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Hedrich R. Ion Channels in Plants. Physiol Rev 92: 1777–1811, 2012; doi:10.1152/physrev.00038.2011.—Since the first recordings of single potassium channel activities in the plasma membrane of guard cells more than 25 years ago, patch-clamp studies discovered a variety of ion channels in all cell types and plant species under inspection. Their properties differed in a cell type- and cell membrane-dependent manner. Guard cells, for which the existence of plant potassium channels was initially documented, advanced to a versatile model system for studying plant ion channel structure, function, and physiology. Interestingly, one of the first identified potassium-channel genes encoding the Shaker-type channel KAT1 was shown to be highly expressed in guard cells. KAT1-type channels from Arabidopsis thaliana and its homologs from other species were found to encode the K⁺-selective inward rectifiers that had already been recorded in early patch-clamp studies with guard cells. Within the genome era, additional Arabidopsis Shaker-type channels appeared. All nine members of the Arabidopsis Shaker family are localized at the plasma membrane, where they either operate as inward rectifiers, outward rectifiers, weak voltage-dependent channels, or electrically silent, but modulatory subunits. The vacuole membrane, in contrast, harbors a set of two-pore K⁺ channels. Just very recently, two plant anion channel families of the SLAC/SLAH and ALMT/QUAC type were identified. SLAC1/SLAH3 and QUAC1 are expressed in guard cells and mediate Slow- and Rapid-type anion currents, respectively, that are involved in volume and turgor regulation. Anion channels in guard cells and other plant cells are key targets within often complex signaling networks. Here, the present knowledge is reviewed for the plant ion channel biology. Special emphasis is drawn to the molecular mechanisms of channel regulation, in the context of model systems and in the light of evolution.

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I. INTRODUCTION

A. Plant Ion Transport History: Darwin Today

The response of the Venus flytrap Dionaea muscipula to mechanical stimulation has been recognized in the 1870s, when Charles Darwin and colleagues measured action potentials in this carnivorous plant (FIGURE 1; Refs. 32, 33, 54). Animal and plant physiologists continued these early physiological studies of electrical activity in the 19th century. Scientists had to find organisms for which electrical responses could be systematically studied. In search for cells, which are large enough to be probed with electrodes and can be isolated from the whole body, the long nerve cells of squids on the animal side and characean algae on the plant side advanced to model systems for membrane excitability. In the early times, Cole and Curtis, pioneers in the electrical excitability of squid neurons, studied, side by side, the squid animal model system and action potentials in the characeae Nitella (51). From their electrical recordings, Cole and Curtis (51) concluded that ions carry electrical currents across membranes and create the action potential. In the next decades, these giant cells were used to identify the ion species involved in the different phases of the action potential. In the squid axon, facing high saline sea water, sodium ions entering the nerve cell were associated with the initial depolarization phase and potassium ion efflux to the subsequent repolarization (51, 134, 135). However, Nitella and Chara, living in (low saline) fresh water, instead release chloride for membrane depolarization and K⁺ to recover the resting potential (for review, see Ref. 15). Apparently, anions rather than sodium represent the ions initiating the depolarization phase, in these giant algae cells.

In 1976, when Erwin Neher and Bert Sakmann invented the patch-clamp technique (112, 243), high-resolution ion-current measurements became feasible with normal-sized cells, characteristic for most multicellular organisms. The first plant cells, for which single channels could be recorded, were guard cells enzymatically isolated from broad bean leaves and cells from photosynthetic active wheat leaves...
Ion Channels in Plants

FIGURE 1. Darwin plant fires action potentials. A flytrap of the Darwin plant *Dionaea muscipula* in the open position with a sketch of Darwin’s head in the left blade and an action potential in the right one. Anatomy and physiology of Venus flytrap’s leaf endings allow the carnivorous plant to catch animals. When a prey touches one of the 3 or 4 sensory hairs per leaf lobe, an action potential is fired that is generated via the concerted action of different ion channels (exemplified by single-channel fluctuations shown below). Touching the sensory hair again or one of the others, a second action potential is elicited that shuts the capture organ entrapping the prey.

(232, 301). Since then, this method enabled single-channel and macroscopic current analyses in a number of cell types and species (for overview, see Ref. 120). Before the late 1980s, ion channel entities could only be distinguished and classified by their selectivity, kinetics, and pharmacology. This situation changed when Pongs et al. (260) and Jan, Jan, and co-workers (250) cloned the first voltage-dependent potassium channel gene, associated with the Shaker muta-

tion of the fruit fly *Drosophila*. These findings also stimulated research in the plant field, and with the help of degenerated primers, plant cDNA libraries were screened for “green” Shaker homologs. However, these first attempts, as well as expression cloning approaches with oocytes from *Xenopus laevis* frogs did not identify plant ion channel genes (see below). Cloning of the first potassium channels KAT1 (potassium channel in *Arabidopsis thaliana*) and AKT1 (Arabidopsis K\(^+\) transporter 1) only was possible after a detour via a “plant gene rescue approach” based on yeast mutants that lack endogenous potassium uptake systems (8, 308). This approach had been proven successful before, when the first plant sucrose transporter from spinach was cloned using a sugar transport-deficient yeast strain (278). After obtaining the potassium channel genes, functional analysis of the voltage-dependent channel activity of KAT1 could be recorded with the *Xenopus* oocyte system (294). Interestingly, the Shaker-type channels, KAT1 from the *Arabidopsis thaliana* and its homolog KST1 from the *Solanum tuberosum*, were found to encode the inward K\(^+\) rectifiers, which represents a major conductance as shown with patch-clamp recordings on guard cells (235, 240).

Following extensive and detailed mutational analyses with Shaker-type channels, a structural model for voltage-dependent animal potassium channels was developed (111). This model was used as a template for structure-function analyses of plant K\(^+\) channels. Later site-directed mutagenesis approaches with plant K\(^+\) channels (53, 68, 332) were further stimulated when the crystal structure of the bacterial potassium channel KcsA (65) as well as that of KvAP, a voltage-dependent Shaker-type K\(^+\) channel from the thermophile bacteria *Aeropyrum pernix*, finally became available (157). Just very recently two plant anion channel families have been identified that have no mammalian counterparts (222, 242, 291, 333). These anion channels, as well as the AKT1-type potassium channels in roots, are now emerging as key targets of plant signaling networks.

B. Plant Cells: Membrane-Sandwiched Life

In contrast to animal cells, mature plant cells are equipped with chloroplasts, a large central vacuole, and a cell wall matrix enclosing the plasma membrane. Due to the differ-
ence in osmotic pressure, low in extracellular space but high in the vacuole lumen, because of high concentrations of stored osmolytes. A turgor pressure pushes the thin cytoplasmic layer against the cell wall. The cytoplasm is thus sandwiched between two extracytoplasmic compartments (FIGURE 2). Solutes taken up from the cell wall space often end up in the vacuole. This kind of movement across two membranes and the compartment in between is equivalent to transepithelial transport (cf. endodermis below) associated with, e.g., the desalting process in kidney cells. Like the apical and basal site of animal epithelia, the plasma membrane and the vacuolar membrane of plants are polarized differentially. The resting plasma membrane potential of plant cells is in the order of $-110$ to $-150$ mV (284, 327), while the vacuolar membrane is only weakly polarized as the membrane voltage, given in accordance with the sign convention for endomembranes (19), ranges from 0 to $-30$ mV (20, 342). In other words, plants operate on the basis of a transcytoplasmic potential of about $-100$ mV. This potential difference is due to the asymmetric distribution and nature of transport proteins in the plasma and the vacuolar membranes. Both membranes are equipped with electro-
genic pumps: the plasma membrane P-type ATPase on the one hand and the vacuolar V-type ATPase together with V-pyrophosphatase (V-PPase) on the other. These pumps are all moving protons out of the cytosol (31). As a result of charge movement, both membranes are polarized, although to a different degree depending on the nature and number of ion channels and \(\text{H}^+\)-coupled carriers which are active in the individual membrane type (FIGURE 2). Excellent reviews exist on proton pumps and the proton-coupled cotransporter; these transporters will therefore not be addressed here (see recent reviews, e.g., Refs. 31, 94, 97, 133, 292, 307).

When working with impalement microelectrodes localized in the vacuole, measurements could be complicated by two membranes with different electrical properties in series (23). However, when the microelectrode tip penetrates the cell wall, the vacuolar membrane often is pushed away, since the vacuole lumen and cytoplasm are isosmotic. As a result, the tip of the microelectrode most often is located in the cytoplasm (214, 228). The plasma- and vacuolar membranes can be individually accessed with patch pipettes using cell wall-free (following enzymatic degradation) plant cells, “protoplasts,” or vacuoles released thereof. In the following section, first the features of individual ion channels will be addressed before they will be discussed as key elements in the biology of integrated cellular systems.

II. PLASMA MEMBRANE: INTERFACE TO ENVIRONMENT

In the resting state, the plasma membrane potential of *Arabidopsis* root cells when cultured in low \(\text{K}^+\) soil can become as negative as \(-200 \text{ mV}\) (132). This state is dominated by the P-type proton pump in media containing 50 \(\mu\text{M}\) potassium or less. However, upon an increase in \(\text{K}^+\) concentration above 100 \(\mu\text{M}\), the “pump state” is switched into the “\(\text{K}^+\) state” where the membrane potential follows the Nernst potential of the alkali ion (16, 132, 163). Patch-clamp studies identified two types of potassium conductances that were denoted as inward and outward rectifying channels (298, 303).

A. Potassium Channels: More Than Housekeepers

The focus of this section is on the features of potassium channels with known molecular nature. When in 2000 the genome of *Arabidopsis thaliana* became available (Arabidopsis Genome Initiative; Ref. 9), the number of afore-identified potassium channels increased to fifteen: 9 Shaker channels, 5 two-pore \(\text{K}^+\) (TPK) channels, and a single IRK-like \(\text{K}^+\) channel (FIGURE 3A) (102, 347). Among them, all Shaker-like channels could be localized to the plasma membrane (120).

1. Shaker \(\text{K}^+\) channels

From the nine plant potassium channels homologous to Shaker, only SKOR and GORK exhibited Shaker-like voltage-dependent gating (FIGURE 3B) (2, 95). GORK is expressed in guard cells and root hairs, where it operates as an outward-rectifying potassium-selective channel (140, 149). SKOR is localized in the vasculature, namely, the xylem parenchyma cells, where it is engaged in solute loading with the xylem network (FIGURE 12) (95). In contrast to most animal Shaker channels, which activate and inactivate in the 10-ms range (105), both GORK and SKOR require seconds to fully activate without pronounced tendency to inactivate (FIGURE 3B). Voltage activation of both outward rectifiers, quantified with respect to the half-maximum activation voltage, shifts negative with the drop in external \(\text{K}^+\) concentration (for review, see Ref. 158), while acidification results in a decrease of the macroscopic \(\text{K}^+\) currents (2, 188). In contrast to GORK and SKOR as well as animal Shaker channels, KAT1 and KAT2 are activated by hyperpolarizing potentials and therefore operate as strict inward rectifiers (FIGURE 3B) (127, 190, 193, 261, 293). KAT1 and KAT2 are predominately expressed in guard cells and the phloem network in an ecotype-dependent manner (150, 240, 257, 321). Both channel types share 79% homology and represent products of recent gene duplication (71). The voltage threshold for KAT1 activation is around \(-80 \text{ mV}\) and independent on the external potassium concentration (29, 139). At extracellular \(\text{K}^+\) concentrations in the millimolar range, KAT1 and -2 will facilitate \(\text{K}^+\) uptake. However, lowering the bath \(\text{K}^+\) content in a way that the Nernst potential for \(\text{K}^+\) \((E_K)\) becomes negative to the activation threshold of KAT1, this channel type also can mediate potassium efflux as long as the voltages are positive of \(E_K\) (29). KAT1 and KST1, the KAT2 homolog from *Solanum tuberosum*, are activated upon extracellular acidification by a positive-going shift in activation potential (141–143, 235). AKT1 is expressed in roots including root hairs, where it forms a complex with AtKC1. AtKC1 represents an electrically silent Shaker-like channel that modulates the voltage dependence of AKT1, when forming heteromeric channel
complexes (98, 149, 272, 345). AKT2, in the literature also referred to as AKT3 or AKT2/3, is expressed in phloem networks (FIGURE 12, A AND C), a low-resistance solute pipeline of interconnected nonvacuolated and nonnucleated cells. In contrast to the KAT1- and AKT1-type Shaker channels, AKT2 was initially identified as a weakly inward-rectifying potassium channel (FIGURE 3B) blocked by protons and calcium ions (167, 215). Comparison of KAT1 and AKT2-type channels, based on biophysical fingerprints of pore-exchange chimera between KST1 and AKT2,
showed that the K⁺ conducting pore is associated with H⁺ and Ca²⁺ action as well as gating (142). In addition, it was shown that phosphorylation-mimicry mutations in the voltage sensor domain (S4) switch AKT2 from a rather voltage-independent channel into a strong KAT1-like inward rectifier (225). One might assume that protein kinase-phosphatase activity addressing AKT2 could switch the channel’s voltage gate on demand (341) (for physiological implications, see sect. IV). AKT5, SPIK, and TPK4 are expressed in the pollen, the male gametophyte of higher plants, and were found to control the membrane potential as well as growth of the pollen tube (14, 80, 234). AKT5 and SPIK (also known as AKT6 or AtKv₉) with a high homology to AKT1 (234) are Shaker-like potassium channels and function as inward rectifiers. In contrast, TPK4 is a member of the AtTPK family (FIGURE 3A) (for TPK4 features, see sect. III) with an animal TASK-like structure displaying no voltage dependence.

2. Gating bias

Plant Shaker-like K⁺ channels appear structurally very similar but exhibit profound differences in their gating properties. Inward-rectifying KAT1- and AKT1-type channels operate over a fixed voltage range. They activate at negative voltages and are largely independent on the concentration of the potassium ion (13, 29, 68, 150, 327). In contrast, gating of outward-rectifying SKOR/GORK-type channels is subject to both voltage and the extracellular K⁺ concentration (2, 95). As a consequence, they activate at voltages positive from the equilibrium voltage for K⁺. As a consequence, they activate at voltages and are largely independent on the concentration of the potassium ion (13, 29, 68, 150, 327). In contrast, gating of outward-rectifying SKOR/GORK-type channels is subject to both voltage and the extracellular K⁺ concentration (2, 95). As a consequence, they activate at voltages positive from the equilibrium voltage for K⁺. Although K⁺- sensitive SKOR gating was studied in detail (158), the key determinants for inward and outward rectification with plant Shaker channels remains yet to be identified (for review, see Ref. 69). How can the gating structure be pinpointed? A potentially promising approach developed by the group of Ingo Dreyer (Universidad Politécnica de Madrid) follows an exclusion strategy. Domains between KAT1 and SKOR are swapped aiming at replacements that do not affect channel-type-specific gating (92, 276). Such experiments currently identify regions that are “not” responsible for inverting gating, narrowing down those areas that are potentially involved. Based on previous results and on the alignment of KAT1- and SKOR-type channels from a number of different plant species, it was possible to generate a functional synthetic inward-rectifying K⁺ channel and an outward-rectifying K⁺ channel that share an identity of 83% at the amino acid level (Ingo Dreyer, personal communication). In comparison, KAT1 and SKOR share identities of only ~30% and 36% at the amino acid level. Remarkably, those residues that are believed to be essential for voltage-dependent gating of plant Shaker-like K⁺ channels (e.g., the charges in the putative voltage sensor S4) are identical in both synthetic channels. In future approaches, computational homology modeling and simulations of molecular dynamics, the group of Ingo Dreyer (personal communication) aims to identify additional regions of KAT1/ SKOR that do not affect the synthetic channel. This iterative domain swap approach, together with computational modeling, is supposed to decompose the site controlling gating direction.

B. Anion Channels: Volume Regulation and Signaling

As in the potassium channel section above, here only the features of anion channels of known molecular nature are described. When the genome of Arabidopsis thaliana was accessible, sequence information did not help much in identifying anion channels because at that time just Torpedo-like CIC channels were known (118, 155) (cf. sect. III on vacuolar channels). At that time, patch-clamp studies on plant cells identified three prominent anion currents classified into S-type, R-type, and Al-sensitive channels (176, 266, 289, 302). Guard cells were known to harbor both S- and R-type anion channels (FIGS. 4–6) (121, 166, 203, 299, 302).

1. SLAC/SLAH family

SLAC1, the first gene encoding S-type anion channels, was identified from an Arabidopsis mutant defective in signal-dependent stomatal closure (242, 333). The SLAC1 gene is expressed in guard cells and shows weak homology to the tellurite-resistance multidrug transporter TehA from Hae- mophilus influenzae (HiTehA) and the malate transporter Mae1 from fission yeast but not human (40). Following expression in Xenopus oocytes together with a distinct plant protein kinase-phosphatase pair (FIGURE 4B) (see sect. IV on guard cells), SLAC1 showed the hallmark characteristics of the S-type channel well known from patch-clamp studies on guard cell (101). This is manifested by a weak voltage dependence and slow deactivation upon hyperpolarization (FIGURE 4, A AND C) (101, 214, 286). In contrast to Mae1 in yeast, SLAC1 did not transport malate, but rather chloride and nitrate (40, 101, 247). Only very recently the bacterial SLAC1 homolog HiTehA was crystallized and the structure of the guard-cell anion channel was modeled accordingly (FIGURE 5A) (40). Thereby a phenylalanine residue was predicted to occlude the anion-conducting pore. Replacing this residue by alanine rendered SLAC1 and even TehA anion-permeable without requirement for preactivation by a protein kinase (see sect. IV). This behavior might indicate that phosphorylation of a cytosolic site, possibly by structural rearrangements, affects the position of the pore-lining F450 in AtSLAC1.

SLAC1 represents the founder of a small gene family comprised of SLAC1 and four SLAC1-homologs (SLAHs) in Arabidopsis (242, 333). SLAH3: among them SLAH3 is expressed in guard cells and able to rescue the SLAC1 mutant phenotype. This SLAC1 homolog exhibits phosphorylation dependence for proper anion transport similar to...
SLAC1, but in addition SLAH3 exhibited pronounced differences (99). 1) Selectivity: since SLAH3 predominately conducts nitrate, in chloride-based media guard cells expressing the SLAH3 but not the SLAC1 gene do not show S-type anion currents. SLAH3 expressing oocytes in chloride-based buffers do not show SLAC1-like anion currents either. However, when in experiments with guard cells and oocytes the halide is replaced by nitrate, anion currents can be elicited (FIGURE 5B) (99). 2) Priming: SLAH3 does not just conduct nitrate but requires about 3 mM of the nitrogen fertilizer for priming (FIGURE 5C). 3) Voltage dependence: in contrast to the leaklike current-voltage curve of SLAC1, SLAH3 in agreement with an outward rectifier is largely inactive at hyperpolarized potentials but opens with positive-going voltages. The half-activation threshold is dependent on the priming nitrate concentration. In the future it will be interesting to learn about the structural basis for differences in selectivity and voltage dependence of the two guard cell anion channels and about new features of the remaining members of the SLAC1/SLAH family.

2. QUAC1 and ALMT family: more than aluminum resistance

The first gene encoding a R-type (for rapid) channel or QUAC (for quick anion channel) was identified in a screen for guard cell localized members (FIGURE 6B) of the ALMT channel family, of which the founding member (ALMT1) was shown to conduct malate (FIGURE 6, C AND D) (109, 266). Mutants lacking the potential malate channel gene ALMT12 were characterized by guard cells partially impaired in signal-dependent volume decrease (222, 291). When the function of the channel gene product was analyzed in Xenopus oocytes, it revealed features known from R-type channels, also named QUAC, in guard cells (222). As a consequence, the nomenclature for current and gene is S-type channel and SLAC, respectively, while the gene encoding R-type channels was named QUAC1. QUAC1 in guard cells is characterized by its 1) voltage dependence: QUAC1 activates upon depolarization with half-activation voltage modulated by extracellular malate level, 2) kinetics: QUAC1 shows fast activation and deactivation time constants (121), and 3) selectivity: QUAC1 predominately conducts malate and sulfate in a feed-forward-type mechanism (83, 124, 126, 222). Thereby, e.g., malate release stimulates malate anion efflux via this channel. Patch-clamp studies with guard cells of Arabidopsis mutants lacking QUAC1 were found to show reduced R-type currents in malate-based media (FIGURE 6C). The remaining R-type currents in this mutant may indicate that other members of the ALMT family encode additional QUACs.

The ALMT family has no relatives in bacteria, yeast, and human. When in aluminium-resistant wheat lines the gene responsible for malate exudation from roots was identified, it was named ALMT for aluminium-induced malate trans-
porter (266). In polluted soils, released malate complexes the toxic Al$^{3+}$. The ALMT1 gene encodes a membrane protein that is constitutively expressed in aluminium-sensing root apices. ALMT1 and QUAC1 share the features of a malate-permeable channel, but neither is ALMT1 voltage-dependent nor is QUAC1 aluminium induced (136, 222). Since ALMT1 homologs from other species than wheat seem either to lack aluminium induction or carry anions other than malate, renaming the family could prevent confusion.

3. TMEM

Recently it has been shown that the transmembrane protein TMEM16A is a calcium-activated anion channel (114, 297). The Arabidopsis genome contains a single-copy gene (At1g73020) the function of which remains to be explored.

C. Calcium Channels: Currents But No Genes

Calcium is a central player in plant signal transduction (64, 282). From patch-clamping plant cells and plant genome sequencing over the years, a detailed knowledge about potassium channels was gained. However, what is known about cation channels permeable for calcium ions? From animal cells two major classes of calcium channels were known that become either activated upon depolarizing voltages or binding of a ligand.
1. Voltage-dependent channels

In search for voltage-dependent plant calcium channels, extracellular calcium levels were varied and changes in the electrical properties of the plasma membrane were monitored. With such electrophysiological studies it came as a surprise that calcium currents, initially recorded in guard cells from *Vicia faba*, were not elicited by depolarizing voltages as in animal cells but rather by hyperpolarization of the plasma membrane (45, 113, 252). This was before genome analyses showed that plants lack genes homologous to the voltage-dependent calcium channels of animals. However, voltage-dependent calcium currents were found in all plant cell types examined (59, 319, 336, 337). Detailed microelectrode studies combined with calcium reporter-dye FURA-fluorometry showed that in guard cells the hyperpolarization-activated calcium channels are under control of the phytohormone abscisic acid (ABA) and require protein phosphorylation (107, 175). This finding is supported by patch-clamping guard cells from *Arabidopsis* (186, 233, 252). These later studies discovered that H$_2$O$_2$, the product of the plasma membrane NADPH oxidase (82), triggers the hyperpolarization-dependent channel. This channel could be classified as a nonselective cation channel permeable to...
K⁺, Ca²⁺, Mg²⁺, and Ba²⁺ (337). In guard cells, ABA can trigger calcium influx and induce anion efflux and volume decrease (discussed below) (214, 300).

2. Ligand-activated channels: CNG channels and GLR channels

Genes with high homology to the animal cyclic nucleotide-gated channel (CNG) and glutamate receptor channels (GLR) were identified in plants already in 1998 (unpublished data). In the laboratory of the author, as well as several other research groups, however, unequivocal data for ion channel function could neither be obtained with plant protoplasts nor with heterologous expression systems. Using a domain exchange approach, Tapken and Holllmann (324) showed that Arabidopsis AtGLR1.1 and AtGLR1.4 have functional Na⁺-, K⁺-, and Ca²⁺-permeable ion pore domains when transplanted into rat α-aminoo-3-hydroxy-5-methyl-4-isoxazole propionate (GluR1) and kainate (GluR6) receptor subunits. Further experiments with growing pollen tubes recently reported promising progress, suggesting that detailed analysis of these tip growing cells will be able to advance this area of research and finally prove that the CNGC genes present in plant genomes indeed are encoding ligand-gated calcium-permeable channels (84, 226). The Arabidopsis CNGC family comprises 20 members. So far, the ability of cyclic nucleotides to activate these ion channels in plants cells has not been shown unequivocally. Distinct members of this family have been associated with pathogen defense and plant immunity (62) as well as biology of the male gametophyte (226). Mutants in some cases exhibit severe dwarf phenotypes (5, 160). What is known about the biology of the ligand? So far, synthesis and metabolism of cyclic nucleotides remain largely unexplored, possibly because proper in-planta-monitoring of this class of second messengers has been problematic (for review, see Ref. 245). One technical progress in recording cGMP changes in planta was recently made by using an endogenous fluorescent cGMP reporter protein on the single-cell level (148). As the CNGC family, the Arabidopsis glutamate receptor-like (GLR) family comprises 20 members (187, 226). The synthesis and metabolism of the amino acid glutamate is known; otherwise, the situation for GLRs seems even less understood than that of the plant CNGCs. GLR mutants studied so far exhibit weak pleiotropic phenotypes only. In pollen tubes, these channels are probably activated by D-serine (226), but an in-depth analysis of the nature of the ligand(s) and pharmacology of this channel type still is lacking at the moment (317).

3. What triggers calcium channels in plant cells?

Blue light perceived via the phototropin receptor activates hyperpolarization-dependent cation channels in photosynthetic cells to optimize chloroplast positioning towards the sun (164, 319). This finding indicates that the chloroplast movement involves cytoplasmic calcium signals. So far, mutant screens regarding stomatal action as well as ROS, and blue light signaling have not yet identified the molecular nature of the hyperpolarization-dependent “calcium” channel.

A) COLD. Rapid cooling of plant cells triggers a transient rise in the cytosolic free calcium concentration of large amplitude. This cold response depends on the availability of calcium ions at the extracellular face of the plasma membrane. With the use of voltage-recording impalement electrodes, studies with the liverworth Conocephalum and higher plants such as Arabidopsis showed that cold induces transient membrane potential depolarizations (37, 181, 182, 351). Patch-clamp studies characterized the currents underlying cold-induced transient potential changes as nonselective cation channels. In contrast to the hyperpolarization-activated channel described above, the “cold” channel was characterized as an outward-rectifying 33 pS nonselective cation channel with a cold-induced transient rise in conductance. The permeability ratio for calcium over cesium was 0.7, pointing to a permeation pore larger than 3.34 Å (37). Thus the features of the cold-sensitive channel in the Arabidopsis leaf are reminiscent of transient receptor potential (TRP) channels in the animal field. In contrast to some unicellular green algae, however, genomes of higher plants do not harbor genes related to TRPs. Likewise, no MEC proteins, found in Caenorhabditis elegans as mechanosensing channel subunits, are present in plants either.

B) MECHANIC FORCES. When attaching a patch pipette to the surface of a cell membrane and in the process of establishing a tight seal between the membrane patch encircled by the tip of the glass capillary, the patch is faced to negative hydrostatic pressure. While establishing high-ohmic seals, most patch clammers have experienced current fluctuations in the picoampere range (45, 63, 216), which disappear when negative pressure is released. These unitary currents elicited by mechanic forces on the membrane were named stretch-activated or mechanosensitive (MS) channels (290). Patch-clamp studies on the plasma membrane of plant protoplasts revealed the existence of MS channels of different ion permeability. In guard cells, three types of MS channels permeable to potassium, chloride, or calcium ions have been recorded (45). What is the level of knowledge about the nature of mechanosensitive channels in general and in plants in particular?

I) MSL. Soon after their discovery in a patch-clamp survey with Escherichia coli (216), cloning of MS channels led to the molecular identification of large- and small-conductance mechanosensitive channels (Mscl and MsCs, respectively) (200, 320). There are 10 bacteria MS-like (MSL) genes in Arabidopsis (231). Among them, MSL2 and MSL3 were found to play a role in chloroplast division (115). In
contrast, MSL9 and MSL10 operate as stretch-sensitive an-
ion channels in the plasma membrane of root cells. Root cell
MSL channel activity was largely reduced in the
msl9/10
double mutants. A quintuple knockout of the
MSL4–6,-9/10
genes abolished mechanosensitive channel activity
completely. However, so far, no clear mechanoresponsive
Arabidopsis phenotype has been found in msl knockout
mutants (116). Are there compensatory MS channel-types?

II) MCA. The yeast mid1 mutant lacks a putative Ca2+-
permeable stretch-activated (SA) nonselective cation chan-
nel component and displays a lethal phenotype in the
presence of mating pheromone (163). The Arabidopsis genes
MCA1 and MCA2 partially rescue the mid1 phenotype
(239). In agreement with its phenotype, MCA1-expressing
yeast shows an increased Ca2+ uptake compared with the
mutant. In line with its role as a putative mechanosensor,
the Arabidopsis mca1 exhibits a defect in mechanical re-
sponsiveness manifested as “roots ability to penetrate a
layer of hard agar”.

E) Piezo. Piezo 1 and 2 represent a new class of MS channels
that are expressed in animal cells (46). Their homologs are
present throughout the kingdoms with Arabidopsis encod-
ing a single Piezo gene (At2g48060). Thus MCA1/2 and
Piezo genes could encode mechanoresponse elements in
plants, yet mechanosensitive channel functions remain to be
demonstrated.

TPKs were shown to activate in response to mechanic forces
(208) but do not appear to be located at the plasma mem-
brane and do not transport calcium ions (338). They repre-
sent green vacuolar membrane-localized relatives of the an-
imal TREK K+ channels. Members of this K2P two-pore K
channel superfamly are sensitive to mechanical membrane
distortion (12). Membrane stretch and osmotic gradients
also alter the activity of TPKs. These two-pore channels
thus seem to operate as intracellular osmosensors that dur-
ing hypoosmotic shock rapidly open and release vacuolar
potassium. Mutant phenotypes suggest that TPK-based os-
mosensing is important for plant cells to tolerate rapid
changes in external osmolarity. TPK4, the only plasma-
membrane-localized TPK family member, is expressed in
pollen tubes (14). It will be interesting to see with this tip-
growing cell system whether TPK4 is operating as a MS
channel, alone or together with MscLs and/or MCAs, to
protect the tension-fragile apical cone from burst in re-
sponse to mechanistic and osmotic changes on its way to meet
the egg cell (66).

III. VACUOLAR MEMBRANE: DOOR TO
THE STORE

Ion channels are not only present in the plasma membrane
but also in the membrane of diverse plant organelles such as
the chloroplast (130, 131, 263, 296) and the large central
vacuole (FIGS. 7A AND 8, A AND B) (122, 128). Besides its
housekeeping role in cell volume and turgor control, the
vacuole serves as a dynamic pool for ions and metabolites
(43, 217, 221). For patch-clamping vacuoles from a cell
wall-free protoplast of choice, endosomes are liberated by
selective osmotic shock lysis (FIGURE 8A) (119). So far, vac-
uoles from all kind of cells and species have been addressed

![FIGURE 7. Calcium regulation of vacuolar two-pore K+ channels. A: two-pore potassium channel TPK1 is localized in the vacuole membrane. Green fluorescence identifies TPK1-GFP fusion constructs (left and right) and red fluorescence RFP fusion construct encoding a cytosolic protein (right) following transient expression in onion epidermis cells. B: in the presence of calcium at the cytosolic face of TPK1, single K+ channels are active in the vacuolar membrane. Upon addition of the calcium chelator EGTA (arrow), TPK1 channel activity vanished. [Modified from Latz et al. (191). Copyright 2007 John Wiley and Sons.] C: constructs with the plasma membrane two-pore potassium channel TPK4 carrying the second pore region of vacuolar TPK2 expressed in Xenopus laevis oocytes (cf. Ref. 73). TPK4-TPK2 chimeras exhibit the hallmark characteristics of this K+ channel subfamily: instantaneous, voltage-independent activation of macroscopic K+ selective currents.](http://physrev.physiology.org/)
The vacuolar membrane is equipped with transport proteins that mediate the passage of a wide spectrum of compounds. Among them, ion channels, H\(^+\) pumps, and H\(^+\)-coupled carriers have been shown to represent major membrane conductances of this organelle (FIGURE 2). Transport processes across the tonoplast are energized by two proton pumps, the vacuolar H\(^+\)-ATPase (V-ATPase) and H\(^+\)-pyrophosphatase (V-PPase). The structure and function of the vacuolar pumps have recently been reviewed (50, 210, 306, 307, 310). Upon pumping cytosolic protons into the vacuolar lumen, a proton gradient and a membrane voltage is generated, enabling the cell to create and maintain ion- or metabolite gradients (180, and references therein). Among H\(^+\)-coupled transporters CIC-type anion carriers, Mg\(^{2+}\), polyol transporters as well as sugar antiporter and symporters have been functionally characterized by direct patch-clamp studies (55, 295, 304, 313, 354). Excellent recent reviews on the biology of proton-coupled electrogenic nutrient and metabolite carriers have been published (34, 56, 217, 221, 244, 292; for recent experimental studies, see Refs. 39, 145, 295, 304, 354).

**A. Vacuolar Cation Channels**

With the use of basically K\(^+\)-based buffers, instantaneous as well as time-dependent cation currents can be observed in the whole-organelle configuration of the patch-clamp technique. These conductances have been named slow vacuolar (SV), fast vacuolar (FV), and vacuolar K\(^+\)-selective (VK) channels, with respect to their kinetics, voltage dependency, (for review, see Ref. 125) including mesophyll cells and guard cells from Arabidopsis (53, 277, 304). Unlike protoplasts, isolated vacuoles are approached with the patch pipette from the cytosolic side. Thus, after establishing the whole-vacuole configuration, the pipette solution is in contact with the vacuolar lumen. According to the sign convention for endomembranes (19), the vacuolar lumen is considered as extracellular space and therefore cation currents flowing from the vacuolar lumen into the cytosol are denoted as negative values.

**FIGURE 8.** TPC1, a vacuolar voltage-dependent two-pore cation channel, is Ca\(^{2+}\) modulated. A: a vacuole liberated by selective osmotic shock from a wild-type A. thaliana mesophyll protoplast. B: a vacuole released from a tpc1–2 mesophyll protoplast via the same procedure as in A but after transient expression of a wild-type TPC1-GFP-fusion construct. Green fluorescence identifies the localization of the GFP-labeled TPC1 channels to the vacuolar membrane (cf. Ref. 53). C: patch-clamp studies on whole vacuoles after transient transformation of tpc1–2 mesophyll protoplasts with wild-type TPC1 channels. Stimulation of typical TPC1 currents was monitored with increasing depolarization (cf. Ref. 53). D. Voltage-dependent gating of TPC1 channels (given as relative voltage-dependent open probability, rel. G) is modulated via different cations such as K\(^+\), Na\(^+\), and Ca\(^{2+}\). A rise in the cytosolic calcium level activates TPC1 by shifting the voltage threshold for channel activation towards the physiological voltage range (indicated by the gray area). [From Hedrich and Marten (125). Copyright 2011 Oxford University Press.] E: luminal calcium blocks single TPC1-channel fluctuations. Current recordings were performed from vacuoles of nontransformed wild-type mesophyll protoplasts in the absence or presence of luminal Ca\(^{2+}\). [From Beyhl et al. (21). Copyright 2009 John Wiley and Sons.]
cation permeability, and sensitivity towards cytosolic Ca$^{2+}$ (6, 7, 30, 128, 328, 348). Among the 15 potassium channel sequences identified in the Arabidopsis genome, four two-pore K$^+$ channels (TPK1, -2, -3, and -5) and a K$_v$-like one (KCO3) (FIGURE 3A) are not located at the plasma membrane.

1. TPKs

When constructs of TPKs or K$_v$ fused to GFP, CFP, or YFP were expressed in plant cells, green, cyan, or yellow protein fluorescence was emitted from the vacuolar membrane with the respective K$^+$ fluorescence was emitted from the vacuolar membrane with the respective K$^+$ channels (FIGS. 7A AND 8B) (72, 103, 339). Among the TPKs, TPK1 is characterized by calcium (EF-hands) and 14–3–3 binding motifs. When TPK1 is expressed in yeast, patch-clamp studies of TPK1 function on vacuoles of transformed yeast cells identified properties of the plant VK channel (22). Well in agreement with the binding motives, TPK1 currents were independent of the membrane voltage but sensitive to cytosolic calcium and 14–3–3 (FIGURE 7B) (22, 103, 191). The plasma membrane localized TPK4 shares with TPK1 the voltage-independent feature (FIGURE 7C) (cf. TPK4; Ref. 14). Based on the properties of K$^+$ channels in their natural environment of the plant vacuole and TPK1 features in yeast vacuole, it was assumed that the TPK1 gene encodes the VK channel characterized in guard cells (348). Indeed, channel mutants show that TPK1 has a role in intracellular K$^+$ homeostasis affecting vacuolar K$^+$ release and thus stomatal closure kinetics (103).

Concerning the targeting of TPK1 to the vacuolar membrane and TPK4 to the plasma membrane, TPK1-TPK4 channel chimeras were generated that were the key to identify a diacidic motif (DLE) required for export of TPK1 from the endoplasmatic reticulum (72) (cf. similar situation with the plasma membrane Shaker K$^+$ channel KAT1; Ref. 227). From these chimeras, however, the assumed target signal(s) that would direct the vacuolar channel to the plasma membrane and vice versa were not identified (72). Plant cells often contain several types of vacuoles that coexist in the same cell. Recently, it was shown in rice that two TPK isoforms, TPKa and TPKb, differentially localize to the large lytic vacuole and small protein storage vacuoles, respectively (146). Essentially, three amino acid residues of the COOH-terminal domain determine differential endomembrane targeting (146).

For studying the K$^+$ transport characteristics of the TPKs, pore-domain swapping experiments between the plasma membrane localized TPK4 and the vacuolar TPKs were performed leading to TPK4 chimeras with the second pore region replaced by that of either TPK2, -3, -5 or the K$_v$-like channel (73). Following expression of these constructs in Xenopus oocytes (FIGURE 7C), the relative permeability and voltage-dependent gating were examined by the two-electrode voltage-clamp technique. The findings indicate that all TPKs function as potassium-selective channels. On the basis of crystal structures, it is generally accepted that four pore regions are needed to form the selectivity filter of a functional potassium channel. In accordance with this feature, based on protein interaction studies, homomeric assembled TPK as well as KCO3 channels (FIGURE 3A) are properly targeted to the vacuolar membrane. In contrast, no convincing evidence exists for the formation of heteromeric channels consisting of TPK/KCO3 subunits (338).

2. TPC1/SV channel

What about the SV channel? As illustrated above TPKs and most likely K$_v$-like channel genes encode the vacuolar VK and FV potassium channels. In patch-clamp studies, the SV channel, however, was identified as nonselective cation channel permeable to physiologically relevant K$^+$ and under salt stress to Na$^+$, too (47, 128, 152, 267). It should be noted that under certain experimental conditions a calcium conductance was observed for the SV channel as well (7, 108, 348; for review, see Ref. 125). To allow channel opening in the physiological vacuolar voltage range, this voltage-dependent, slow-activating channel type of large unitary conductance requires an elevated cytosolic calcium level (FIGURE 3A, C–E) (128). Therefore, it was proposed that this vacuolar cation channel type is involved in calcium-induced calcium release (CICR) (348). This notion, however, is not in line with the fact that cytosolic calcium transients induced by external calcium upshocks do not require functional TPC1/SV channels (147). Thus direct evidences for TPC1/SV channel-catalyzed cytosolic Ca$^{2+}$ signals in planta are still awaited (for review, see Ref. 125).

Screening of the Arabidopsis databases identified the TPC1 gene with two fused subunits in tandem, each subunit related to a voltage-dependent Shaker channel monomer, and two cytosolic EF hands in between (FIGURE 3A) (90). Later, patch-clamp studies with tpc1 loss-of-function mutants proved that TPC1 encodes the SV channel (254). TPC2 represents the TPC1 counterpart in animal systems, where TPC2 localizes in lysosomes (for reference and comparison of TPC1 and TPC2 biology, see review in Ref. 125). The gene expression profile in the tpc1 loss-of-function mutant is reminiscent of profiles that were obtained under potassium limitation (27). This issue and the potassium-dependent features under physiological conditions indicate that the TPC1/SV channel controls the potassium homeostasis of the plant cell. TPC1 is broadly expressed in Arabidopsis, and SV channel channels were found to be active in all plant cell types and species looked at, including early land plants (53, 119). As a great surprise plants lacking TPC1 function seem not to be impaired in growth, development, and physiology (254, 267). This may indicate that the channel is closed most of the time and only opens on demand. Consequently, SV channel mutants that do not properly control the closed state will show a phenotype.
Fou2 represents such a gain-of-function tpc1 mutant. A point mutation results in a hyperactive SV channel (21, 27) with an altered voltage-dependent gating behavior. Voltage-dependent activation of the fou2 channel occurred at \( \sim 30 \text{ mV} \) less depolarized voltages increasing the probability of the channel to be open under physiological vacuolar potentials. As a consequence of a “leaky” channel, the proton pump-derived membrane polarization in fou2 vacuoles should appear to be limited. Mutant plants behave as progressively wounded by, e.g., herbivores foraging. fou2 contains elevated levels of the stress hormone jasmonate and adopts an epinastic phenotype (26).

The fou2 mutation D454N is located in the region between the transmembrane segment 7 and 8, probably facing the vacuolar lumen. Elevated luminal calcium levels block the SV channel but not the fou2 channels (21). Structural modeling and side-directed mutagenesis, followed by patch-clamp analyses, showed that the mutated glutamate at position 454 in fou2 is located within a calcium-binding tetrad of three glutamates and an aspartate. Mutations within this tetrad affect the luminal calcium-binding site of the SV channels and affected sensing of the vacuolar level of this cation as well as its voltage dependence. In line with plants monitoring vacuolar and cytoplasmatic calcium in an independent fashion, the fou2-side mutations leave the cytoplasmic EF-hand-based calcium sensor unaffected (53, 305).

**B. Vacuolar Anion Channels**

In the process of releasing patch-clamp-ready vacuoles from their native environment, where they are embedded in the biochemistry of the cytoplasm, their regulation by proteins such as kinases interacting will “run down” in time. Application of a recombinant calcium-dependent protein kinase (CDPK), however, seems to restore anion channel activity in vacuoles isolated from guard cells of broad bean (253). S-type anion current channels in guard cells as well as that of SLAC/SLAH channels expressed in *Xenopus* oocytes requires the activity of certain protein kinases (100, 101, 233). In patch-clamp studies with the plasma membrane of plant cells, initially anion currents have been observed only rarely (166). The situation changed when the cytosolic free calcium level was elevated or when the external load with weakly buffered cellular calcium, the driving force for divalent cation to enter the cell, was increased (121, 299). Thus anion channels in the plasma and vacuole membranes appear to be tightly controlled by the biochemistry of the cell. To spot anion channels with vacuoles isolated from rather specialized cell types is also a question of using the right anion. In plants, nitrate and malate are required for redox control, pH homeostasis, and even as osmoticum (221). When these physiological-relevant anions were used in the context of the proper cell type, distinct vacuolar anion conductances could be analyzed.

**1. ClCs**

ClCs have been found to operate as anion channels in one system, whereas they function as proton-chloride antiporters in another (55). The *Arabidopsis* genome contains seven genes encoding ClCs. Among them four members, ClCa-c, and ClCg localize to the vacuolar membrane and/or to prevacuolar compartments, while ClCd and ClCf reside in the Golgi apparatus and ClCe was shown to be localized to chloroplasts (206, 212, 358). Lack of ClCa function with *Arabidopsis* mutants has been shown to affect plant nitrate management (96). Patch-clamp studies finally documented that the vacuolar ClC antiporter activity couple transport of nitrate to export of protons from the vacuole (55). Despite that ClCa and -b in the vacuolar membrane work as anion carriers (55, 340), it cannot be excluded that ClCe encodes an anion channel in the chloroplast (262, 296) operating in a Torpedo-like channel mode (262).

**2. ALMT6**

Recently, ALMT6 was found to be expressed in *Arabidopsis* guard cells where it was localized to the vacuolar membrane (223). Patch-clamp studies with vacuoles isolated from wild-type cells identified malate channels, but these were absent in the ALMT6 mutants. Besides malate these hyperpolarization-activated channels are permeable to fumarate, which is present in guard cells in large quantities. ALMT6 mutants did not show a phenotype under standard growth conditions, similar to the mutant lacking TPC1 function (267). This indicates that the channel is only active when transport of malate or fumarate is demanded.

**IV. ION CHANNELS INTEGRATED**

In the previous section, all plant ion channels with known molecular structure have been discussed on the basis of their individual transport properties. The following cell-based systems have been selected to exemplify how plant ion channels are integrated into often-complex functions of cells and tissues. Since our knowledge about cell specificity and abundance of vacuolar ion channels is limited, the focus of this section is on the plasma membrane only. Progress in system biology is tightly coupled to model plants and model cell types.

Currently the major plant model is the weed *Arabidopsis thaliana*, not just because among plants its genome was identified first, but because of the short life cycle and small size, large populations can be screened, and a large collection of mutant lines is available (http://www.arabidopsis.org/). In addition to *Arabidopsis*, major crops such as the gramineae rice, maize, barley, and wheat as well as the solanaceae potato and tobacco have been used for ion channel work.
In terms of model cells, two cell types in the outer layer of the leaf and root should be highlighted in particular: guard cells and root hairs. In the past, numerous screens addressed number, shape, and performance of guard cells and root hairs as well as patterns that they form with common epidermal cells (17, 18, 256). Concerning cell type-specific ion channel biology, mutants impaired in guard cell function and root hair outgrowth and polarity became most interesting.

A. Guard Cells

Guard cells represented the system of choice for the first patch-clamp measurements because they operate isolated. Arranged in pairs within the “stomata” (FIGURE 9A), their ion-based volume regulation system controls water loss of leaves. The current picture about ion channel function and regulation in stomatal action got inputs from two sides: electrophysiological studies with single guard cells (FIGURE 6) and mutants in stomatal performance. Guard cell defects that result in a kind of locked-open stomata phenotype like aba2, ost1, ost2, and abi1 were identified on the basis of a rapidly wilting phenotype when facing drought (219, 238, 355). Mutant plants impaired in stomatal closure could be identified from a population of mutagenized seeds. This open stomata phenotype was easily detectable by an infrared camera because mutants with elevated transpiration appeared cooler than wild type (238, 346). Ion fluxes in guard cells are triggered by a set of signals from the environment such as changes in light quality and quantity, CO₂ concentration, and humidity as well as the water stress hormone ABA received from the plant body during soil drying.

FIGURE 9. ABA activates anion channels in the plasma membrane of guard cells. A: impalement of a triple-barrelled microelectrode for voltage- and current-clamp recordings (Iₘ, Vₘ) into a guard cell that forms with a sister cell a unique stoma in the leaf epidermis (see also D). The third barrel can be used for loading the guard cell with a fluorescent dye (shown here) or with ABA (as done in C, indicated by ABAᵦᵣᵳ) via iontophoresis. B: reversible changes in the free-running membrane potential Vₘ of a guard cell exposed to 20 μM ABA and darkness (as indicated by the bars below the trace). Note that K⁺ conductances were eliminated with Ba²⁺ in the external solution and Cs⁺ in the pipette. C: guard cell current response to externally and cytoplasmically applied ABA (ABAᵦᵣᵳ, ABAᵦᵣᵢ, respectively). Recorded ABA-induced anion current transients were triggered with 20 μM ABA externally, as indicated by the black bar over the trace, and subsequently through cytosolic injection of ABA. ABA was current-injected via the third barrel, filled with 100 μM ABA, during a 30 s loading pulse (arrows). Note that during injection the membrane potential remained clamped at −100 mV, the inward loading current via the third barrel therefore is compensated by an outward current. [B and C modified from Levchenko et al. (199), with permission from the National Academy of Sciences.] D: ABA is perceived in guard cells via an intracellular receptor (R) leading to release of anions.
Taking advantage of open stomata mutants in combination with guard cell-specific signals, guard-cell ion channels and signaling pathways were analyzed.

1. Signaling elements

A) ABA SYNTHESIS. One of the first wilting mutants identified such as aba2 and -3 could be associated with defects in synthesis of the water stress hormone ABA (177, 178). Soil drying or drop in relative humidity triggers the synthesis of ABA (312, 353). When ABA is received by guard cells, it induces release of potassium salts and in turn loss of water, leading to reduced guard cell volume and hence to stomatal closure (268). As a result, transpirational water loss is terminated. However, this process does not take place with plants unable to produce ABA or abi/ost mutants that are deficient in sensing the stress hormone.

B) ABI PROTEIN PHOSPHATASE. ABA via gene expression is controlling plant development that includes adaptation of stomatal performance to changes in the environment in the long term as well as fast stomatal movement to sudden changes in signal strength. This paragraph is restricted to the latter gene expression-independent fast ABA signaling (for reviews on ABA and gene expression, see Ref. 117). ABA-insensitive mutants, found to be severely impaired in stomatal closure, were abi1 and abi2. A point mutation associated with the abi1 phenotype results in a gain-of-function of a PP2C-type protein phosphatase (178, 196, 198, 220). In contrast, abi1 loss-of-function mutants appear to be hypersensitive to ABA (106, 218).

C) OST1 PROTEIN KINASE. The open stomata mutant ost1 is lacking a SnRK-type (OST1/SnRK2.6/SnRK2E) protein kinase (10, 238). GCA2 (growth control exerted by ABA) represents a mutant likely resulting from the impaired activity of a calcium-dependent protein kinase.

D) ABA RECEPTOR. The ABA receptor was discovered by two approaches: 1) a screen for ABI1 interacting proteins (207) and 2) chemical genetics using the ABA agonist pyrobactin (251). The identified cytosolic ABA receptors derive from a subfamily of putative ligand-binding proteins in the START domain superfamily. PYR/RCAR-PP2C complex formation leads to inhibition of PP2C activity (52, 159), thereby allowing activation of SnRK2s (89).

2. Protons and ion channels: pumps and leaks

While the mutants addressed above are defective in detecting the input signal ABA, here mutants impaired in guard-cell osmolyte balance will be addressed. The major ions contributing to volume and turgor changes in guard cells are represented by potassium and the anions chloride, nitrate, malate, and even sulfate. In some situations sugars instead or in addition to ions might serve to power the osmotic motor (323).

A) PROTONS: OST2. With guard cells in particular and plant cells in general, the plasma membrane P-type ATPase pumps protons out of the cytosol. The activity of the proton pump generates a membrane potential (24, 126) and acidiﬁes the cell wall space around the guard cell. Besides serving housekeeping functions, the guard cell H⁺ pump is targeted by blue light (BL) signaling (11, 213). Upon BL stimulation, BL receptor kinases phototropin 1 and 2 trigger phosphorylation of the H⁺-ATPase (169, 170). Phosphorylation of a distinct motif of the P-type ATPase triggers binding of 14–3-3 proteins and increases proton-pumping activity. When analyzing the defect associated with the ost2 mutant, a guard cell expressed P-type ATPase AHA1 was identiﬁed (219). Due to mutations, this pump appeared to be hyperactive, likely arresting the plasma membrane in a far-hyperpolarized state.

B) POTASSIUM: KINLESS AND GORK. Membrane hyperpolarization of guard cells activates inward-rectifying potassium currents (270, 301). Guard cells express the Shaker channels KAT1, AKT1/AtKCl1, and AKT2 (FIGURE 3, A AND B) (321) known to shuttle potassium into hyperpolarized cells. Among them KAT1, or in some Arabidopsis ecotypes Kat2 (151), is assumed to comprise the major component of the guard cell inward rectiﬁer. The KAT1::En-1 knock-out mutant, however, did not appear to be impaired in stomatal opening (321). In patch-clamp studies, the guard cell inward rectiﬁer of wild-type plants, but not of AKT2 loss-of-function mutants, was sensitive towards block by external calcium loads (151). This behavior of the mutant suggests that different plant Shaker subunits form a functional heteromeric K⁺ channel (70, 192). This notion is underlined by the fact that coin expression of KAT1 together with the AKT2 homolog VFK1 and mutated KAT1 results in channels carrying properties of both Shaker-types (1, 67). Kinless is the name of a transgenic Arabidopsis kat2–1 knockout line which under a guard cell-specific promoter expresses a nonfunctional KAT2 subunit. This dominant-negative approach resulted in guard cells that neither show inward potassium currents nor drive proper stomatal opening (193).

C) CHLORIDE AND NITRATE: SLAC1 AND SLAH3. Single Shaker channel subunit mutations have not been detected in screens for improper stomatal action, because of redundancy with respect to KAT1, AKT2, and AKT1/ATKC1.
expressed in guard cells. The SLAC1 mutant, however, was identified as an essential element for ozone-insensitive guard cell performance. Patch-clamp studies with slac1–3 mutants revealed that guard cells lack S-type anion currents under chloride-based conditions (333), but not in the presence of nitrate that is carried by SLAH3 (FIGURE 5B) (99). To activate SLAC1 in the Xenopus oocyte system, the calcium-independent SnRK protein kinase OST1 or the calcium-dependent kinase CPK23 or CPK21 have to be present (100, 101). SLAH3 in oocytes does not respond to OST1 but to phosphorylation by CPK23 and -21, two kinases showing differential sensitivity towards cytosolic calcium changes (41, 318). The respective phosphorylation sites on SLAC1 and SLAH3 have been identified. Furthermore, oocyte studies and in vitro analyses have shown that ABI1 suppresses kinase action with SLAC1 and SLAH3 (99, 100).

### D) MALATE, SULFATE, AND QUAC1
Malate is the product of CO2 fixation based on PEP carboxylase activity (249, 271). During stomatal closure, the organic anionic osmolite is either released from guard cells or degraded (335). Malate and sulfate are anions transported by QUAC1, and mutants lacking QUAC1 function appear to be impaired in stomatal closure (222). QUAC1 represents a component of the R-type current in guard cells, involved in transducing CO2 changes in the atmosphere to changes in stomatal aperture. R-type channels are sites that feed forward “malate release-induced” malate channel activation (124, 126).

### 3. Stomatal movement

#### A) OPENING.
The outer layer of leaves is sealed by a gas tight wax called “cuticle.” Guard cell pairs form “stomata, microscopic little sphincters, for exchange of gases across the epidermis (FIGS. 68 AND 9A). Embedded in the epidermis that covers the inner photosynthetic tissue, stomata control the entry of CO2, the building block of carbon fixation. Opening and closing of the stomata dramatically change the resistance for the entry and release of gases but do not alter the selectivity of these pores. Thus the intake of CO2 is inevitably coupled to “transpirational” loss of water vapor from the leaf. Changing the stomatal aperture as a response to changes in the environment, plants can optimize the gain of carbon and loss of water (water use efficiency).

The ratio of wavelength associated with blue and red spectrum of the sunlight changes in a diurnal fashion. Triggers for stomatal opening cover two types of signals: 1) the spectral densities and the intensity of the sunlight and 2) current status of photosynthetic apparatus of CO2-fixing cells inside the leaf. Guard cells sense blue and red light in an independent manner. Changes in BL are directly monitored in guard cells by a pair of phototropins (169). The sites of red light sensing are the chlorophylls embedded in the photosynthetic membrane of the chloroplast. Fluctuations in red light intensity as they occur between day and night directly affect CO2 fixation in the leaf. Guard cells sense the change of CO2 concentration in the substomatal cavities, a measure for red-light-use efficiency of the photosynthetic common leaf cells, and adjust the stomatal aperture accordingly (285). In this respect, the signal “low CO2 level” within the leaf, photosynthesis running out of substrate, triggers stomatal opening. This signal is strong, since stomata of darkened leaves open at low CO2 levels in the atmosphere (269). To date, the CO2 sensor of guard cells remains unknown, but a distinct carboanhydrase appears to play a central role in this process (144).

Exposure to BL sensed via the phototropins leads to activation of guard cell P-type H+-ATPase and inhibition of SLAC/SLAH-type anion channels. Increasing the pump activity and closing the membrane leaks causes the plasma membrane to hyperpolarize (213, 284). As a matter of fact, KAT/ALK1-type channels open as a function of voltage changes and potassium moves along its electrochemical gradient. Potassium transport is accompanied by proton-coupled chloride, nitrate, and sulfate uptake as well as synthesis of the divalent organic anion malate (110, 248, 271). Following the accumulation of potassium salts and osmotic water, guard cells swell along their axis, pushing each other apart thereby opening the stomatal pore between them (the online version of this article includes supplemental data; see supplement movie 1).

#### B) CLOSURE.
Stomatal closure in terms of ion movements represents a guard cell-operated process inverse to the one underlying the opening of the stomatal pore. In this respect, the light-dark transition and increase in intercellular CO2 concentration are closing signals reflecting an idle or inefficient-working photosynthetic apparatus. Following the strategy “no photosynthesis why losing water”, deflation of paired guard cells causes the closure of the stomatal pore. During soil drying, the limited water availability is reported to guard cells via the stress hormone ABA (312, 353). Water loss to the atmosphere, i.e., “transpiration,” is driven by the water-vapor gradient across the open stomata. Guard cells, placed in the interface between leaf and atmosphere, are the first to experience changes in water vapor content of the air. Apart from soil drying, water stress resulting from a drop in humidity represents a closing signal as well (355), which depends on the ability of guard cells to generate ABA autonomously (H. Bauer, University of Würzburg, personal communication).

#### C) ABA SIGNALING.
ABA is sensed by a receptor Pyr/Pyl/RCAR (here named RABA) localized in the cytoplasm (FIGURE 10A) (207, 251; for review, see Ref. 159). Guard cells can recognize changes in signal intensity received from roots and leaves, but ABA has to cross the plasma membrane and meet the guard cells’ RABA (for ABA transport; see Refs. 162, 185). In impalement-microelectrode studies, cytosolic application of ABA activates guard cell anion channels without delay, while external hormone stimulation delays
this process (FIGURE 9, B AND C). When \( R_{ABA} \) binds the hormone, it becomes activated and inactivates the signaling protein phosphatase ABI1 (FIGURE 10A, LEFT) (255). ABI1 controls a set of protein kinases in a way that they remain inactive in the absence of ABA. In the process of ABA signaling through ABI1 inhibition OST1 on the one hand and CPK23/21 on the other are released. As a result, SLAC1 is addressed by the SnRK kinase in a calcium-independent manner, but by CPKs in a calcium-dependent one (FIGURE 10A, LEFT) (100, 101). SLAH3, when primed by increasing nitrate levels received from the root, also is addressed by calcium-dependent protein kinases, but not by OST1 (FIG-
brane NADPH oxidase AtrbohF, which together with AtrbohD represent the two major NADPH oxidases responsible for H₂O₂ production in guard cells (186, 246, 315). Therefore, this SnRK could bridge the calcium-dependent and -independent branch of fast ABA signaling in guard cells (186). It is thus tempting to speculate that ABA induces a background oxidase activity via OST1. The initial ROS signal in turn can activate the calcium channel, and the second messenger enters the cytoplasm to meet the EF hands of the NADPH oxidase. In a kind of feedforward response, the calcium transients are coupled to ROS production and vice versa. This model puts OST1 into a kind of key position, explaining the fact that mutants lacking OST1 function are unable to close their stomata, because both the calcium-independent as well as -dependent ABA signaling for anion channel activation is blocked (FIGURE 10A).

When SLAC/SLAH anion channels open, the membrane depolarizes (FIGURE 10A, LEFT) and activates QUAC1 and GORK. As a result of a concerted action, K⁺ and anions are released and guard cells deflate. Feeding ABA via the petiole of intact leaves, stomatal action sets in when the hormone arrives at the guard cells. Following stimulus onset, stomata half-close within 5–10 min and significantly reduce transpiration.

B. Root hairs

Root hairs in particular and cells in the root epidermis and cortex in general are involved in nutrient uptake from the soil (195). The outgrowth of root hairs occurs in a polar fashion, just as in pollen tubes. It is guided by an external electrical field, with proton and calcium influx accompanied by anion efflux at the tip and inverse fluxes at a more basal part (for review, see Ref. 224). Polar growth depends on the electrochemical potential of the transported ion species and localization of the respective ion channel, carrier, or pump relative to apical-basal topology of the cell. Thus ion-channel function and targeting likely control outgrowth and shape of a root hair (35). Growth velocity and final length of root hairs and of the entire root are dependent on the concentration of nutrients such as potassium (3, 279). Recently, glutamate receptor-like genes were shown to guide the polar growth of pollen tubes (226). Because of the similarity in growth patterns, it is feasible that GLR channels are also important for shaping root hairs.

1. Potassium channels

In patch-clamp studies, the potassium-dependent conductance of root hairs can be decomposed into the activity of an inward and outward rectifier (149). Arabidopsis root hairs express three genes encoding the Shaker channel isoforms AKT1, AtKC1, and GORK. When expressed in Xenopus oocytes they give rise to hyperpolarization-activated K⁺ currents on the AKT1/AtKC1 side and depolarization-activated K⁺ currents on the GORK side. While functional expression of GORK in animal cells does not require proteins interacting with the outward rectifier, AKT1 is only active when coexpressed with a calcineurin B-like CBL calcium sensor and CBL-interacting protein kinase CIPK (98, 201, 356). CIPK23 was identified in Arabidopsis screens for low potassium tolerance, and CBL1 and -9 were found to interact with CIPK23 in the yeast-2-hybrid system. Mutants for both CIPK23 and AKT1 have similar potassium starvation phenotypes.

With calcium present in the soil or growth medium, plants have been shown to better tolerate low potassium levels (76, 311). Why? Like all plant cells, root hairs operate a hyperpolarization-activated, Ca²⁺-permeable, and H₂O₂-sensitive channel (ROSICa) of presently unknown molecular nature. It is assumed that upon potassium starvation H₂O₂ is generated, which induces a channel-mediated calcium influx (60). Elevated cytoplasmic calcium doses should be sensed by CBL, and in turn the calcium-bound form would activate the associated protein kinase (FIGURE 10B). Upon assembly with their interacting CIPK, the CBL/CIPK complex is targeted to the plasma membrane to activate the AKT1 channel in a phosphorylation-dependent manner (FIGURE 10B) (123, 194, 356). While AKT1 phosphoryla-

FIGURE 10. ABA signaling. A: fast ABA signaling in guard cells. Schematic view of the fast ABA signal transduction pathway, including ABA receptor (PYR/PYL/RCAR, here abbreviated as RABA), PP2C phosphatase ABI1 and 2, SnRK2 kinase OST1, Ca²⁺-dependent kinases CPKs, S-type anion channel SLAC1 and SLAH3, as well as R-type anion channel QUAC1. The PYR/PYL/RCAR receptor family is homologous to the Bet v 1-fold and START domain proteins. Mechanics of ABA binding to PYR/PYL/RCAR receptors bear some resemblance to the binding of gibberellins to GID1 receptors. In the absence of ABA, the PP2C phosphatases inhibit the activity of protein kinases, and anion channels remain silent. In the presence of ABA, however, the stress hormone is recognized by cytosolic ABA receptors. ABA bound to the receptors in turn inhibits the activity of PP2C phosphatases relieving the inhibition of OST1 and CPKs. Specific phosphorylation events activate anion channels. Anion efflux through SLAH3, SLAC1, and QUAC1 leads to depolarization of the guard cell membrane potential and in turn stimulates the outward-rectifying potassium channel GORK to release K⁺, leading to loss of turgor pressure in the guard cells and stomatal closure. The scheme also illustrates the slow ABA-induced expression of drought stress-related genes. In the absence of ABA, the receptor cannot bind to the 2C phosphatases that prevent both autophosphorylation and cross-phosphorylation of the SnRK2 protein kinase. In the presence of ABA, RABA binds PP2C phosphatases and covers the phosphatase’s active site. This permits the autophosphorylation of OST1 and phosphorylation of transcription factors (TF). In its active state TF binds to an ABA-responsive element (ABRE) in the promoter of ABA-responsive genes. B: schematic view of “low K⁺ signaling” in roots. Upon K⁺ depletion, a calcium signal is triggered that activates distinct CBLs. Calcium-bound CBLs target individual CIPK to the plasma membrane and address AKT1/AtKC1 channel complexes. Following CIPK-dependent phosphorylation, AKT1 becomes active and gates open by hyperpolarization. nm, Nuclear membrane; pm, plasma membrane.

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tion leads to channel activation, channel dephosphorylation by the PP2C phosphatase AIP1 inactivates AKT1 (194). On the basis of patch-clamp studies on root hair protoplast from wild-type as well as AKT1 and AtKC1 mutants, AKT1 can be classified as voltage-dependent inward rectifier (similar to KAT1) (FIGURE 3A) and AtKC1 as its corresponding regulatory α-subunit (FIGURE 11A) (98, 272). Voltage-dependent activation of AKT1 sets in at about −80 mV, and it is only weakly dependent on the K⁺ gradient. Under potassium starvation, the equilibrium potential (E_K) is far negative of AKT1’s activation threshold (with 100 μM K⁺_soil and 100 mM K⁺_cyt, E_K is about −180 mV). Under these conditions, voltage-dependent activation of AKT1 elicits a pronounced K⁺ efflux. In planta, however, heteromeric assembly of AKT1/AtKC1 shifts the activation threshold of the root K⁺ uptake channel negative of E_K. Therefore, potassium loss to potassium-depleted soils is prevented (98, 154, 272, 345). What happens when root hairs adapted to this situation face heavy rain falls that suddenly shift the root’s environment from high to low salinity and osmolality? The growing tip of the root hair is protected from burst by a thin wall only. To limit osmotic uptake of rainwater, root hairs have to release osmolites. Here potassium release by GORK channels is required (FIGURE 11A). What about the release of anions accompanying potassium? In line with previous studies (61, 281) in the author’s group, recent patch-clamp studies have identified S-type and R-type anion currents and gene expression of SLAH-type and QUAC-type genes.

C. Long Distance Transport

Two systems have been chosen as examples to integrate ion channel properties into cellular function: guard cells exposed to the atmosphere, and root hair cells in direct con-

![FIGURE 11](http://physrev.physiology.org.org)

**FIGURE 11.** Radial ion transport in root. The cartoon on the right shows simplified transverse section through an *Arabidopsis* root. A: root hair equipped with the inward-rectifying K⁺ channel unit AKT1/AtKC1, the outward K⁺ rectifier GORK, and SLAH-type anion channels. B: polar transport across the endodermis cell layer. Note that a trans-endodermis potential difference exists between the apical and basal membrane. Endodermis cell carrying AKT1/AtKC1 and NO₃⁻/H⁺ symporters (NRTs) for K⁺ and NO₃⁻ uptake from the soil side and SKOR together with SLAH-type channels for their transport towards the xylem.
tact with the soil. How is transport of solutes and signals from root to shoot and vice versa accomplished? Given that even in a small plant like Arabidopsis guard cells on the outer leaf surface and hairs in the root’s periphery are several centimeters and cell layers apart, long distance transport is required.

1. Radial root transport

Even in a simple structure such as the Arabidopsis root, nutrient ions have to pass several cell layers to reach the xylem, vessels for unidirectional long distance transport of water and solutes (FIGURE 11). Transport occurs through the rhizodermis, several layers of cortex cells, the endodermis, as well as parenchyma cells facing the vascular tissue. According to the symplast theory (48), cells are connected via plasmodesmata, forming a continuum that extends from the rhizodermis into the stele and allows radial diffusion of nutrients from the site of uptake to the site of release into the xylem vessels. Solutes can travel from cell to cell but also among the cell wall compartment, the apoplast. Crossing the plasma membrane, for entry into the root cell cytoplasm (symplasm), represents the major barrier for any solute. Solutes traveling the cell wall route, however, finally face the endodermis as major barrier (FIGURE 11A). The “Casparian strip” (287) renders a cell wall compartment of cells juxtaposed within the ring of endodermis cells impermeable (287). Thus molecules have to pass the plasma membrane barrier at the outer surface of this cell layer. At the level of the endodermis, ions taken up from the soil thus have to be loaded into the cytoplasm. To finally enter the xylem pipeline, cargo has to exit across the plasma membrane of parenchyma cells surrounding the vessels. To reduce complexity of the radial transport system, let us focus on the steps on-going in the endodermis. The endodermis is constructed analogous to a tight polar epithelium (4): on the apical “soil” side solutes are taken up, and on its basal “xylem” side they are released. Transepithelial transport in animal systems is accomplished by polarization, in terms of differential furnished membranes at both sides of the cell (189). The process of crossing the two membranes and cytosol in between is comparable to the transcellular movement from the cell wall space to the vacuole lumen (CF. FIGS. 7A AND 11B). Indeed, polar localization of carrier proteins was demonstrated for proteins engaged with transport of boron, silicon, as well as Casparian strip formation (287, 322, 357). To enable transport of molecules of opposite charge, one has to assume for the apical side that 1) the plasma membrane is hyperpolarized; 2) K⁺ is driven by the electrical field and transported by channels, while one cannot exclude that K⁺ is transported in cotransport with protons as well (44); and 3) anions enter this side via H⁺-coupled cotransporters (326). However, to release cations and anions at the basal side, one has to assume that this plasma membrane is depolarized and therefore able to drive transport of both ion species via channels on the basis of the electrochemical gradient. Is there evidence for a transepi-

thelia potential? The voltage drop between the xylem and the bath has been named “trans-root potential” (349). It can be determined, by placing electrodes into a xylem vessel and a cortical cell. The trans-root potential thus represents the potential difference between the two membrane sides of the endodermis layer, which are of opposite polarity (74). Based on such microelectrode recordings, potential differences of up to −70 mV were obtained, depending on the plant species studied (349).

What about the ion channels involved? When root samples are treated with cell wall-digesting enzymes, the cells lose their shape and polarity. Inward- and outward-rectifying currents were recorded with cell wall-free protoplasts, obtained from xylem parenchyma cells, when patch clamped with K⁺-based buffers. As mentioned above, the major root inward-rectifying channel is encoded by the AKT1, together with the AtKC1 gene product. It is thus tempting to speculate that AKT1/AtKC1 mediates K⁺ influx on the apical side of the endodermis cell (FIGURE 11B). Nitrate uptake into the root in general and the stele in particular is mediated by NRT1.1 (104, 184). Interestingly, another nitrate transporter, NRT2.4, showed a polar localization in the lateral root epidermis (168). The fact that in Arabidopsis SKOR has been shown to be expressed in parenchyma cells facing xylem vessels and skor mutants appear to be impaired in potassium delivery to the shoot, would indicate that the SKOR gene encodes the stelar K⁺ outward rectifier (95). On the basis of the same line of evidence, S-type nitrate currents in xylem parenchyma (281) could result from SLAH3-like channels (99).

2. Root-shoot transport

Besides potassium and other major and minor nutrients, leaves receive ABA and nitrate via the xylem network (49, 309). ABA is reporting about the water status of the root and shoot system (352), and the nitrate-glutamine ratio reflects the current capacity for nitrate reduction (42, 137, 171, 202, 264). Stomata appear to be separated from the endings of vascular stands by the substomatal cavity. This large intercellular gas space represents an aerosol fed by xylem water and sensitive to transpiration. Guard cells receive potassium and anions from the root via the transpiration stream and solute exchange with neighboring cells. Sugars are taken up by guard cells from the exudate of photosynthetic cells, which are in close contact with the stomatal complex. Mature leaves are often regarded as “source” for carbohydrates, since they export sugars to “sinks” such as roots. The surplus sugar produced by mature leaves is channelled in the form of the disaccharide sucrose to the sinks via the phloem network (FIGURE 11, A AND G) (292, 334). In contrast to the water flow in the xylem (258), the phloem transport is bidirectional in nature (78). Volume flow is driven by osmotic forces generated in the source phloem (236, 331) that pumps sugar into the

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system. This sweet load in sink phloem is released for maintenance of cell viability and cell proliferation.

A) PHLOEM LOADING. Apoplastic phloem loading is accomplished by a module located in the companion cells feeding sugar into the interconnected sieve tubes (FIGURE 12A). The module is based on three components such as 1) the ATP-powered H⁺ pump AHA3 generating the proton gradient and electrical field that drive 2) a SUC2-type sucrose carrier as a symporter coupling sucrose flux to the proton-motive force PMF (FIGURE 12C) (28, 36). The acidic pH of the loading site at the plasma membrane of the companion cell and its negative membrane potential provide a proton-motive force that enables more than 100-fold accumulation of sucrose. In sieve tubes of the source phloem, a sucrose content of ~1 M or more has been measured (204, 205). In the long run, however, SUC2 activity directing sucrose together with protons into the companion cell will depolarize the phloem potential (FIGURE 12B). 3) The third component of the sugar loading module, the Shaker potassium channel AKT2, is required to prevent membrane depolarization (57). When this Shaker channel stays in the nonrectifying “leaky” mode rather than in the inward-rectifying state, the membrane potential is clamped near the Nernst potential of potassium. This potassium battery is assumed to drive sucrose loading under conditions when ATP is limiting (93).

B) PHLOEM UNLOADING. At the site of sucrose unloading in sinks, the external sugar level is extremely low, the cell wall pH is less acidic, and the membrane potential more depolarized compared with the source site (1, and references cited therein). These conditions favor release of sucrose from the sieve tubes. How is this achieved? ZmSUT1, the SUC2-type carrier from Zea mays, has been shown to operate as a perfect thermodynamic machine: under source conditions, ZmSUT1 mediates sucrose

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**FIGURE 12.** Phloem transport. A: fluorescence image: Arabidopsis leaf expressing GFP under control of the promoter of a phloem-localized sucrose carrier (AtSUC2). B: phloem loading reconstituted in Xenopus laevis oocytes by expression of sucrose transporters (ZmSUT1) and K⁺ channels (KZM1 or ZMK2) from maize. Upon application of sucrose with just ZmSUT1 expressed oocytes, H⁺-coupled sucrose uptake depolarizes the membrane. When ZmSUT1 was expressed together with the AKT2-orthologous K⁺ channel ZMK2, the depolarization is prevented, but not with the hyperpolarization-activated inward rectifier KZM1. [From Philippar et al. (259), Copyright 2003 American Society for Biochemistry and Molecular Biology.] C: sucrose (S) loading into companion cells creates an osmotic potential for volume flow of the sugar moiety along a given phloem pipeline. Depicted are cell-cell bridges (plasmodesmata) that interconnect sieve tube elements and companion cells with the latter equipped with the P-ATPase (AHA), sucrose/H⁺-coupled symporter (SUC), and AKT2 channel. Sieve plates between the sieve tubes provide for low resistance volume flow of the K⁺-rich sugar sap from source to sink region of the phloem networks.
loading, while under sink conditions this transporter can release the disaccharide (36).

3. Electrical signaling

**A) SIEVE TUBES: AN AXONLIKE SYSTEM.** In the process of sieve tube development, the sieve-element mother cells degrade vacuoles and other major organelles (334, and references therein). Coupled to the companion cells but otherwise symplastically isolated from the surrounding tissue, sieve elements do not only enable the bulk flow of sucrose and electrolytes but also transmit electrical signals. In analogy to an axon, adjacent sieve tube elements, containing a high saline cytoplasm, are interconnected by “sieve plates.” This low-resistance pipeline, filled with 80 mM K+/H11001- based electrolyte and separated by the plasma membrane from the extra-tube low-saline-fluid, provides for proper electrical conductance in and transmission along the “green axon” (85, 86, 275, 343, 344) (cf. Chara; Ref. 91). Note that in response to wounding, action potentials are transmitted in the phloem cables (86–88).

**B) MICROBES POLARIZE.** Ion movements and electrical signals accompany the interaction of plants with their biotic environment (FIGURE 13A). Thereby, bacteria and fungi are recognized by conserved microbe-associated molecular patterns (MAMPs) such as flagellar proteins, elongation factors, or chitin fragments (25). In the process of invading the host, MAMPs are recognized by cell surface receptors (e.g., flagellin Flg) by the receptor kinase FLS2. Binding of FLS2 to the Flg22 epitope triggers gene expression associated with the plant innate defense response (FIGURE 13A) (359). Bacterial attack results in change of pH and an oxidative burst (FIGURE 13A) (58, 241). In animal cells, outward-directed electron pumping of the NADPH oxidase is compensated by proton release into the pH-neutral extracellular environment via depolarization-activated Hv1-type proton channels (75). Plant cells live in an acidic environment due to proton pumping via the P-type ATPase. When facing pathogens, the oxidative burst with plant cells is accompanied by alkalinization of the external compartment (81). Plants have Hv1-type channels, too (316, 325), but so far it is not known whether they mediate proton influx into a rather pH neutral cytoplasm. The early steps in signaling, however, are associated with an increase in cytosolic calcium and concomitant depolarization of the host cell (FIGURE 13) (157). The depolarizing ionic conductance very likely represents an anion channel. A similar “elicitor-induced channel type” was triggered by Elf18, an active epitope of the elongation factor Tu, and by damage-associ-
ated molecular pattern (DAMP) peptide 1 and 2 (PEP1 and PEP2) (183). These observations provide the basis for the speculation that signals derived from different molecular patterns are directed via a variety of receptors to the “elicitor channel,” leading to pathogen recognition and defense. The nature of the channel is not known yet, but guard cells suffering from infection with powdery mildew fungus *Blumeria graminis* activate S-type anion channels (174).

V. COEVOLUTION OF CHANNELS AND SIGNALING

Did ion channels and the signaling pathways that are regulating them coevolve? This issue is exemplified on the basis of guard cell anion channel SLAC1 and water stress signaling. When during evolution plants transitioned from an aqueous environment to land, they were faced with drought periods. To survive such episodes of water depletion, they had to develop strategies to overcome drought stress. The first land plants were mosses that evolved 400 million years ago (FIGURE 14). The genome of the model moss *Physcomitrella patens* has been sequenced and compared with that of the higher plant *Arabidopsis* (273). Furthermore, knock-out/down approaches for both moss and higher plant exist. Thus related genes can be easily transformed into an *Arabidopsis* null background, providing a platform for functional analyses of ion channels via cross-species exchange (cf. Ref. 53). In terms of evolutionary distance, *P. patens* is related to the flowering plants as *Drosophila melanogaster* is to humans. Plants appear to be composed of two major lineages, which branched from a unicellular processor (FIGURE 14). One of these major lineages contains the land plant *Arabidopsis*, the moss *Physcomitrella*, as well as the green alga *Chara*. For mosses, the need for desiccation tolerance was particularly critical for survival on land. They evolved an ABA-based strategy to overcome dehydration.

Gene homologs to SLAC/SLAH and GORK/SKOR, which control anion and potassium release in *Arabidopsis* guard cells, can be identified in *Physcomitrella* (www.plantgdb.org/PpGDB). Furthermore, genes homologous to PYR/PYL/RCAR, ABI1, and OST1 encoding regulatory components of guard-cell ABA signaling in *Arabidopsis* were identified in *P. patens* (38). In early land plants, these are likely to induce transcriptional changes that direct the synthesis of dehydration protectants named late embryogenesis abundant (LEA) proteins (FIGURE 10A, RIGHT) (172, 209). In vascular plants, the LEA proteins have been found to be associated with salt and osmotic stress, and some of these genes also appear to be upregulated in response to ABA treatment (79, 161, 197, 237, 350). In *Arabidopsis*, as well as the moss *P. patens*, ABI3 genes represent transcriptional regulators of ABA-dependent LEA gene expression (211). This finding may indicate that ABA signaling, concerning formation of drought protectants in moss and higher plant is conserved.

**FIGURE 14.** ABA signaling, in the light of plants’ evolution. Phylogenetic tree of plant life: lineages of aquatic algae, bryophytes, and vascular plants. Stomata arose early in the evolution of land plants and optimized CO$_2$ uptake and transpirational water loss. Stomatal aperture is controlled by ABA, a hormone associated with dehydration tolerance, already before evolution of stomata.
What about stomata? While Physcomitrella carries some on the sporophyte (38), the gametophyte of Selaginella shows stoma densities (153) and stomatal responses to ABA that are comparable to those of higher plants (288). Thus stomata-controlled plant water balance occurred after the emergence of mosses. The role of the few guard cell pairs at the bottom of the sporophytes, however, is not yet fully understood, but components of guard-cell ABA signaling in Arabidopsis seem to share functions with related gene products in P. patens. The fact that the ABA signaling kinase PpOST1 from the moss rescues the Arabidopsis ost1 mutant seems to indicate that the molecular and guard cell-directed function of OST1 has been conserved since the divergence of vascular plants and mosses (38). In Arabidopsis, OST1 activates SLAC1 triggering stomatal closure (101). To unequivocally demonstrate the guard cell function of the moss OST1, one thus has to demonstrate that PpOST1 activates the P. patens SLAC1 homolog via phosphorylation. Given that core regulatory components involved in guard-cell ABA signaling of vascular plants are operational in mosses, they most likely originated in the last common ancestor of these lineages, prior to the evolution of ferns.

How do you find the last common ancestor? Does this stoma-free ancestor contain ABI1, OST1, and SLAC1 already? Within the bryophytes liverworts are phylogenetically older than the mosses. Liverworts do not yet have stomata (265), but multicellular macroscopic pores that cannot be closed (274). From an ongoing genome-sequencing project with liverworts for Marchantia polymorpha, a representative of the phylum, ESTs are available (http://www.marchantia.org/). Recently, the ABI1 gene has been found in Marchantia (329). When expressed in the moss Physcomitrella patens, the liverwort protein phosphatase was able to regulate ABA-sensitive LEA genes (FIGURE 10A, RIGHT). Before the patch-clamp era, the classical giant green alga Chara was in most cases the system of choice for detailed plant ion channel studies. Chara fires action potentials in response to electrical stimulation or salt stress (314) that is based on sequential activation of anion channels and potassium channels (91, 138, 179). While Chara has an aquatic life-style only, the Klebsormidium algae (FIGURE 14) occur in a very wide range of freshwater and terrestrial habitats (280). These algae of simple filamentous morphology are assumed to have adapted to enter the land. Systematic analysis of Marchantia, Klebsormidium, and Chara genomes will show if they already encode major ABA signaling elements. What if Chara has a SLAC/SLAH-like anion channel already and, Klebsormidium evolved ABA-associated genes to survive dry periods? Then the obvious question is: When was the first SLAC/SLAH-like anion channel co-opted by ABA signaling pathways that evolved to survive desiccation. If so, was OST1 optimized to phosphorylate SLAC/SLAHs or did the prototypic anion channel have to develop an OST1 side? Finally, one would ask how ABA regulation of the anion channel was recruited by guard cells for active control of water balance.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

What is the nature of the plant calcium channel? In plants, as in all living creatures, calcium represents a central element in transduction of a large variety of signals. Without knowing the molecular nature of calcium channels in plasma and endomembrane systems, the knowledge of most signaling cascades will remain incomplete. Plant calcium channels are apparently not related to voltage-dependent calcium channels known from the animal field. Thus ongoing sequencing plant genomes are unlikely to identify calcium-permeable channels. The voltage-dependent plasma membrane calcium channel is activated by ROS and gated open by hyperpolarization. What about the GRL-like and 30 CNCG-like genes? How do you assign them a function? To identify this channel, one might think about a mutant screen suited to detect seedlings lacking H2O2-induced membrane polarization. This screen could take advantage of the genetic membrane potential sensors of the Mermaid type (330), combined with genetic calcium reporters such as aequorin or cameleon (173, 229). Aequorin-expressing plants were shown to be suitable reporters for cytoplasmic calcium changes, associated with elicitor-triggering innate immunity responses. Thus ROS and strong elicitors such as flagellin with calcium signal screens could serve as triggers for calcium channel activation and molecular identification.

Back to the future: sequencing plant genomes will provide information about the ion channel makeup of plants that show outstanding ion channel features. One such plant is the carnivore Dionaea muscipula, “the Darwin plant.” Living on nutrient-poor soils, this plant, also known as “the Venus flytrap,” evolved a bilobed leaf structure for catching animals (FIGURE 15A). The trap closes within a fraction of a second, fast enough to capture flies or other small animals (FIGURE 15B). Sensory hairs on the inner surface of the two lobes recognize creatures visiting the flytrap (FIGURE 15A). When a prey such as an ant touches a sensory hair, an action potential is fired and speeds along the lobe (FIGURE 15, A AND C; see supplemental movie 2). After touching the same, or another sensory hair, twice, in a time window of ~20 s, the second action potential shuts the trap (FIGURE 15B). After having used their mechanosensors and electrical signaling to get the prey, the flytrap explores the potential nutrient source, by taking advantage of its chemosensors (FIGURE 15D). Depending on the taste of the individual prey, a cocktail of lytic enzymes is secreted to degrade it. In case that the object entrapped was not eatable, or after all nutrients have been extracted, the trap will open again, ready to go for a new round. Several obvious questions have remained open, ever since Darwin described the action of the Venus flytrap.

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Klebsormidium

Chara
trap. Is the mechanosensor on the basis of the sensory organ (FIGURE 15C) operated by MS channels? Which ion channels constitute the action potential? How did the trap get so fast? Are the flytrap’s ion channels co-opted by incorporation of prey genes? How are the taste buds and the endocrine system working?

It is time to demystify the Darwin plant!

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