2008). The plasma membrane protein SLAC1 plays an essential role in stomatal closure in response to CO_2, ABA, ozone, darkness, humidity reduction, Ca ions, hydrogen peroxide, and nitric oxide (Negi et al. 2008; Vahisalu et al. 2008). Loss-of-function mutations in *SLAC1* are accompanied by an overaccumulation of osmoregulatory anions in guard cell protoplasts (Negi et al. 2008). T-DNA insertion and point mutations in the *SLAC1* gene led to abrogation of S-type anion channels in guard cells (Vahisalu et al. 2008). Interestingly however, R-type anion channels were intact in *slac1* mutant guard cells. SLAC1 shows homology to a yeast and a bacterial malate transporter. The permeability of S-type anion channels to anions and the increased trapping of malate in *slac1* guard cells suggest that SLAC1 encodes the anion conducting subunit of S-type anion channels (Negi et al. 2008; Vahisalu et al. 2008). *Slac1* mutants provide strong evidence for the model that anion channels represent central mechanisms in mediating stomatal closing. Interestingly, a different type of malate transporter, AtABC14 has been identified as a malate import protein mediating malate uptake from the cell wall into guard cells (Lee et al. 2008) and thus distinct channels and transporters are now known that mediate anion efflux and uptake in guard cells.

Aluminum is the third most abundant element in the Earth's crust. In acidic soils aluminum (Al^{3+}) is solubilized and Al^{3+} is toxic to plants. However, plants release organic acids, including malate and citrate from their roots, to chelate free aluminum (Al^{3+}) in acidic soil (Ma et al. 2001; Kochian et al. 2004). Al^{3+} activates anion

channels in the plasma membrane of wheat roots (Ryan et al. 1997). Genes were identified in genetic studies and named *ALMTs* for Al^{3+}-activated malate transporters, since they play important roles in this Al^{3+} resistance response (Sasaki et al. 2004). ALMT expression in *Xenopus* oocytes is sufficient for Al^{3+}-activated anion channels, showing that ALMTs appear to function as a type of Al^{3+} receptor (Pineros et al. 2008). TaALMT1 mediates transport of malate, and to a lesser extent nitrate/chloride based on electrophysiological measurements (Pineros et al. 2008; Zhang et al. 2008).

Furthermore, Al^{3+}-activated citrate transporters (HvAACT1) (Furukawa et al. 2007) and (SbMATE) (Magalhaes et al. 2007) belong to the multidrug resistance transporter family and also function in aluminum tolerance in acid soils. The Al^{3+} resistance-associated anion transporters show no homology to the above SLAC1 anion channel from guard cells.

In animals, chloride channels of the ClC family have been characterized. Bacterial CLC homologues however function as 2 $Cl^-/1H^+$ exchangers (Accardi and Miller 2004; Picollo and Pusch 2005; Miller 2006). The functions of the homologous genes in *Arabidopsis* and tobacco have largely remained unknown (Hechenberger et al. 1996; Lurin et al. 1996). However, in 2006 the AtCLCa transporter was characterized as a NO_3^-/H^+ exchanger in the vacuolar membrane of *Arabidopsis* cells (De Angeli et al. 2006). *Atclca* knockout mutants provide evidence that AtCLCa functions in nitrate accumulation into vacuoles in *Arabidopsis thaliana* (Geelen et al. 2000). AtCLCd and AtCLCe are targeted to the thylakoid membranes in chloroplasts and AtCLCf was localized in Golgi membranes (Marmagne et al. 2007). Further studies on the subcellular localizations of AtCLCs may illuminate intracellular anion transport mechanisms in plant cells.

5 Ca^{2+} Channels and Intracellular Ca^{2+} Elevations

Stimulus-induced changes in the Ca^{2+} concentration in the cytoplasm of plant cells are triggered by many diverse stimuli (Hetherington and Brownlee 2004). Intracellular Ca^{2+} concentration changes in guard cells were identified using fluorescent Ca^{2+} indicators, Fura-2 (McAinsh et al. 1990; Schroeder and Hagiwara 1990), and Fluo-3 (Gilroy et al. 1990). Patch clamp analyses showed the presence of Ca^{2+}-permeable channels in the plasma membrane of guard cells (Schroeder and Hagiwara 1990; Hamilton et al. 2000; Pei et al. 2000). ABA-induced intracellular Ca^{2+} elevations have been extensively studied (Allan et al. 1994; Grabov and Blatt 1998; Allen et al. 1999a; Staxen et al. 1999). The pH-independent, green florescent protein-based Ca^{2+} indicators yellow cameleon 2.1 and 3.6 were applied for monitoring cytoplasmic free Ca^{2+}, $[Ca^{2+}]_{cyt}$, in *Arabidopsis thaliana* (Allen et al. 1999b; Miyawaki et al. 1999; Yang et al. 2008). Studies using low concentration cameleon or fura2-based Ca^{2+} reporters have revealed that repetitive spontaneous Ca^{2+} transients occur in plant cells (Grabov and Blatt 1998; Allen et al. 1999a; Staxen et al. 1999; Wais et al. 2000; Young et al. 2006; Yang et al. 2008). Furthermore, experimentally imposing Ca^{2+} oscillations, by repetitive depolarizations and

hyperpolarizations of the plasma membrane, showed that independent of the Ca^{2+} elevation pattern, Ca^{2+}-induced a rapid stomatal closure which was named the "Ca^{2+} reactive" stomatal closing response (Allen et al. 2001). In addition to this Ca^{2+} reactive response, it was revealed that the pattern of experimentally-induced $[Ca^{2+}]_{cyt}$ elevations controls the ability of stomata to re-open after the initial stomatal closing response, even when the $[Ca^{2+}]_{cyt}$ elevations are terminated (Allen et al. 2001; Li et al. 2004). This long-term Ca^{2+} pattern inhibition of re-opening of stomatal pores, was named the "Ca^{2+} programmed" response and is impaired in glutamate receptor overexpressing guard cells (Cho et al. 2009). Thus $[Ca^{2+}]_{cyt}$ oscillation kinetics in guard cells can function in maintaining steady-state stomatal closing. Organelles in plant cells serve as intracellular stores for Ca^{2+}. A Ca^{2+} sensing receptor, CAS, was isolated via a functional expression screening approach using heterologous expression (Han et al. 2003). Recent work shows that CAS1 is localized in thylakoid membranes (Nomura et al. 2008; Weinl et al. 2008) and functions in extracellular Ca^{2+}-induced, transient cytosolic Ca^{2+} increases, which lead to stomatal closure (Han et al. 2003; Nomura et al. 2008; Weinl et al. 2008).

6 Gene Candidates for Plasma Membrane Ca^{2+} Channels

Several classes of Ca^{2+} permeable channels have been characterized in the plasma membrane of plant cells, including depolarization-activated Ca^{2+} channels (Thuleau et al. 1994a, b; Miedema et al. 2008) and hyperpolarization-activated Ca^{2+} influx channels (Gelli and Blumwald 1997; Hamilton et al. 2000; Pei et al. 2000; Demidchik et al. 2002). In general, plant Ca^{2+} channels are not entirely Ca^{2+} selective but also show permeabilities to other cations (Schroeder and Hagiwara 1990; Thuleau et al. 1994a, b; Pei et al. 2000; Demidchik et al. 2002). However, the genes encoding plasma membrane Ca^{2+} channels remain less well-clarified. Two gene families are likely to provide possible candidates. One family includes 20 genes in the *Arabidopsis* genome and encodes homologs to "ionotropic" glutamate receptors, which encode receptor ion channels in animal systems (Lam et al. 1998; Kim et al. 2001). Research has shown that glutamate application to roots causes $[Ca^{2+}]_i$ elevations that are disrupted in knock-out mutants in the *Glr3.3*, glutamate receptor gene (Qi et al. 2006). A second candidate family of plant Ca^{2+} permeable channels is cyclic nucleotide-gated channel homologs. In *Arabidopsis*, 20 different cyclic nucleotide-gated channel genes (*CNGCs*) are present, and several individual channels have been analyzed. Voltage dependent K^+ channels, including KAT1 and AKT1 have corresponding cyclic nucleotide binding sites in the C-terminal regions (Hoshi 1995). However, CNGC channels do not include the typical "GYG" K^+ selectivity signature sequence of K^+ channels (Ward et al. 2009). Studies analyzing CNGC functions after heterologous expression in yeast indicate that they may encode Ca^{2+} permeable channels (Kohler et al. 1999; Leng et al. 1999), although this may not apply to all members of the CNGC family. Genetic analysis showed that both AtCNGC11 and AtCNGC12 are positive mediators of resistance signaling pathways activated by pathogen infection (Yoshioka et al. 2006). Future research

into the physiological functions of this large gene family may reveal new and unexpected ion channel functions.

7 Properties of Vacuolar Cation Channels

Plant vacuoles often take up more than 90% of the cell volume, and thus the channels mediating K^+ transport across the vacuolar membrane (tonoplast) may be of relevance to cell volume regulation and storage of this nutrient. Three classes of cation channel, SV (Slow Vacuolar), VK (Vacuolar K), and FV (Fast Vacuolar), have been named based on the endogenous K^+ channel activities identified by patch clamp studies. FV channels mediate K^+ transport at very low concentrations of cytosolic Ca^{2+} (Hedrich and Neher 1987; Allen and Sanders 1996). SV channels are activated by elevation in the cytosolic Ca^{2+} concentration (Hedrich and Neher 1987; Pei et al. 1999). SV channels were initially reported to be anion permeable channels (Hedrich et al. 1986). However, later studies revealed that SV channels are Ca^{2+} permeable cation channels that do not significantly conduct anions (Ward and Schroeder 1994; Ward et al. 1995; Allen and Sanders 1996). A third class of vacuolar cation channels are the Ca^{2+}-activated channels, named VK channels, which are highly K^+ selective channels (Ward and Schroeder 1994). The determination of genome sequences of *Arabidopsis* and reverse genetic approaches have led to the identification of the genes encoding SV channels (Peiter et al. 2005) and VK channels (Gobert et al. 2007). The AtTPC1 protein is targeted to the vacuolar membrane and these proteins encode SV channels (Peiter et al. 2005). The genes encoding two-pore K^+ channels (TPKs) include two repeats of membrane-pore-membrane domains (Czempinski et al. 1997, 2002; Kaplan et al. 2007). AtTPK1, 2, 3, and 5 are tonoplast K^+ channels (Voelker et al. 2006), whereas AtTPK4 is located in the plasma membrane (Becker et al. 2004). AtTPK1 was shown to encode the VK channel (Gobert et al. 2007). Functional characterization of NtTPK1, located in tobacco tonoplasts, shows K^+ currents induced by cytosolic acidification, indicating the presence of other types of vacuolar K^+ channels that differ from the above vacuolar channel types (Hamamoto et al. 2008).

8 Sodium Transport Systems in Plants

Sodium (Na^+) is not categorized as an essential nutrient in higher plants, and excessive Na^+ leads to detrimental effects on plant growth. Several distinct classes of Na^+ transporters mediate Na^+ homeostasis (Fig. 3). After Na^+ entry into the cytoplasm of root cells, Na^+ is loaded into the xylem (de Boer 1999). The presence of a Na^+/H^+ exchange activity at the xylem/symplast interface of soybean roots (Lacan and Durand 1996) and Na^+-permeable nonselective ion channels in the plasma membrane of barley root xylem parenchyma cells (NORC) (Wegner and

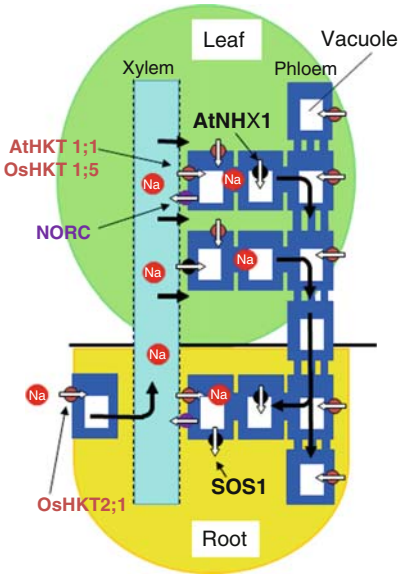

Fig. 3 Simplified model for mechanisms of Na^+ absorption, recirculation, and extrusion by different classes of Na^+ channels/transporters, including Na^+ loaded into xylem vessel by non-selective outwardly rectifying cation conductance, NORC (Wegner and De Boer 1997), Na^+ influx mediated by HKT transporters (Uozumi et al. 2000; Mäser et al. 2002a; Sunarpi et al. 2005), plasma membrane Na^+ extrusion via SOS1 antiporters (Shi et al. 2000), and tonoplast Na^+ sequestration by NHX antiporters (Apes et al. 1999). AtHKT1;1, and OsHKT1;5 are present in the plasma membrane of xylem parenchyma cells, and mediate unloading of Na^+ from xylem vessels into xylem parenchyma cells, thus protecting leaves from Na^+ overaccumulation and Na^+ damage (leaf Na^+ exclusion) (Berthomieu et al. 2003; Sunarpi et al. 2005; Ren et al. 2005). In the case of K^+ starvation in soils, rice roots take up Na^+ at low extracellular Na^+ levels via OsHKT2;1 (Horie et al. 2007). Na^+ is sequestered in vacuoles by AtNHX1. Excessive Na^+ in the cytosol is transported out of cells by SOS1

De Boer 1997) and in wheat and Arabidopsis root cortex and epidermis (NSC) (Tyerman et al. 1997; Buschmann et al. 2000; Davenport and Tester 2000; Demidchik and Tester 2002) have been reported.

The exclusion of Na^+ from plant cells and the sequestration of Na^+ in vacuoles alleviate sodium stress under saline conditions. The plasma membrane Na^+/H^+ antiporter named SOS1 (Shi et al. 2000), was identified in an *Arabidopsis* mutant, *sos1*, that shows a salt oversensitive phenotype (Wu et al. 1996). SOS1-mediated Na^+/H^+ transport activity is modulated by a Ca^{2+} sensor/protein kinase complex CBL4 (SOS3)/CIPK24 (SOS2) (Wu et al. 1996; Shi et al. 2002; Zhu 2002). Na^+/H^+ antiporters were also identified which are targeted to the vacuole-membrane. The first functionally-characterized member of this gene family, AtNHX1, contributes to Na^+ and monovalent cation sequestration in plant vacuoles. Overexpression of AtNHX1 was shown to increase salt tolerance in *Arabidopsis* (Apse et al. 1999).

In contrast to these Na^+ transporters that remove Na^+ from the cytoplasm, molecular identification of plasma membrane Na^+ influx systems into plant cells has also been achieved. Na^+ uptake transporters in wheat HKT1 also named, TaHKT1 (TaHKT2;1) (Schachtman and Schroeder 1994; Rubio et al. 1995; Gassmann et al. 1996) and in *Arabidopsis thaliana* AtHKT1 (AtHKT1;1) were identified (Uozumi et al. 2000). The first HKT gene, TaHKT1 (TaHKT2;1), was originally cloned from wheat and shown to mediate K^+ and Na^+ co-transport in yeast and *Xenopus* oocytes (Schachtman and Schroeder 1994; Rubio et al. 1995; Gassmann et al. 1996). Further extensive studies on HKT structure and function demonstrated that HKTs include 4 domains that resemble the K^+ permeation pore of a K^+ channel tetramer (Durell et al. 1999; Kato et al. 2001; Mäser et al. 2002a; Tholema et al. 2005; Gambale and Uozumi 2006) and HKT transporters have indeed been proposed to mediate channel-like transport (Gassmann et al. 1996; Corratge et al. 2007). Note that the term, transporter or channel has been used interchangeably for HKT transporters, and HKTs provide an interesting model to explore the shrinking distinctions between co-transporters and ion channels. Whereas some HKT transporters change their K^+ and Na^+ selectivities depending on the ionic conditions, similar to multi-ion channel pores (Schachtman and Schroeder 1994; Rubio et al. 1995; Gassmann et al. 1996; Horie et al. 2001), the only HKT transporter encoded in the *Arabidopsis* genome, AtHKT1, was found to be more Na^+ selective (Uozumi et al. 2000). Further studies showed that HKT transporters fall into either of these two cation selectivity HKT subfamilies (Horie et al. 2001, 2006). Research identified an amino acid residue that contributes to the distinction of these two cation selectivities of HKT transporters: AtHKT1;1 has a Ser instead of Gly in the first pore loop region which reduces K^+ selectivity. In contrast, TaHKT1 lacks this residue and is more Na^+ selective (Durell et al. 1999; Mäser et al. 2002a; Tholema et al. 2005; Gambale and Uozumi 2006). The nomenclature of HKT transporters cloned from various plants has been divided into two distinct groups, which also largely separate these subfamilies by their Ser or Gly in the selectivity filter, with the exception of OsHKT2;1 (Horie et al. 2001). Bacterial HKT homologs, Trk, or Ktr transporters, function as major K^+ uptake systems (Gaber et al. 1988; Ko et al. 1990; Schlosser et al. 1995; Nakamura et al. 1998; Matsuda et al. 2004). K^+ uptake is stimulated by Na^+ in the cyanobacterial Ktr homologues of this family and significantly contributes to adaptation to hyperosmolar shock (Matsuda et al. 2004).

The question why plants express Na^+ selective Na^+ influx transporters such as AtHKT1;1 remained. Null mutations or those that reduce activity in the Na^+ transporter AtHKT1;1 (Mäser et al. 2002b; Gong et al. 2004; Berthomieu et al. 2003) resulted in Na^+ overaccumulation in leaves of these plants. The AtHKT1;1 transporter was immuno-localized in the plasma membrane of xylem parenchyma cells (Sunarpi et al. 2005). The Na^+ hypersensitive phenotype of *Athkt1;1* mutants (Mäser et al. 2002b) is due to the lack of Na^+ retrieval from xylem vessels by AtHKT1;1, leading to toxic Na^+ overaccumulation in leaves (Sunarpi et al. 2005). Mapping of a salt tolerance quantitative trait locus (QTL) from rice led to the isolation of OsHKT1;5, which is expressed in xylem parenchyma cells (Ren et al. 2005) and thus AtHKT1;1 and OsHKT1;5 have analogous functions in Na^+

retrieval from the xylem sap (Ren et al. 2005; Sunarpi et al. 2005). Interestingly, this HKT transporter-mediated exclusion of Na^+ accumulation in *Arabidopsis* and rice leaves via Na^+ removal from the xylem, has more recently been found to be the underlying mechanism of three major salinity tolerance QTLs in wheat (Byrt et al. 2007), providing an example of transfer of knowledge from model plants such as *Arabidopsis* and rice (Uozumi et al. 2000; Mäser et al. 2002b; Ren et al. 2005; Sunarpi et al. 2005), to applications in the field.

In contrast to the above discussed sodium toxicity at high Na^+ concentrations, low concentrations of Na^+ (e.g. < 5 mM) support growth of many plant species when K^+ is deficient. The Na^+ transporter OsHKT2;1 (previously named OsHKT1) is strongly induced in rice roots in response to K^+ starvation (Horie et al. 2001). Three loss of function mutant lines in *OsHKT2;1* exhibited substantial reduction in Na^+ influx into plant roots, showing that rice plants use Na^+ as a nutrient in the medium for their survival and growth under K^+ starvation and low Na^+ conditions (Horie et al. 2007). Thus several classes of Na^+ transporters and exchangers exist in plants and each class has unique roles in mediating sodium tolerance.

9 Future Prospects

Starting 25 years ago the study of plant transport moved into the era of identifying and characterizing individual ion channels and transporters. Such studies have benefited from several independent technical innovations including patch clamping, heterologous expression in yeast, oocytes, *E coli* and animal cells, ion sensitive fluorophores for imaging, biophysical structure-function analyses, forward and reverse genetic analyses, and the sequencing of reference plant genomes. However, the genes encoding some of the known channels/transporters remain to be identified. Additional approaches will aid in their identification including genetic studies of natural variation, systems biology, in silico analyses and proteomics. Abiotic stress and biotic stress continuously influence the plant body. Plants have developed an adaptive response to them; for example, reactive oxygen species have been used as intracellular and extracellular signals, which regulate membrane transport system, and coregulate Ca^{2+} signaling (McAinsh et al. 1996; Pei et al. 2000; Foreman et al. 2003; Demidchik et al. 2007).

Interestingly, almost every characterized plant ion channel and transporter class was found to have unique and intriguing properties, which have required new concepts and interdisciplinary analyses for their characterizations. These unique properties are often intimately related to their physiological functions and remain a basis for further analyses in the future. These advances are also contributing to the derivation of fundamental principles on the relationship of channels and transporters in all organisms. Moreover, many of the identified plant ion channels and transporters are linked to major environmental stresses that are directly relevant for the challenges facing humanity in the present century, including drought resistance, desiccation

avoidance, salt tolerance, aluminum resistance, pathogen responses, and water use efficiency. These pressing global needs will require further creative, interactive, and dynamic research efforts by the community of plant ion transport researchers. In particular, new knowledge will lead to the selection and generation of elite crops.

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