Plant Molecular Biology **26**: 1637–1650, 1994.
© 1994 *Kluwer Academic Publishers. Printed in Belgium.*

Green circuits – The potential of plant specific ion channels

Rainer Hedrich* and Dirk Becker
*Institut für Biophysik, Herrenhäuser Strasse 2, 30419 Hannover, Germany (*author for correspondence)*

Received 11 October 1994; accepted in revised form 11 October 1994

Introduction

In 1983 when visiting the German Botanical Congress in Vienna to present out initial studies on the identification of the first plant K^+ channel [73], the previous speaker finished his talk with the conclusion that unlike animals which use channels like drums, plants are more sophisticated since like playing a melody on a piano they are able to bring various carrier types and pumps into play.

In the light of the rapid progress in the field of plant membrane transport/biology during the past decade both statements turned out to be incorrect:

1) both animal and plant cells take advantage of ion channels, carriers, and pumps and
2) plant and animal K^+ channels are structurally closer related than was ever expected [93].

Because of this similarity on one hand and the presence of action potentials in both branches of the evolutionary tree on the other, one might suggest that with respect to function a common set of membrane elements has been evolved. The 'green circuits', however, differ anatomically from their animal counterparts and with regard to their composition in electrogenic elements. Although higher plants contain nerves, they are generally not concerned with the transmission of action potentials. These transport elements mediate the long distance water and nutrients supply of the various plant tissues such as roots, shoot, leaves, flowers, and developing buds.

The first models which have been constructed following electrophysiological recordings from 'sensitive' plants like *Mimosa pudica*, *Samanea saman*, or *Dionaea muscipula* to describe the peculiar fast transmission of electrical signals initiated at the site of stimulation spreading out all over the plant were applied to other plants, including vegetables. These models generated according to that of Hodgkin and Huxley [36] on the ionic basis of action potentials and its propagation within nervous systems involve conductive elements and shields. In nerve cells the cable is presented by the intracellular/-axonal electrolytes. The plasma membrane isolating the conductive cytoplasm is equipped with ion channels often located at specialized regions such as the nodes of Ranvier along the axon. Activation of voltage-dependent ion channels allows the permeation of charged molecules along their electrochemical gradients and thus propagation of an electrical signal.

In 1984 ion channels have been discovered in plants, too [54, 73]. Since then, however, these electroenzymes have neither been correlated to conductive macrostructures like the xylem or phloem nor have clusters of extremely high channel density been described in excitable plant cells.[1] Therefore four questions are still matter of debate:

1. Do electrical signals travel along the xylem and/or phloem or are other not yet defined structures involved?
2. Do isolators, functional equivalent to Schwann cells, such as polymeres (wax or

[1] Evidence for a high anion channel density has recently been reported for *Chara*, a giant green alga [96].

suberines) shield entire excitable plant cells or tissues to form a cable-like structure?
3. Is excitability in animals and plants founded by a similar set of ion channels or is there evidence for 'green circuits'?
4. Besides excitability, are 'green' ion channels required to fulfil plant specific tasks?

Summarizing the progress in the field of plant ion transport, this review will concentrate on 'green circuits' and plant specific ion channels or properties which trigger them.

A. The plant action potential

In contrast to animal cells Na^+ does not play a fundamental role in excitability; for non-halophytes Na^+ is even toxic with respect to growth and development. Instead of using Na^+ channels to depolarize the plasma membrane the 'green circuits' take advantage of voltage-dependent anion channels [7]. Furthermore, the uptake of sugars and amino acids or even ions in cotransport with Na^+ in their 'red' counterparts, is coupled in plants to the free enthalpie of the H^+ gradient [66, 23, 46, 99, 58, 69]. In the context of

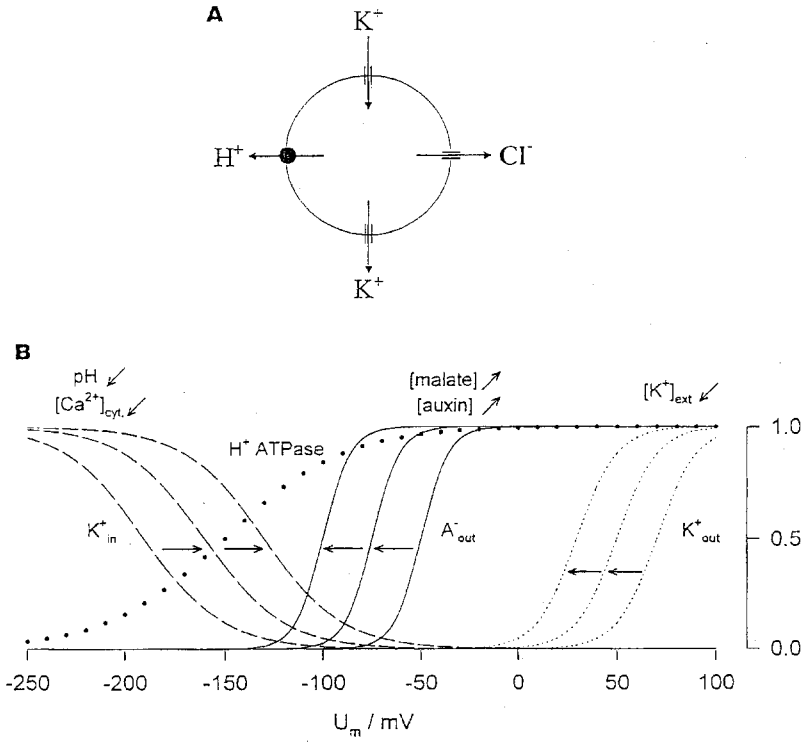

Fig. 1. Voltage dependent ion transporters in the plasma membrane of higher plant cells. A. Physiological direction of ion fluxes through K^+ uptake and K^+ release channels, anion channels and the H^+-ATPase. B. Activation curves, representing the relative conductance as a function of voltage for the ionic pathways shown in A (0 = closed channel; 1 = open channel). Voltage range fractionation, shift in the activation curve and its direction along the voltage axis is indicated by horizontal arrows. Upward and downward arrows on top of the activation curves for the individual ion channels behind the effectors indicate the direction of concentration change able to modify the membrane property in the given manner. Following resting levels for the various effectors were assumed: 30 mM K^+, pH 7.0, <100 nM Ca^{2+}, 0 mM auxin and malate. Depending on the effector concentration the working range of guard cell anion channel 1 (GCAC1) is overlapping with K^+ uptake and K^+ release channels. Note, that the activation curve for the H^+-ATPase overlaps with each ion channel. Simplified activation curves were constructed from single Boltzman distributions which correlate quantitatively to data in the given literature.

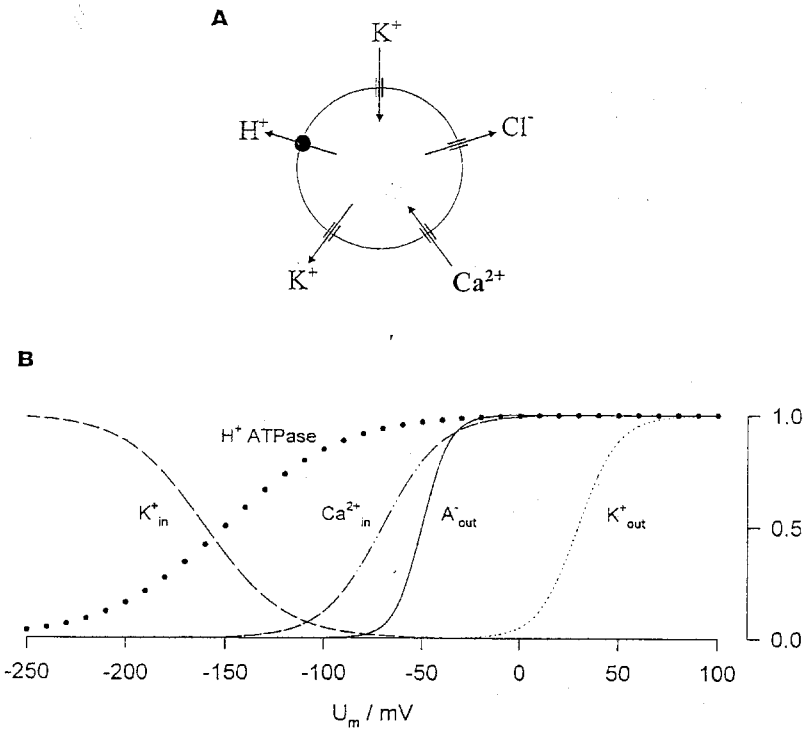

Fig. 2. Voltage dependent ion transporters in the plasma membrane of higher plant cells. A. Physiological direction of ion fluxes through K^+ uptake K^+ release channels, *Ca^{2+} channels*, anion channels and the H^+-ATPase. B. Activation curves, change in the relative conductance as a function of voltage for the ionic pathways given in A (0 = closed channel; 1 = open channel). In the absence of gating modifiers for the anion channel (cf. Fig. 1B), a Ca^{2+} channel with an activation threshold in the range of the resting potential upon stimulation could provide the initial depolarizing conductance of the plasma membrane. The resting potential is assumed to be located in the range of −250 to −150 mV.

cotransport it should be mentioned, however, that some archaebacteria can use the H^+ gradient as well as the Na^+ gradient to create energy [37].

The sequence of events during the plant action potential might include the activation of Ca^{2+}-, voltage-, and time-dependent anion channels [28, 33 and references therein]. Opening of anion channels will depolarize the plasma membrane towards the activation threshold of voltage-dependent K^+ channels which in turn will repolarize the membrane [78]. The shape of the action potential and its kinetics is determined by the relative contribution of the ionic conductances at rest – such as the inward-rectifying K^+ channels and the H^+ ATPase, responsible for the often very negative resting potentials. In the face of time-dependent (inactivating) anion channels [28] the termination of the action potential does, however, not consequently involve outward-rectifying K^+ channels. This is even more pronounced in the presence of hormones or signal metabolites [53, 29], which would separate the working range of anion channels from that of K^+ release channels.[2] This situation is given when ligands such as auxin or malate shift the activation curve of the *g*uard *c*ell *a*nion *c*hannel 1 (GCAC1) negative, towards the resting potential of the cell (Fig. 1B) and/or inactivation is completed before voltage- and time-dependent K^+ release channels open [75, 43].

[2] Note, that cell types such as guard cells or suspension cultured cells are equipped with anion channels which differ in voltage-dependence, unit conductance, and ligand sensitivity [108, 28, 53].

Table 1. K^+ uptake and release channels in the plant plasma membrane.

Plant	Tissue	Conductance	Selectivity	Modulation	Reference(s)
Vicia faba	guard cell	20 pS sym. K^+ [105 mM]	$K^+ > Na^+$ 17:1 $K^+ > Ca^{2+}$ 1:0.3 $K^+ > Rb^+ > Na^+$ $> Li^+ \gg Cs^+$	activation by hyperpolarization; block by Ba^{2+}, $Ca^{2+}_{ext.}$ and Al^{3+}; inhibition by GTPγS; Ca^{2+}_{cyt} and modulation by phosphorylation	[73, 74, 75, 76, 77, 79, 19, 20, 50]
Vicia faba	guard cell	20 pS sym. K^+ [105 mM]	$K^+ > Na^+$ 8:1 $K^+ > Rb^+ > Na^+$ $> Li^+ \gg Cs^+$	activation by depolarization ($V_m > -20$ mV); block by $Ba^{2+}_{ext.}$	[73, 74, 78]
Vicia faba	guard cell	40 pS $K^+_{ext.}$ [100 mM] $Na^+_{int.}$ [100 mM]	$K^+ > Na^+$ 20:1	activation by depolarization ($V_m > +70$ mV); activation by ABA; block by $TEA_{ext.}$ and $Cs^+_{int.}$	[70]
Vicia faba	guard cell	14 pS $K^+_{int.}$ [100 mM] $K^+_{ext.}$[10mM]		activation by depolarization, blocked by Protons	[39]
Zea mays	shoot suspension culture	40 and 125 pS $K^+_{ext.}$ [75 mM] cell attached	$K^+ > Cl^-$ 1:0.4	activation by depolarization, block by Cd^{2+}, verapamil and TEA, internal Ca^{2+} shifts voltage dependence	[18, 41, 42]
Arabidopsis thaliana	mesophyll	44, 66 and 109 pS $K^+_{int.}$ [220 mM] $K^+_{ext.}$ [50 mM]	$K^+ > Cl^-$	activation by depolarization and ATP	[89, 90]
Arabidopsis thaliana	tissue culture	63 pS sym. K^+ [105 mM]	$K^+ > Cl^-$	activation by depolarization ($V_m > 0$ mV), $K^+_{int.}$ modulates conductance	[47]
Asclepias tuberosa	suspension culture	40 pS $K^+_{ext.}$ [100 mM] $Na^+_{int.}$ [100 mM]	$K^+ > Cl^-$	activation by depolarization ($V_m > 0$ mV)	[71]
Samanea saman	extensor and flexor cells	20 pS $K^+_{int.}$ [125 mM] $K^+_{ext.}$ [25 mM]	$K^+ \gg Cl^-$ 100:3 $K^+ > Rb^+ > Na^+$ $= Li^+ = Cs^+$	activation by depolarization ($V_m > -30$ mV); block by TEA and quinine; voltage dependent block by Cs^+ and Ba^{2+}; block by Gd^{3+} and La^{3+}	[54, 55]

Table 1 (Continued).

Haemanthus albiflos, carriage return, H. katherinae, Clivia	endosperm	34 pS sym K^+ [100 mM]	$K^+ > Rb^+ = Na^+ = Li^+ = Cs^+$	activation by depolarization ($V_m > +80$ mV); activation by internal Ca^{2+} and Ba^{2+}	[92]
Dionaea muscipula	trap-lobe cells	3.3 pS sym. K^+ [30 mM]	$K^+ > Na^+$	activation by depolarization ($V_m > 0$ mV)	[38]
Pisum sativum	epidermal cells	35 pS sym. K^+ [100 mM]	$Na^+ > Li^+ > K^+$ 5:2:1	activation by internal Ca^{2+}	[17]
Plantago media	root	8 to 133 pS	$K^+ > Cl^-$	11 different cation-selective channel types 6 activated by depolarization, 5 activated by hyperpolarization	[101]
Hordeum vulgare	aleurone	35 pS $K^+_{int.}$ [100 mM] $K^+_{ext.}$ [10 mM]	$K > Na^+$ 35:1	activation by hyperpolarization	[14]
Hordeum vulgare	root xylem parenchyma	21 pS sym K^+ [100 mM]	$K^+ > Mg^{2+}$ $Ca^{2+} > K^+ \approx Na^+$	Two channel types, activated by depolarization; one channel type TEA insensitive	[103]
Hordeum vulgare	root xylem parenchyma	30 pS sym. K^+ [100 mM]	$K^+ > Rb^+ = Cs^+ > Li^+ = Na^+$	activation by hyperpolarization, permeable to Cs^+, voltage-dependent block by La^{3+}	[104]
Triticum spec.	root	32 pS sym. K^+ [100 mM]	$K^+ > Na^+$ 30:1	activation by depolarization	[67]
Triticum spec.	root	115 and 450 pS $K^+_{int.}$ [60 mM] $K^+_{ext.}$ [105 mM]	$K^+ > Cl^-$	activation by hyperpolarization	[22]
Avena sativa	mesophyll	15 pS sym. K^+ [100 mM]	$K^+ > Na^+$	activation by hyperpolarization, voltage-dependent block by Na^+ and Cs^+	[44]

Since voltage-dependent anion channels in plants require elevated cytoplasmic Ca^{2+} levels, the activation of Ca^{2+} permeable channels in the plasma membrane and/or the vacuolar membrane are proposed to represent an initial step within an action potential [33]. In line with this prediction several kinds of Ca^{2+} permeable channels have been found in both membranes, the activation of which is still under investigation [15, 102, 83, 98]. Equivalent to the heart muscle action potential voltage-dependent, slowly inactivating Ca^{2+} channels may dominate the depolarization phase. Indeed, evidence for the existence of L-type like Ca^{2+} channel in coexistence with a voltage-dependent anion channel has been reported recently for carrot cells [98, 5].

Voltage- and ligand-dependent anion channels in conjunction with K^+- and Ca^{2+} channels as well as an electrogenic H^+-ATPase may allow specialized plant cells or plant cells within their developmental program to respond to the variability in the environmental conditions by changes in the electrical activity of the plasma membrane (Fig. 2). The transduction of signals and information could hence be encoded by the shape of a single action potential or frequence of firing. Besides their macroscopic organization a characteristic feature of the 'green cables' is the absence of voltage-dependent Na^+ channels in favour of voltage- and ligand-sensitive Cl^- channels.

B. Plant specific properties of 'green ion channels'

Whereas excitability and consequently the presence of voltage-gated ion channels in animals is restricted to only a few, highly differentiated cell types, this class of channels was found throughout all plant species, cell types and developmental stages studied so far (Tables 1–3 and [27]). Compared to animals, plants are omnipotent and can adapt more easily to limitations in their neighbourhood since programs for pattern formation and e.g. differentiation are redundant. Therefore a plant is more robust, which is an important property in the light of their inability to flee from unfavourable environmental conditions. During their life cycle plants have to overcome periods where water supply is limiting (water/drought stress), the Na^+, Cl^- and pH in the soil is increasing (salinity stress) or is characterized by the presence of toxic cations released from heavy metall containing minerals by acid rain (e.g. Al^{3+}-toxicity). Omnipotence and adaption is therefore based on the ability of almost all plant cells and/or tissues to de-differentiate before individual clones start their developmental programs again. Germination, root- or shoot formation, and reproduction requires the differentiation into specialized cell types with distinct tasks such as

secretion of lytic enzymes, slime, sugars and salt	aleuron cells of growing seeds or cells in the root tip or gland cells
photosynthesis	mesophyll cells
movement (turgor-driven)	guard cells and cells in the pulvinus, modified leaf cells in carnivorous plants
microbe/pathogen interaction	root-hair cells
uptake, release and long distance transport of nutrients and water	cells in xylem and phloem

We have just started to gain new insights into the abundance, distribution, function and molecular structure of the various channel types in cells performing different tasks, we will focus on three voltage-dependent channel types for the following reasons:

K^+ channels	– a family of voltage-gated, inward-rectifying channels of known function and molecular structure
Anion channels	– a diverse class of ion channels where at least the functional properties of a voltage-gated one in guard cells has been investigated in detail.
Channels of the slow vacuolar (SV-) type	– a voltage-gated channel type found in all plants and cell types looked at.

K^+ uptake channels seem to represent a general feature of plant cells (see Table 1). Voltage-dependent K^+ uptake channels slowly activate when the plasma membrane is hyperpolarized towards potentials more negative than −80 to −100 mV [27, 4]. Upon prolonged stimulation by voltage this K^+ channel does not inactivate. Un-

like its functional counterpart in animal cells and outward-rectifying depolarization activated potassium release channels in plants, the voltage-dependence of the inward rectifier is insensitive towards changes in extracellular K^+ concentration [35, 45, Bertl, pers. communication]. The substrate dependence of the current amplitude, however, is characterized by a Michaelis-Menten kinetics with an K_m of 3–4 mM [80].

Very negative membrane potentials and K^+ uptake are generally accompanied by a high H^+-ATPase activity [49], proton release and subsequent acidification of the extracellular/cell wall space [87]. In line with its supposed physiological function this channel is sensitive to pH changes. At neutral pH the K^+ current amplitude is small. Upon acidification the threshold potential of activation shifts more positive and consequently current amplitude and kinetics increase [12, 31, 56]. The plant-specific properties of this K^+ channel, provided by sustained activity upon voltage activation, K^+ selectivity within the range for the K^+ concentration found in the extracellular space of plants,[3] and linkage to the chemiosmotic motor via its voltage- and pH dependence, are in agreement with its physiological role: K^+ uptake and regulation of turgor and volume.

When the first molecular structures of K^+ uptake channels from *Arabidopsis thaliana* (KAT1, AKT1) appeared they where surprisingly homologous to those of the *Shaker* family of voltage-dependent outward-rectifying channels rather than to their physiological animal equivalents [86, 3, 35, 45]. Following functional expression in *Xenopus* oocytes, insect cell lines (e.g. *Sf9*) and yeast the gene product indeed carried the characteristic features of plant inward rectifiers [68, 31, 32]. Since its location within the plant is unknown its cellular function is still an open question. Because of the striking similarities in the electrophysiological fingerprint of KAT1 and the guard cell inward rectifier, KST1, its related gene in *Solanum tuberosum*, has been isolated from this cell type by heterologous screening [56]. Molecular localization and comparison of its *in vivo* (guard cell protoplasts) and *in vitro* (functional expressed in oocytes) properties indicated that inward K^+ currents in potato guard cells seem to result from the activity of the KST1 gene product only, even though a low expression of the potato guard cell AKT1-homologue (SKT1) could be determined (Müller-Röber, pers. communication). Future analysis of the phenotype of transgenic plants with regard to K^+ channel expression may allow a more detailed understanding of its physiological contribution during growth, development (specialization), movement, and reproduction. Analysis of the subunit composition (monomers, homo-, heterooligomers) and stochiometry or presence of regulators within an individual cell type or tissue together with its electrophysiological fingerprint should give new insights towards the understanding of the basis of functional diversity. The clarification of its plant cell-specific differences, such as selectivity, susceptibility to blockers, and threshold potential of voltage activation (Table 1) together with the analysis of mutants and structural chimera between 'red' and 'green' members of K^+ channel families, will provide the missing link between their structure and function. Within this context the question about the molecular structure of the 'green' outward rectifier and whether or not it is related to *Shaker* is of prime interest.

Anion channels are characterized by a great diversity in both branches of the phylogenetic tree of life (for plants see Table 2). This fact as well as the lack of any structural information prevents the identification of the individual counterparts in each phyla.

Nevertheless a plant specific anion channel represents the voltage- and Ca^{2+}-dependent *g*uard *c*ell *a*nion *c*hannel 1 (GCAC1). Its physiological role, a depolarizing activity (see above), and its electrophysiological properties resembles those of voltage-dependent Na^+ channels in animal nerve- or muscle cells [34, for review]. In guard cells and plant cells in general the opening of

[3] For K^+ uptake at nM K^+ concentrations from the soil see K^+/H^+ symporters; [69].

Table 2. Comparison of the basic characteristics of plant anion channels.

Plant	Membrane	Conductance	Selectivity	Activation	Reference(s)
Suspension cels *Asclepias tuberosa*	PM	100 pS	$Cl^- > K^+$	hyperpolarization	[71]
Suspension cells *Amaranthus tricolor*	PM	200 pS	$NO_3^- > Cl^- > K^+ > Asp^-$	hyperpolarization	[95]
Suspension cells *Nicotiana tabacum*	PM	15 pS		depolarization ATP	[108]
Roots *Triticum aestivum* *Triticum turgidum*	PM	4 pS	$NO_3^- \geq Cl^- > I^- \gg PO_4^{3-}, ClO_4^-$		[88]
Mesophyll cells *Peperomia metallica*	TM	65–150 pS	$NO_3^- > Cl^-$	depolarization	[72]
Cotyledons *Arabidopsis thaliana*	PM	5–40 pS		voltage-independent	[47]
Stem cells *Nicotiana tabacum*	PM	86; 146 pS	$Cl^- > K^+$	stretch	[21]
Epidermal cells *Pisum sativum*	PM	300	$NO_3^- > Cl^- = Br^- > I^- > F^- > Mal^{2-}$	hyperpolarization	[17]
Guard cells *Commelina communis*	PM	34 pS; 59 pS	$A^- > K^+$	stretch	[74]
Guard cells *Vicia faba* *Xanthium strumarium*	PM	24–39 pS	$NO_3^- \geq I^- > Br^- > Cl^- > Mal^{2-}$	depolarization Ca^{2+}, ATP	[40, 28, 48, 29, 16]
Guard cells *Vicia faba*	PM	1;33 pS		depolarization Ca^{2+}	[81, 82]
Guard cells *Vicia faba*	PM	27 pS; 13 pS	$Cl^- > K^+$	stretch depolarization	[15]

anion channels results in anion release from the cytoplasm into the extracellular space. Anion efflux is driven by the negative membrane potential and outward-directed anion gradient. The cytoplasmic concentration of e.g. Cl^- is in the order of 50 mM. In contrast to animal cells, where the Cl^- extracellular concentration exceeds that of the cytoplasm, plant cells are exposed to pond water-like media of low ionic strength (2–6 mM Cl^-; [91]). Therefore energy-coupled anion uptake systems, taking advantage of the plasma membrane proton gradient were postulated [94, for recent progress in the conformation of the cotransport hypothesis see 23, 64, 65]. Under conditions of extreme salt stress where the chloride concentration reaches sea water levels in conjunction with membrane potentials far more positive than −100 mV, anion influx mediated by anion channels is thermodynamically possible, only [100].

So far, a detailed analysis of cell-type specific functional properties of plant anion channels has only been provided for guard cells [for review see 33]. In this paragraph we will thus concentrate on GCAC1 located in the plasma membrane of guard cells. Pairs of this cell type, the stomata, form hydrodynamic, turgor-driven valves which are concerned in the control of water loss during photosynthetic CO_2 uptake [62]. Electrically and metabolically isolated from other cells, guard cells

receive signals from the environment and within the plant (e.g. hormones and metabolites/ions, reflecting the growth rate, water status/salinity or metabolic status). Given the number and nature of stimuli affecting stomatal movement, guard cells have to perceive and integrate them, possibly through a change in electrical activity (movement of charges, excitability, or single transient/prolonged potential changes). Thereby coordinated changes in volume (mass flow of K^+ and anions) are used to adjust stomatal aperture to improve water use efficiency [for review see 62].

Within this circuit GCAC1 is supposed to present an essential element in membrane polarization as well as mediation of large and rapid anion fluxes.[4] This voltage-gated anion channel is modulated by extracellular hormones (auxin), the photosynthate malate [28] salinity changes (Cl^- concentration) [53, 29, 30] as well as cytoplasmic Ca^{2+} and nucleotides [28]. These ligands are capable to control the activation status (number of active channels and/or probability of opening), transport capacity (such as apoplastic Cl^- concentration affects its unit conductance), position of the voltage sensor, and consequently the voltage threshold of activation (auxin and malate; see Fig. 1).

The latter, modifiers of gating, enable guard cells to shift the working range of anion channel activity along the voltage axis. In this resting position the activity of GCAC1 overlaps with the voltage range of activity of K^+ release channels (Fig. 1B, dotted lines on the right hand side), allowing salt release and down regulation of turgor and volume, a pre-requisite for stomatal closure. Whereas the simultaneous voltage activation of both channels requires a pre-depolarization, such as opening of Ca^{2+} channels (Fig. 2), the presence of extracellular gating modifiers will shift the activation curve of GCAC1 towards the resting potential of the cell to activate this particular anion channel (Fig. 1). Separation of the activation curves for GCAC1 and the K^+ release channel will excite the plasma membrane, a property essential for rapid transduction of changes in the environmental conditions. Range fractionation, with respect to the membrane potential has also been found for K^+ uptake and K^+ release channels (Fig. 1). Triggers like changes in the cytoplasmic Ca^{2+} concentration, H^+-ATPase activity (ΔpH), and in the extracellular K^+ concentration shift the activation threshold of the individual channels along the voltage axis (Fig. 1B; [9, 76, 13, 33], Bertl pers. communication).

Activation and modulation of anion channels through modifiers of gating hence allow to repetitively or sustained interconvert the electrical properties and the resting potential of the plasma membrane from a hyperpolarized state (dominated by the K^+ uptake channel and the H^+-pump) into a depolarized state (dominated by GCAC1 and the K^+ release channel). The maxima and minima of the two extremes [97] might therefore depend on K^+ supply (nutrition), H^+-ATPase activity (energy charge [59]), and Ca^{2+} conductances [49].

Thus GCAC1 might be classified as a 'green' channel, since in contrast to animal ion channels it is gated by voltage as well as ligands the combination of which provides for its plant/guard cell specific properties.

SV-type channels are located in the membrane of vacuoles, the major intracellular store of plants for K^+, Na^+, Ca^{2+} salts, metabolites and lytic enzymes. This organelle with its transport systems embedded in the vacuolar membrane is involved in turgor-formation, the driving force for cell expansion, growth and development. In the vacuole as well as in the lysosomal compartments of 'red' cells V-type H^+-ATPases of highly conserved molecular structure have been detected [61, 57]. This finding, besides others, has led to the assumption that these endosomal organelles share functional properties. Because of the difference in size, up to 90% of the total cell volume

[4] For interconversion between rapid (R-type) and slow (S-type) gating modes of GCAC1 or different anion channels in favour of charge flow on one hand and mass flow on the other, see [48, 81, 16].

Table 3. Slow vacuolar SV-type channels* in the vacuolar membrane of various plant cells.

Plant	Tissue	Conductance	Solution/ mM	Selectivity	Modulation	Reference(s)
Hordeum vulgare	aleurone	26 pS	[100 KCl]	$K^+ \gg Cl^-$	Ca^{2+}- and CaM-activated; blocked by W-7 and TFP	[11]
Beta vulgaris	suspension culture	51–68 pS	[100 KCl]	$K^+ > Cl^-$	Ca^{2+}-activated	[60]
Nicotiana tabacum	mesophyll	60–80 pS	[100 KCl]			[26]
Beta vulgaris conditiva	hypocotyl root	65 pS	[100 KCl]	$K^+ > Cl^-$	Ca^{2+}-activated	[2]
Plantago media Plantago maritima	root	60–70 pS	[100 KCl]	$K^+ = Na^+ > Cl^-$	Ca^{2+}-activated	[51]
Chenopodium rubrum	suspension culture	70 pS	[100 KCl]	$K^+ > Cl^-$	Ca^{2+}-activated; blocked by CTX, (+)tubocurarine, W-7, W-5	[8, 105, 106, 107, 63]
Vigna unguiculata	stem	102 ± 4 pS	[100 KCl]	$K^+ \approx Na^+ > Cl^-$	blocked by a vacuolar factor	[52]
Riccia fluitans	thallus	120–140 pS	[200 KCl]			[26]
Beta vulgaris	taproot	120–160 pS	[200 KCl]	$K^+ = Na^+ > Ac^- > NO_3^- > Mal^{2-} > Cl^-$	Ca^{2+}-activated, blocked by cytosolic and vacuolar H^+; blocked by DIDS, Zn^{2+}	[24, 25, 83]
Allium cepa	guard cells	210 ± 17 pS	[200 KCl]	$Na^+ > K^+ > Rb^+ > Cs^+ \gg Cl^-$		[1]
Vicia faba	guard cells	281 ± 20 pS	[200 KCl]	$K^+ > TEA^+ > Cl^- \gg Ca^{2+} \gg Gluc^-$	Ca^{2+}-activated, blocked by cytosolic H^+; blocked by Zn^{2+}, W-7, TFP, calmidazolium	[83, 85]

* All SV-type channels are voltage-gated outward rectifiers.

in plants compared to 10–20% in some chromaffine cells but generally less than 10% in animal cells, patch clamp studies on the ion channel composition and the properties of individual channel types have been restricted to 'green' vacuoles. Consequently, we are unable to decide whether the features of the vacuolar ion channels correspond to the basic task of the lysosomal compartment or exhibit plant specific characters. In Table 3 we hence present cell specific differences of a channel common to plant vacuoles [26].

The slow vacuolar (SV-type) channel, named after its slow voltage-dependent kinetics [24], is activated by depolarized potentials (for use of the new convention see 10) in the presence of elevated cytoplasmic Ca^{2+} only (see [27] for summary). Depending on the cell type and experimental conditions this channel is permeable to cations such as K^+, Na^+, Ca^{2+} and even anions [24, 102, 83]. Even though patch-clamp studies on vacuoles released from their natural habitats became feasable [32], taking into account the activity and current direction of the H^+-pumping V-type ATPase and PP_iase, we still lack conclusive information about its short- and long-term electrical behaviour and the gradients for the various charge carriers *in vivo*. Therefore the alignment of channel properties to their physiological roles is still a problem. Depending on the plant, tissue or cell type, vacuolar ion fluxes may change direction and amplitude within minutes (guard cells, motor

cells), hours to days and even month (storage cells in roots or fruits). We thus predicted that e.g. the guard cell plasma membrane and/or vacuolar membrane is equipped with ion transporters of high abundance or transport capacity [6, 83]. Indeed, when comparing the single channel amplitude (turn over) of the SV-type channels (Table 3) the guard cell representative is by far the most conductive. Permeable to ions stored in the vacuole, outward-rectifying SV-type channels might represent release channels for K^+ salts during stomatal closure. The serial arrangement of two membranes and the coordinated regulation of ion channels in the plasma membrane (K^+ release and anion channels) and in the vacuolar membrane (SV-channels) mediates transcellular (vacuolar to extracellular) ion efflux [84] and might display part of a green circuit as well.

Conclusion

To introduce the reader into the molecular biology and biophysics of plant ion channels we have selected three examples for primarily voltage-gated ion channels. Their 'green' features were discussed with respect to our current understanding of structure and function, physiology and plant/cell specificity. Unlike the voltage-gated ion channels in animal cells, plant channels are able to respond to changes within the cell, plant and the environment. Following the analysis of the electrical properties, channel structure and the identification of potential regulators, future studies will be directed towards the understanding of cell specific elements, the expression and assembly of different channel types or subunits to gain heterooligomerous channels with new properties.

Acknowledgements

We thank Heiner Busch, Petra Dietrich, Barbara Schulz-Lessdorf and Ingo Dreyer for assistance in preparing the manuscript. Experimental studies which provided the background for this review were supported by DFG grants to RH.

References

1. Adomeo G, Zeiger E: A cationic channel in the guard cell tonoplast of *Allium cepa*. Plant Physiol 105: 999–1006 (1994).
2. Alexandre J, Lassalles JP, Kado RT: Opening of Ca^{2+} channels in isolated red beet root vacuole membrane by inositol 1, 4, 5-trisphosphate. Nature 343: 567–570 (1990).
3. Anderson JA, Huprikar SS, Kochian LV, Lucas WJ, Gaber RF: Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 89: 3736–3740 (1992).
4. Assman SM: Signal transduction in guard cells. Annu Rev Plant Physiol 9: 345–375 (1993).
5. Barbara J-G, Stoeckel H, Takeda K: Hyperpolarisation-activated inward chloride current in protoplasts from suspension cultured carrot cells. Protoplasma 180: 136–144 (1994).
6. Becker D, Zeilinger C, Lohse G, Depta H, Hedrich R: Identification and biochemical characterization of the plasma-membrane H^+-ATPase in guard cells of *Vicia faba* L.. Planta 190: 44–50 (1993).
7. Beilby MJ: Electrophysiology of giant algal cells. Meth Enzymol, 174: 403–443 (1989).
8. Bentrup F-W: Cell physiology and membrane transport. Progress in Botany 51: 70–79 (1989).
9. Bertl A, Slayman CL: Complex modulation of cation channels in the tonoplast and plasma membrane of saccharomyces cerevisiae: Single-channel studies. J Exp Biol 172: 271–287 (1992).
10. Bertl A, Blumwald E, Coronado R, Eisenberg R, Findlay G, Gradmann D, Hille B, Köhler K, Kolb H-A, MacRobbie E, Meissner G, Miller C, Neher E, Palade P, Pantoja O, Sanders D, Schroeder J, Slayman C, Spanswick R, Walker A, and Williams A: Electrical measurements on endomembranes. Science Letters 258: 873–874 (1992).
11. Bethke PC, Jones RL: Ca^{2+}-Calmodulin modulates ion channel activity in storage protein vacuoles of barley aleurone cells. Plant Cell 6: 277–285 (1994).
12. Blatt MR: Ion channel gating in plants: Physiological implications and integration for stomatal function. J Membr Biol 124: 95–112 (1991).
13. Blatt MR: K^+ Channels of stomatal guard cells. Characteristics of the inward rectifier and its control by pH. J Gen Physiol 99: 615–644 (1992).
14. Bush DS, Hedrich R, Schoeder JI Jones RL: Channel-mediated K^+ flux in barley aleurone protoplasts. Planta 176: 368–377 (1988).
15. Cosgrove DJ, Hedrich R: Stretch-activated chloride, potassium, and calcium channels coexisting in the plasma membranes of guard cells of *Vicia faba* L.. Planta 186: 143–153 (1991).
16. Dietrich P, Hedrich R: Conversion of fast and slow

gating modes of GCAC1, a *g*uard *c*ell *a*nion *c*hannel. Planta, in press (1994).
17. Elzenga JTM, Van Volkenburg E: Characterization of ion channels in the plasma membrane of epidermal cells of expanding pea (*Pisum sativum* arg) leaves. J Membr Biol 137, in press (1994).
18. Fairley K, Laver D, Walker NA: Whole-cell and single-channel currents across the plasmalemma of corn shoot suspension cells. J Membr Biol 121: 11–22 (1991).
19. Fairley-Grenot KA, Assmann SM: Evidence for G-protein regulation of inward K^+ channel current in guard cells of fava bean. Plant Cell 3: 1037–1044 (1991).
20. Fairley-Grenot KA, Assmann SM: Permeation of Ca^{2+} through K^+ channels in the plasma membrane of *Vicia faba* guard cells. J Membr Biol 128: 103–113 (1992).
21. Falke L, Edwards KL, Pickard BG, Misler SA: A stretch-activated anion channel in tobacco protoplasts. FEBS Lett 237: 141–144 (1988).
22. Findlay GP, Tyerman SD, Garrill A, Skerrett M: Pump and K^+ inward rectifiers in the plasmalemma of wheat root protoplasts. J Membr Biol 139: 103–116 (1994).
23. Frommer W, Hummel S, Riesmeyer J: Expression cloning in yeast of a cDNA encoding a broad specifity amino acid permease from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 90, 5944–5948 (1993).
24. Hedrich R, Neher R: Cytoplasmic calcium regulates voltage dependent ion channels in plant vacuoles. Nature 329: 833–835 (1987).
25. Hedrich R, Kurkdjian A: Characterization of an anion-permeable channel from sugar beet vacuoles: Effect of inhibitors. EMBO 7: 3661–3666 (1988).
26. Hedrich R, Barbier-Brygoo H, Felle H, Flügge UI: General mechanisms for solute transport across the tonoplast of plant vacuoles: a patch-clamp survey of ion channels and proton pumps. Bot Acta 101: 7–13 (1988).
27. Hedrich R, Schroeder JI: The physiology of ion channels and electrogenic pumps in higher plant. Annu Rev Plant Physiol 40: 539–569 (1989).
28. Hedrich R, Busch H, Raschke K: Ca^{2+} and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. EMBO J 9: 3889–3892 (1990).
29. Hedrich R, Marten I: Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells. EMBO J 12: 897–901 (1993).
30. Hedrich R, Marten I, Lohse G, Dietrich P, Winter H, Lohaus G, Heldt HW: Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO_2 concentration. Plant J 6: 741–748 (1994).
31. Hedrich R, Moran O, Conti F, Busch H, Becker D, Gambale F, Dreyer I, Küch A, Neuwinger K, Palme K: Voltage-dependence and high-affinity Cs^+ block of a cloned plant K^+ channel. Eur J Biophys in press (1994).
32. Hedrich R: Technical approaches to studying specific properties of ion channels in plants. In: Neher E, Sakmann B (eds) Single Channel Recordings II. Plenum Press, NY, in press (1994).
33. Hedrich R: Voltage-dependent chloride channels in plant cells: Identification, characterization, and regulation of a guard cell anion channel. In: Guggino WB (ed) Current Topics in Membranes 42, Chloride Channels, pp. 1–34. Academic Press, San Diego (1994).
34. Hille B: Ionic channels of excitable membranes. Sinauer Assoc., Sunderland, MA (1992).
35. Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanazirska MV, Herbert SC: Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature 362: 31–38 (1993).
36. Hodgkin HL, Huxley AF: A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117: 500–544 (1952).
37. Hoffmann A, Laubinger W, Dimroth P: Sodium-coupled ATP synthesis in *Propionigenium modestum*: is it a unique system? Biochim Biophys Acta 1018: 206–210 (1990).
38. Iijima T, Hagiwara S: Voltage-dependent K^+ channels in protoplasts of trap-lobe cells *Dionaea muscipula*. J Membr Biol 100: 73–81 (1987).
39. Ilan N, Schwartz A, Moran N: External pH effects on the depolarization-activated K channels in guard cell protoplasts of *Vicia faba*. J Gen Physiol 103: 807–831 (1994).
40. Keller BU, Hedrich R, Raschke K: Voltage-dependent anion channels in the plasma membrane of guard cells. Nature 341: 450–453 (1989).
41. Ketchum KA, Shrier A, Poole RJ: Characterization of potassium dependent currents in protoplasts of corn suspension cells. Plant Physiol 89: 1184–1192 (1989).
42. Ketchum KA, Poole RJ: Cytosolic calcium regulates a potassium current in corn (Zea maize) protoplasts. J Membr Biol 119: 227–288 (1991).
43. Kolb HA, Marten I, Hedrich R: GCAC1 a guard cell anion channel with gating properties like the HH sodium channel. J Membr Biol in press (1994).
44. Kourie J, Goldsmith MHM: K^+ channels are responsible for an inwardly rectifying current in the plasma membrane of mesophyll protoplasts of *Avena sativa*. Plant Physiol 98: 1087–1097 (1994).
45. Kubo Y, Baldwin TJ, Jan YN, Jan LY: Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 362: 127–133 (1993).
46. Kwart M, Hirner B, Hummel S, Frommer WB: Differential expression of two related amino acid transporters with differing substrate specificity in *Arabidopsis thaliana*. Plant J 4: 993–1002 (1993).
47. Lew RR: Substrate regulation of single potassium and chloride ion channels in *Arabidopsis plasma* membrane. Plant Physiol 95: 642–647 (1991).
48. Linder B, Raschke K: A slow anion channel in guard cells, activating at large hyperpolarization, may be principal for stomatal closing. FEBS Lett 313: 27–31 (1994).
49. Lohse G, Hedrich R: Characterization of the plasma

membrane H^+-ATPase from *Vicia faba* guard cells. Modulation by extracellular factors and seasonal changes. Planta 188: 206–214 (1992).
50. Luan S, Lee W, Rusnack F, Assmann SM, Schreiber SL: Immunosuppressants implicate protein phsphatase regulation of K^+ channels in guard cells. Proc Natl Acad Sci USA 90: 2202–2206 (1993).
51. Maathuis FJM, Prins HBA: Patch clamp studies on root cell vacuoles of a salt-tolerant and a salt-sensitive *Plantago* species. Plant Physiol 92: 23–28 (1991).
52. Maathuis FJM, Prins HBA: Inhibition of inward rectifying tonoplast channels by a vacuolar factor, Physiological and kinetic implications. J Membr Biol 122: 251–258 (1991).
53. Marten I, Lohse G, Hedrich R: Plant growth hormones control voltage-dependent activity of anion channels in plasma membrane of guard cells. Nature 353: 758–762 (1991).
54. Moran N, Ehrenstein G, Iwasa K, Mischke C, Bare C, Satter RL: Potassium channels in motor cells of *Samanea saman*. A patch-clamp study. Plant Physiol 88: 643–648 (1988).
55. Moran N, Fox D, Sutter RL: Interaction of the depolarization-activated K^+ channel of *Samanea saman* with inorganic ions: A patch-clamp study. Plant Physiol 94: 424–431 (1990).
56. Müller-Röber B, Busch H, Ellenberg J, Becker D, Dietrich P, Provart N, Hedrich R, Willmitzer L: Cloning and electrophysiological characterisation of a voltage-dependent K^+ channel predominantly expressed in potato guard cells. EMBO J, submitted (1994).
57. Nelson N, Taiz L: The evolution of H^+-ATPases. Trends Biochem Sci 14: 113 (1989).
58. Ninnemann O, Jauniaux JC, Frommer WB: Identification of a high affinity ammonium transporter from plants. EMBO J 13: 3463–3471 (1994).
59. O'Rourke B, Ramza BM, Marban E: Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells. Science 265: 962–966 (1994).
60. Pantoja O, Dainty J, Blumwald E: Cytoplasmic chloride regulates cation channels in the vacuolar membrane of plant cells. J Membr Biol 125: 219–229 (1992).
61. Pederson PL, Carafoli E: Ion motive ATPases. II Energy coupling and work output. Trends Biochem Sci 12 (5): 186–189 (1987).
62. Raschke K: Movements of stomata. In: Haupt W, Feinleib ME (eds) Encyclopedia of Plant Physiology, Bd. 7, Physiology of Movements. Springer Verlag, Berlin (1979).
63. Reifarth FW, Weiser T, Bentrum F-W: Voltage- and Ca^{2+}-dependence of the K^+ channel in the vacuolar membrane of *Chenopodium rubrum* L. suspension cells. Biochim Biophys Acta 1192: 79–87 (1994).
64. Riesmeier JW, Willmitzer L, Frommer WB: Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J 11: 4705–4713 (1992).
65. Sauer N, Tanner W: Molecular biology of sugar transporters in plants. Bot Acta 106: 277–286 (1993).
66. Sauer N, Baier K, Gahrtz M, Stadler R, Stolz J, Truernit E: Sugar transport across the plasma membranes of higher plants. Plant Mol Biol 26: 1671–1679 (1994).
67. Schachtman DP, Tyerman SD, Terry BR: The K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. Plant Physiol 97: 598–605 (1991).
68. Schachtmann DP, Schroeder JI, Lucas WJ, Anderson JA, Gaber RF: Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. Science 258: 1654–1658 (1992).
69. Schachtmann DP, Schroeder JI: Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plant. Nature 370: 655–658 (1994).
70. Schauf CL, Wilson KJ: Effects of ABA on K^+ channels in *Vicia faba* guard cell protoplasts. Biochem. Biophys Res Com 145: 284–290 (1987).
71. Schauf CL, Wilson KJ: Properties of single K^+ and Cl^- channels in *Asclepias tuberosa* protoplasts. Plant Physiol 85: 413–418 (1987).
72. Schönknecht G, Hedrich R, Junge W, Raschke K: A voltage-dependent chloride channel in the photosynthetic membrane of a higher plant. Nature 336: 589–592 (1988).
73. Schroeder JI, Hedrich R, Ferandez JM: Potassium-selective single channels in guard cell protoplasts of *Vicia faba*. Nature 312: 361–362 (1984).
74. Schroeder JI, Raschke K, Neher E: Voltage-dependence of K^+ channels in guard-cell protoplasts. Proc Natl Acad Sci USA 84: 4108–4112 (1987).
75. Schroeder JI: K^+ transport properties of K^+ channels in the plasma membrane of *Vicia faba* guard cells. J Gen Physiol 92: 667–683 (1988).
76. Schroeder JI, Hagiwara S: Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. Nature 338: 427–430 (1989).
77. Schroeder JI, Hedrich R: Involvement of ion channels and active transport in osmoregulation and signalling of higher plant cells. Trends Biochem Sci 14: 187–192 (1989).
78. Schroeder JI: Quantitative analysis of outward rectifying K^+ currents in guard cell protoplasts from *Vicia faba*. J Membr Biol 107: 229–235 (1989).
79. Schroeder JI, Hagiwara S: Voltage-dependent activation of $Ca2^+$-regulated anion channels and K^+ uptake channels in *Vicia faba* guard cells. In: Leonard RT, Hepler PK (eds) Current Topics in Plant Physiology 4: Calcium in Plant Growth and Development, pp. 144–150. American Society of Plant Physiologists, Rockville, Maryland (1990).

80. Schroeder JI, Fang HH: Inward-rectifying K^+ channels in guard cells provide a mechanism for low-affinity K^+ uptake. Proc Natl Acad Sci USA 88: 11583–11587 (1991).
81. Schroeder JI, Keller B: Two types of anion channel currents in guard cells with distinct voltage regulation. Proc Natl Acad Sci USA 89: 5025–5029 (1992).
82. Schroeder JI, Schmidt C, Sheaffer J: Identification of high-affinity slow anion channels blockers and evidence for stomatal regulation by slow anion channels in guard cells. Plant Cell 5: 1831–1841 (1993).
83. Schulz-Lessdorf B, Hedrich R: Protons and calcium modulate SV-type channels in the vacuolar-lysosomal compartment – Interaction with calmodulin antagonists. J Gen Physiol, submitted (1994).
84. Schulz-Lessdorf B, Dietrich P, Marten I, Lohse G, Busch H, Hedrich R: Coordination of plasma membrane ion channels during stomatal movement. In: Leigh RA (ed) The SEB Symposium 48, Membrane Transport in Plants and Fungi. The Company of Biologists Limited, Cambridge (1994).
85. Schulz-Lessdorf B, Hedrich R: pH and Ca^{2+} modulate the activity of ion channels in the vacuolar membrane of guard cells – possible interaction with calmodulin. Poster Abstract [377], Botanikertagung Berlin, FRG (1992).
86. Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon J-M, Gaymard F, Grignon C: Cloning and expression in yeast of a plant potassium ion transport system. Science 256: 663–665 (1992).
87. Shimazaki K, Iino M, Zeiger E: Blue light-dependent proton extrusion by guard cell-protoplasts of *Vicia faba*. Nature 319: 324–326 (1986).
88. Skerrett M, Tyermann SD: A channels that allows inwardly directed fluxes of anions in protoplasts derived from wheat roots. Planta 192: 295–305 (1994).
89. Spalding EP, Goldsmith MHM: Activation of K^+ channels in the plasma membrane of *Arabidopsis* by ATP produced photosynthetically. Plant Cell 5: 477–484 (1993).
90. Spalding EP, Slayman CL, Goldsmith MHM, Gradmann D, Bertl A: Ion channels in *Arabidopsis plasma* membrane. Plant Physiol 99: 96–102 (1992).
91. Speer M, Kaiser WM: Ionic relations of symplastic and apoplastic space in leaves from *Spinatia oleracea* L. and *Pisum sativum* L. under salinity. Plant Physiol 97: 990–997 (1991).
92. Stoeckel H, Takeda K: Calcium-activated, voltage-dependent, non-selective cation currents in the endosperm plasma membrane from higher plants. Proc R Soc Lond B 237: 213–231 (1989).
93. Sussmann MR: Shaking *Arabidopsis thaliana*. Science 256: 619 (1992).
94. Sze H: H^+-translocating ATPases. Advances using plasma membrane vesicles. Annu Rev Plant Physiol 36: 175–208 (1985).
95. Terry BR, Tyerman SD, Findlay GP: Ion channels in the plasma membrane of Amaranthus protoplasts: One cation and one anion channel dominate the conductance. J Membr Biol 121: 223–236 (1991).
96. Thiel G, Homan U, Gradmann D: Microscopic elements of electrical excitation in chara: Transient activity of Cl^- channels in the plasma membrane. J Membr Biol 134: 53–66 (1993).
97. Thiel G, McRobbie EAC, Blatt MR: Membrane transport in stomatal guard cells. Importance of voltage control. J Membr Biol 126: 1–18 (1992).
98. Thuleau P, Ward JM, Ranjeva R, Schroeder JI: Voltage-dependent calcium-permeable channels in the plasma membrane of carrot suspension cells. EMBO J 13: 2970–2975 (1994).
99. Tsay Y-F, Schroeder JI, Feldmann KA, Crawford NM: The herbicide sensitivity gene *CHL1* of *Arabidopsis thaliana* enodes a nitrate-inducible nitrate transporter. Cell 72: 705–713 (1993).
100. Tyerman SD: Anion channels in plants. Annu Rev Plant Physiol Mol Biol 43: 351–373 (1992).
101. Vogelzang SA, Prins HBA: Patch clamp analysis of the dominant plasma membrane K^+ channel in root cell protoplasts of *Plantago media* L. Its significance for the P and K state. J Membr Biol 141: 113–122 (1994).
102. Ward JM, Schroeder JI: Ca-activated K^+ channels and Ca-induced Ca release by slow vacuolar ion channels in guard cells vacuoles implicated in the control of stomatal closure. Plant Cell 6: 669–683 (1994).
103. Wegner LH, Raschke K: Ion channels in the xylem parenchyma of barley roots. Plant Physiol 105: 799–813 (1994).
104. Wegner LH, DeBoer AH, Raschke K: Properties of the K^+ inward rectifier in the plasma membrane of xylem parenchyma cells from Barley roots: Effects of TEA^+, Ca^{2+}, Ba^{2+}, and La^{3+}. J Membr Biol, in press (1994).
105. Weiser T, Bentrup F-W: (+)-Tubocurarine is a potent inhibitor of cation channels in the vacuolar membrane of *Chenopodium rubrum* L. FEBS Lett 277: 220–222 (1990).
106. Weiser T, Bentrup F-W: Pharmacology of the SV channel in the vacuolar membrane of *Chenopodium rubrum* suspension cells. J Membr Biol 136: 43–54 (1993).
107. Weiser T, Blum W, Bentrup F-W: Calmodulin regulates the Ca^{2+}-dependent slow-vayacuolar ion channel in the tonoplast of *Chenopodium rubrum* suspension cells. Planta 185: 440–442 (1991).
108. Zimmermann S, Thomine S, Guern J, Barbier-Brygoo H: An anion current at the plasmamembrane of tobacco protoplast shows ATP-dependent voltage regulation and is modulated by auxin. Plant J 6: 707–716 (1994).