ELSEVIER

Biochimica et Biophysica Acta 1564 (2002) 299–309

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Review

Genes for calcium-permeable channels in the plasma membrane of plant root cells

Philip J. White [a,*], Helen C. Bowen [a], Vadim Demidchik [b], Christopher Nichols [b], Julia M. Davies [b]

[a] *Department of Plant Genetics and Biotechnology, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK*
[b] *Department of Plant Sciences, Downing Street, Cambridge CB2 3EA, UK*

Received 5 February 2002; received in revised form 20 June 2002; accepted 26 June 2002

Abstract

In plant cells, Ca^{2+} is required for both structural and biophysical roles. In addition, changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) orchestrate responses to developmental and environmental signals. In many instances, $[Ca^{2+}]_{cyt}$ is increased by Ca^{2+} influx across the plasma membrane through ion channels. Although the electrophysiological and biochemical characteristics of Ca^{2+}-permeable channels in the plasma membrane of plant cells are well known, genes encoding putative Ca^{2+}-permeable channels have only recently been identified. By comparing the tissue expression patterns and electrophysiology of Ca^{2+}-permeable channels in the plasma membrane of root cells with those of genes encoding candidate plasma membrane Ca^{2+} channels, the genetic counterparts of specific Ca^{2+}-permeable channels can be deduced. Sequence homologies and the physiology of transgenic antisense plants suggest that the *Arabidopsis AtTPC1* gene encodes a depolarisation-activated Ca^{2+} channel. Members of the annexin gene family are likely to encode hyperpolarisation-activated Ca^{2+} channels, based on their corresponding occurrence in secretory or elongating root cells, their inhibition by La^{3+} and nifedipine, and their increased activity as $[Ca^{2+}]_{cyt}$ is raised. Based on their electrophysiology and tissue expression patterns, *AtSKOR* encodes a depolarisation-activated outward-rectifying (Ca^{2+}-permeable) K^+ channel (KORC) in stelar cells and *AtGORK* is likely to encode a KORC in the plasma membrane of other *Arabidopsis* root cells. Two candidate gene families, of cyclic-nucleotide gated channels (*CNGC*) and ionotropic glutamate receptor (*GLR*) homologues, are proposed as the genetic correlates of voltage-independent cation (VIC) channels.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Arabidopsis thaliana*; Ca^{2+} channel; Cyclic nucleotide; Gene; Glutamate; K^+ channel; Plasma membrane; Root

1. The physiology of Ca^{2+} channels in the plasma membrane of root cells

Calcium is one of the most abundant elements in plants. It is an essential macronutrient that plays key structural, metabolic and signalling roles. Plant cells maintain a cytoplasmic Ca^{2+} activity ($[Ca^{2+}]_{cyt}$) in the submicromolar range. Hence, Ca^{2+} influx to root cells from the soil solution, which generally has millimolar Ca^{2+} concentrations, occurs down its electrochemical gradient. Specialised transport proteins, namely cation channels, catalyse Ca^{2+} influx, which not only supplies the Ca^{2+} for metabolic and structural needs but also acts in many signalling cascades. Transient elevations in the $[Ca^{2+}]_{cyt}$ of root cells initiate cellular responses to a variety of environmental, developmental and pathological challenges. In many cases, this occurs through Ca^{2+} influx across the plasma membrane via Ca^{2+} channels [1]. Until recently, reviews of Ca^{2+} channels in plants were restricted to a description of their electrophysiological characteristics, because the genes encoding plant Ca^{2+} channels were unknown [1,2]. However, in the past 2 years, a number of genes (and gene families) encoding putative plasma membrane Ca^{2+} channels have been identified and proposed as

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; BMAA, *S*(+)-β-methyl-α,β-diaminoproprionic acid; CNGC, cyclic nucleotide gated channel; cNMP, cyclic nucleotide monophosphate; DNQX, 6,7-dinotropuinoxaline-2,3(1*H*,4*H*)dione; E_K, Nernst potential for K^+; iGluR, ionotropic glutamate receptor; KORC, outward-rectifying K^+ channel; NMDA, *N*-methyl-D-aspartate; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; NORC, nonselective outward-rectifying channel; NSC, nonselective cation channel; PLB, planar lipid bilayer; P_o, probability of being open; P_X, permeability to ion X; REZ, root elongation zone; VIC, voltage-independent cation channel

* Corresponding author. Tel.: +44-1789-470382; fax: +44-1789-470552.
E-mail address: philip-j.white@hri.ac.uk (P.J. White).

0005-2736/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0005-2736(02)00509-6

the genetic counterparts to the Ca^{2+} channels previously studied electrophysiologically [3]. These include genes encoding outward-rectifying K^+ channels (e.g. *AtSKOR* and *AtGORK*; [4,5]), a wheat Ca^{2+} transporter (*TaLCT1*; [6]), homologues of two pore channels (e.g. *AtTPC1*; [7]), homologues of cyclic nucleotide-gated channels (e.g. *AtCNGC1-20*; [8]), homologues of ionotropic glutamate receptors (e.g. *AtGLR1-3*; [9]) and annexins (e.g. *AnnAt1-7*; [10]). In this article, the electrophysiological characteristics and putative physiological roles of Ca^{2+} channels in the plasma membrane of root cells will be reviewed. These will be compared with information on the pattern of expression of genes encoding putative Ca^{2+} channels within the root, the membrane location of their gene products, functional assays of Ca^{2+} transport in heterologous expression systems and insights to their physiological roles obtained through the use of transgenic plants.

2. Types of Ca^{2+}-permeable channels in the plasma membrane of root cells

Roots are complex structures designed to explore and exploit the soil volume. Their anatomy and architecture respond to both environmental cues as well as innate developmental programmes. A typical transverse section through a root reveals concentric layers of cells [11]. In younger roots, the outer (epidermal and cortical) cell layers are involved in the acquisition of water and solutes. Long root hairs may develop from the epidermal cell layer to increase the surface area available for solute uptake. Solutes must pass either through or around the epidermal and cortical cell layers to enter the stele for transport to the shoot. The endodermal cell layer separates the root cortex from the stele. During root development, a waxy structure termed the Casparian band develops initially between, and eventually over, the endodermal cell layer. This not only acts as a barrier to the extracellular movement of solutes [12], but also prevents Ca^{2+} influx from the extracellular space to mature endodermal cells [13]. Immediately within the stele is a layer of pericycle cells. Both they, and other cells within the stele, are devoted to the loading, unloading and long-distance transport of solutes in the xylem and phloem.

Calcium-permeable channels have been identified in the plasma membrane of root cells by both biochemical and electrophysiological techniques [1,2,14]. Although a role in catalysing nutritional fluxes has been envisaged, their principal roles appear to be in cell signalling [1,2,12]. In addition to depolarisation-activated [1,14] and hyperpolarisation-activated [15–17] 'Ca^{2+} channels', Ca^{2+}-permeable 'voltage-independent' cation (VIC) channels [18] and Ca^{2+}-permeable outward-rectifying K^+ channels [3,19–22] have also been observed in the plasma membrane of root cells.

3. Depolarisation-activated Ca^{2+} channels

A classical depolarisation-activated Ca^{2+} current was identified in protoplasts of root cells from *Arabidopsis thaliana* (Table 1; [23]). This Ca^{2+} current was activated by membrane depolarisation to voltages more positive than about −140 mV, and exhibited slow and reversible inactivation at extreme negative voltages. The channel mediating this current was permeable to divalent cations, including Ca^{2+}, Ba^{2+}, Sr^{2+} and Mg^{2+} [23,24], and had a unitary conductance of approximately 13 pS with 40 mM calcium

Table 1
Calcium-permeable channels in the plasma membrane of root cells

Channel	Permeable cations	Inhibitors of Ca^{2+} flux	Unitary conductance	Expression	Key references
Depolarisation activated	Ca, Ba, Mg		13 pS (40 mM Ca)	Root Root hair	[23,24]
VDCC2	Ca, Ba Cs, K, Rb, Na	La^{3+}, TEA^+, verapamil	40 pS (100 mM Ca) 170 pS (100 mM K)	Root	[1]
rca	Ba, Sr, Ca, Co, Mg, Zn, Mn, Ni, Cd, Cu Na, Cs, K, Li, Rb	Al^{3+}, La^{3+}, Gd^{3+}, Ni^{2+}, TEA^+, verapamil, diltiazem, ruthenium red	29 pS (40 mM Ca)	Root	[27]
Maxi	Ba, Sr, Mn, Mg, Co, Ca K, Rb, Cs, Na, Li	ruthenium red	135 pS (100 mM Ca) 450 pS (100 mM K)	Root	[1]
Hyperpolarisation activated	Ca	Al^{3+}, La^{3+}, Co^{2+}, verapamil	–	Endodermis, REZ cortex, tip epidermis	[15]
	Ba, Ca, Mg, Mn	Al^{3+}, Gd^{3+} La^{3+}	22 pS (10 mM Ca)	Root hair	[16]
	Ca, Sr, Ba Cs, Na, K, TEA	Gd^{3+}	–	Root stele	[17]
VIC/NSC	NH_4, Rb, K, Cs, Na, Li Ca	Gd^{3+}, La^{3+}, Ba^{2+} (Quinine)	5 pS (100 mM Ca) 40 pS (100 mM K)	Roots	[18,21,56]
KORC	K, Rb, Cs, Na, NH_4 Ca	Ba^{2+}, TEA^+	30 pS (100 mM K)	Epidermis, cortex, stele, xylem parenchyma	[19–22]
NORK	Ca, Mg K, Na, Rb, Cs, Li		14–97 pS (120 mM K)	Epidermis, xylem parenchyma	[20,21]

in the external solution (cf. Ref. [25], suspension cells). Unfortunately, depolarisation-activated Ca^{2+} channels occur infrequently, pass little current and their activity is short-lived following the establishment of the whole-cell patch-clamp configuration, since they enter a quiescent state [23]. However, quiescent channels can be fully recovered by depolarisation to extreme positive voltages in a phenomenon known as 'recruitment'. In addition, pharmaceuticals that disorganise microtubules, such as colchicine and oryzalin, stabilise depolarisation-activated Ca^{2+} currents and these currents appear to be constitutively recruited in protoplasts from the *Arabidopsis ton2* mutant, which has a defect in microtubule organisation [23].

Two distinct depolarisation-activated Ca^{2+}-permeable channels have been recorded when plasma-membrane vesicles from cereal roots were incorporated into artificial planar lipid bilayers (PLB). These are termed the *rca* or VDCC2 channel, and the maxi cation channel [1,14,21]. The *rca*/VDCC2 channel opens upon plasma membrane depolarisation to allow Ca^{2+} influx to root cells. It inactivates upon prolonged depolarisation, but this inactivation is slower with increasing depolarisation. Thus, Ca^{2+} influx parallels the extent of plasma membrane depolarisation. In the absence of Ca^{2+}, the *rca*/VDCC2 channel is permeable to other monovalent and divalent cations (Table 1). It has a unitary conductance of about 40 pS in symmetrical 100 mM $CaCl_2$, and a predicted unitary conductance of 34 pS under the ionic conditions employed in patch-clamp experiments with 30–40 mM extracellular calcium [26]. The *rca*/VDCC2 channel is inhibited by a range of pharmaceuticals including micromolar concentrations of Al^{3+}, La^{3+}, Gd^{3+}, verapamil, diltiazem and ruthenium red, but it is insensitive to 1,4-dihydropyridines and bepredil [1,27].

The kinetics of the maxi cation channel resemble those of the *rca*/VDCC2 channel [28]. The channel is closed at the resting potential of the cell, but activates rapidly upon plasma membrane depolarisation. It inactivates upon prolonged depolarisation, with the rate of inactivation slowing with increased plasma membrane depolarisation. The maxi cation channel is permeable to a wide variety of monovalent and divalent cations (Table 1). It has a unitary conductance of 135 pS in symmetrical 100 mM $CaCl_2$, and Ca^{2+} influx dominates the current under physiological ionic conditions [28]. The channel exhibits a complex pharmacology. The inward Ca^{2+} flux through the maxi cation channel is inhibited by 50 μM ruthenium red, but diltiazem, verapamil and quinine at micromolar concentrations and TEA^{+} at millimolar concentrations inhibit only the outward K^{+} flux through this channel [1].

It has been speculated that depolarisation-activated Ca^{2+} channels transduce general stress-related signals [1,14]. This follows since depolarisation of the plasma membrane is not only a nonspecific response to many stimuli, and may occur by one of many diverse mechanisms, but also because it is likely to increase $[Ca^{2+}]_{cyt}$ throughout the cell periphery. A specific role for depolarisation-activated Ca^{2+} channels in the acclimation of chilling-resistant plants to growth at low temperatures has been proposed [28,29]. Such channels may also have a role in microbial interactions. Addition of Nod factors to legume root hairs causes membrane voltage depolarisation and initiation of complex $[Ca^{2+}]_{cyt}$ changes [30]. There is a compelling case for the involvement of a Ca^{2+}-permeable depolarisation-activated channel in this system and it may be significant that the root hair of *Arabidopsis* has been found to contain one [24].

4. Two pore (Ca^{2+}) channel homologues

Candidate genes for depolarisation-activated Ca^{2+} channels are now emerging. A single gene in *A. thaliana* (At4g03560) encodes a protein with homology to the α1

Pore sequence I

Arabidopsis thaliana	ILFTTSNNPDVWIPAY	(AF360372)
Sorghum bicolor	VLFTTSNNPDVWVPAY	(BE597573)
Glycine max	LLFTTSNNPDVWVPAY	(AW234659)
Medicago truncatula	LLFNTANNPDVWVPAY	(AW687005)

Pore sequence II

Arabidopsis thaliana	NGMVTLFNLLVMGNWQVWMESY	(AF360372)
Gossypium arboreum	NGMVTLFNLLVMGNWQVWQESY	(BF272765)
Zea mays	SGMVTLFNLLVMGNWQVWMESY	(AW146773)
Sorghum bicolor	SGMVTLFNLLVMGNWQIWMESY	(BE597150)
Lotus japonicus	NGMVTLFNLLVSEVWLELMESY	(BI418571)
Glycine max	NGIVTLFNFLVTATWDEVMTSY	(BE556570)

Fig. 1. Amino acid sequences in the two pore loops of *A. thaliana* AtTPC1 and its homologues in other plant species. The GenBank accession numbers are given in parentheses.

subunit of animal voltage-dependent Ca^{2+} channels [31] and the yeast plasma membrane Ca^{2+} channel ScCCH1 [32–34]. This gene has been called *AtTPC1*, for *t*wo *p*ore *c*hannel [7] and its homologues are present in many plant species. It is predicted to have 12 transmembrane domains (which compares with the 24 transmembrane domains found in animal voltage-dependent Ca^{2+} channels and ScCCH1), with two internal repeats (S1-6 and S7-12). There is a cytoplasmic loop between S6 and S7 that contains two Ca^{2+}-binding EF-hand motifs, suggesting that $[Ca^{2+}]_{cyt}$ might regulate its activity. Both S4 and S10 contain charged residues and may function as voltage sensors. Pore loops occur between S5 and S6 and S11 and S12, but their amino acid sequences differ from those of any known channel (Fig. 1), making the selectivity of AtTPC1 difficult to predict.

Circumstantial evidence suggests that *AtTPC1* encodes a depolarisation-activated Ca^{2+} channel [7]. Heterologous expression of *AtTPC1* in a yeast mutant lacking ScCCH1 enhances both its Ca^{2+} uptake and growth rate [7]. In *Arabidopsis*, reduced expression of *AtTPC1* prevents the rise in leaf $[Ca^{2+}]_{cyt}$ induced by feeding the plant sucrose, and its overexpression augments it. Since the sucrose-induced rise in $[Ca^{2+}]_{cyt}$ is reduced when antisense H^+/sucrose symporter genes (*AtSUC1* and *AtSUC2*) are expressed, Furuichi et al. [7] have speculated that Ca^{2+} influx through AtTPC1 is activated by plasma membrane depolarisation. *AtTPC1* is expressed highly in green siliques and developing seeds, but it is also present in leaf, stem and root tissues [7].

5. Hyperpolarisation-activated Ca^{2+} channels

Hyperpolarisation-activated cation channels, which are significantly more permeable to Ca^{2+} than to K^+, have been recorded in protoplasts from various root cell types using patch-clamp electrophysiological techniques [15–17]. They have been observed in the apical plasma membrane of *Arabidopsis* root hairs [16], in protoplasts from the endodermis, from the cortex of the root elongation zone (REZ) and from the epidermis of the growing root tip of *Arabidopsis* roots [15] and in protoplasts from the stele of maize roots [17]. Their electrophysiological properties resemble the well-characterised hyperpolarisation-activated Ca^{2+} channels recorded in guard cells [1,35–38]. At physiological $[Ca^{2+}]_{cyt}$, the hyperpolarisation-activated Ca^{2+} channel from root hairs activates at voltages more negative than about -100 mV, but increasing $[Ca^{2+}]_{cyt}$ shifts its activation potential to more positive voltages [16]. The channel is selective for $Ba^{2+}>Ca^{2+}=Mg^{2+}>Mn^{2+}$, as determined from measurements of single-channel conductance, and cation influx is blocked completely by submillimolar concentrations of Al^{3+}, Gd^{3+} and La^{3+}, and slightly by 100 μM nifedipine. This pharmacology is consistent with the dissipation of a $[Ca^{2+}]_{cyt}$ gradient focussed at the apex of root hairs and the consequential inhibition of root hair elongation [39,40]. The hyperpolarisation-activated Ca^{2+} current from cortical cells of the root REZ is similarly activated at voltages more negative than about -100 mV and is inhibited completely by 100 μM verapamil, 100 μM Al^{3+}, 100 μM La^{3+} or 1 mM Co^{2+} [15]. The hyperpolarisation-activated Ca^{2+} current in stelar cells is activated by 20 μM ABA [17]. The selectivity of the Ca^{2+} channel underlying this current is $Ca^{2+}>Sr^{2+}>Ba^{2+}\gg Cs^+$, Na^+, K^+, TEA^+. This current is inhibited by 15 μM Gd^{3+}, and its activation by ABA is prevented by 400 μM Mg-ATP.

It is likely that Ca^{2+} influx through hyperpolarisation-activated Ca^{2+} channels is responsible for the elevated $[Ca^{2+}]_{cyt}$ required for both general and directional elongation growth of root cells, which often have extremely negative membrane potentials. In addition to a possible role in signal transduction, Ca^{2+} influx through these channels may contribute to the depolarisation of the root plasma membrane and to the opening of depolarisation-activated ion channels, including depolarisation-activated Ca^{2+} channels. This would be required, for example, for solute efflux during turgor regulation. The co-localisation of both hyperpolarisation-activated and depolarisation-activated Ca^{2+} channels in the plasma membrane of root cells would certainly provide them with a greater physiological "flexibility", but to date, there is only one report of such co-localisation [24]. The interplay of both channel types, and its consequences for the regulation of membrane voltage, has been considered by Miedema et al. [2]. Almost paradoxically, it is possible that the outward rectifying K^+ channels (which themselves could permit some Ca^{2+} influx, see below) are involved in repolarising the membrane voltage, thereby closing the depolarisation-activated Ca^{2+} channels.

6. Outward-rectifying K^+ channels

Two classes of outward-rectifying K^+ channels (NORC and KORC), and possibly several distinct K^+ channels within each class have been identified in root cells (reviewed in Ref. [21]). The NORC select poorly between cations and anions and are observed less frequently than KORC [20–22]. They activate at extreme positive voltages and, even though increasing $[Ca^{2+}]_{cyt}$ from 150 nM to 5 μM shifts their activation potential to more negative voltages [20], they are unlikely to open under normal physiological conditions.

The KORC are relatively selective for K^+, but also conduct other monovalent and divalent cations, including Ca^{2+} [19–21]. Similar channels have been reported in both leaf mesophyll cells [41] and guard cells [42]. They activate at low positive voltages with a sigmoidal time course and are present in all root cells exhibiting outward-rectifying K^+ currents [21]. The KORC do not inactivate. The voltage-dependent opening of KORC follows changes in

the Nernst potential for potassium (E_K), with the voltage at which $P_o = 0.25$ remaining about 80 mV more positive than E_K at physiological $[Ca^{2+}]_{cyt}$ and a cytoplasmic pH of 7.2 [21,22]. In addition, the unitary conductance of KORC increases anomalously as extracellular K^+ ($[K^+]_{ext}$) is increased, perhaps through an allosteric interaction [19,20]. An increase in $[Ca^{2+}]_{cyt}$ reduces the occurrence of KORC indirectly, possibly through the actions of Ca^{2+}-dependent kinases or phosphatases [20,43], and decreasing cytoplasmic pH shifts their activation potential to more positive voltages [43]. In protoplasts from root cells of most plant species, KORC are inhibited by Ba^{2+} and TEA^+, but not by Ca^{2+}, Cs^+ or quinine, at millimolar concentrations in the extracellular medium [21]. They are also inhibited by 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and niflumic acid at low micromolar concentrations [44].

A gene encoding a KORC (*AtSKOR*) was identified some time ago in *A. thaliana* [4]. This channel has the hallmark GYGD amino acid sequence found in the pore of 'highly K^+-selective' channels. Several pieces of evidence suggest that *AtSKOR* is the genetic correlate of KORC in the stele of the root [4,45]. (1) *AtSKOR* is expressed in the pericycle and xylem parenchyma cells of roots. (2) Its expression is downregulated upon the application of ABA to roots, which is consistent with the reduced appearance of KORC in stelar cells following ABA treatment [22,43]. (3) When expressed heterologously in *Xenopus* oocytes, AtSKOR activates with sigmoidal kinetics, and its activation potential shifts with E_K. (4) AtSKOR is permeable to both monovalent cations and to Ca^{2+}. (5) The unitary conductance of AtSKOR (23 pS in extracellular/cytoplasmic 10:100 mM K^+) is similar to that observed for KORC in protoplasts from the stele of *Arabidopsis* roots assayed under similar ionic conditions [46]. (6) Decreasing cytoplasmic pH reduces AtSKOR-mediated currents. An *Arabidopsis* mutant lacking AtSKOR has lower shoot K content and lower xylem sap K concentration than wildtype plants, indicating a physiological role of this channel in xylem loading. Recently, a second gene encoding a KORC (*AtGORK*) was identified in *Arabidopsis* [5]. In contrast to *AtSKOR*, *AtGORK* is expressed in cell types throughout the root, including root hairs [47]. When expressed heterologously in *Xenopus* oocytes, AtGORK activated with sigmoidal kinetics and its activation shifted with E_K. The unitary conductance of AtGORK (13 pS in 30:100 mM K^+) was comparable to that observed for KORC in protoplasts from the cortex of *Arabidopsis* roots assayed under similar ionic conditions.

7. VIC channels

VIC channels have been observed in all root cell-types studied [21,48,49]. They are permeable to a wide range of monovalent cations, with a general selectivity sequence $NH_4^+ > Rb^+ \geq K^+ > Cs^+ > Na^+ > Li^+$ [21]. However, the order of Cs^+ and Na^+ is occasionally reversed, suggesting the possibility of distinct VIC channel subtypes [50]. Recently, they were shown to be permeable to Ca^{2+} also [18], and a unitary conductance of 5 pS has been predicted for these channels in symmetrical 100 mM $CaCl_2$ [51]. At physiological voltages more positive than about -120 mV, VIC channels have a high probability of being open (P_o) of between 0.20 and 0.80. However, the exact voltage dependence of the P_o of VIC channels is unclear. Some studies indicate a slight increase P_o at extreme negative voltages [52,53], whereas other studies indicate a decline [18,54,55]. Thus, VIC channels are probably better described as non-selective cation channels (NSC) [18,56]. Cation influx through VIC channels is insensitive to TEA^+, verapamil and nifedipine, but is partially inhibited by Ba^{2+}, Gd^{3+}, La^{3+} and, in some plant species, quinine [21,53]. Demidchik and Tester [53] observed that the sensitivity of VIC currents to these pharmaceuticals differed between protoplasts, again suggesting the existence of distinct VIC channel subtypes. The nonselective cation permeability, voltage independence and pharmacology of VIC channels resemble the biophysical properties of cyclic nucleotide gated channels (CNGC) in animals [57]. Genes homologous to animal *CNGC* have been identified in plants [8] and it has been suggested that these are the genetic correlates of VIC channels [58]. Supporting this contention, some VIC channels in protoplasts from *Arabidopsis* roots appear to be inactivated directly by cAMP or cGMP, but these channels have low unitary conductance (8 pS in symmetrical 50 mM KCl) and are insensitive to Cs^+, TEA^+ and quinidine [55]. Another alternative, that homologues of animal ionotropic glutamate receptors (*iGluR*) encode VIC channels, is supported by the observation that glutamate activates VIC channels in protoplasts from *Arabidopsis* root, which are permeable both to Ca^{2+} and to monovalent cations (Na^+, Cs^+, K^+) and sensitive to Gd^{3+}, La^{3+} and quinine [56].

Recent modelling of cation fluxes through VIC channels in the plasma membrane of wheat roots suggests that VIC channels provide a weakly voltage-dependent inward Ca^{2+} current under physiological ionic conditions [51]. This lack of voltage-dependence led White and Davenport [51] to suggest that VIC channels provide a background Ca^{2+} influx required to offset the constant Ca^{2+} efflux from the cytoplasm and, thereby, effect $[Ca^{2+}]_{cyt}$ homeostasis during steady-state.

8. Cyclic nucleotide-gated channel homologues

The voltage-independent, CNGCs of animals are permeable to monovalent and divalent cations, including Ca^{2+} [57]. Homologues of animal *CNGC* genes have been identified throughout the plant kingdom [8,59,60], and an increase in cytoplasmic cAMP or cGMP elicits both Ca^{2+} influx and a rise in $[Ca^{2+}]_{cyt}$ in plant cells [61,62]. In *Arabidopsis*, 20 genomic *CNGC* sequences have been identified, and the CNGC proteins have been divided into

five different subgroups (Fig. 2; [8]). The CNGC proteins contain six putative transmembrane domains (S1–S6) and a binding site for calmodulin within a C-terminus binding site for cyclic nucleotide monophosphates (cNMP). Thus, they can potentially integrate signals from two intracellular signal transduction pathways. The characteristic quartet of amino acids present in the pore of K^+-selective channels (GYGD) is absent from plant CNGC. It is replaced by the motifs GQGL, GQNL, GQNI, GQSL, ANDL, GN-L, AGNL and AGNQ (Fig. 2). The pore structure of plant CNGC also differs from that of animal CNGC, which contains the motif GETP [31]. Thus, the selectivity of plant CNGC cannot be predicted from sequence homologies alone. Charged residues are present in the S4 domain, but it has been argued that this domain is unlikely to function as a voltage sensor in AtCNGCs [56]. By analogy with animal CNGCs, it is thought that the functional channel will be tetrameric.

Several *AtCNGC* genes are expressed in roots. These include *AtCNGC1-3* [63,64], *AtCNGC14*, *AtCNGC18* and *AtCNGC19* (TIGR: The Institute for Genomic Research, Rockville, MD, USA). The membrane location of AtCNGC in root cells has not been determined. However, NtCBP4 (an ortholog of AtCNGC1) was detected immunologically in plasma membrane fractions from roots of both wildtype and transgenic tobacco overexpressing this protein [60] and when a fusion protein of *HvCBT1* (also an ortholog of *AtCNGC1*) and green fluorescent protein was expressed in barley aleurone cells, fluorescence was located at the plasma membrane [59].

Plant CNGC appear to be permeable to both monovalent and divalent cations. Both *AtCNGC1* and *AtCNGC2* complemented a yeast (*Saccharomyces cerevisiae*) mutant lacking endogenous high-affinity K^+ transporters [65,66], and the expression of *AtCNGC3* increased the Na^+ content, and impaired the growth, of a yeast mutant defective in Na^+-export in Na^+-replete media [63]. Calcium permeates AtCNGC2, and human embryonic kidney cells (HEK293) transfected with *AtCNGC2* cDNA show increased Ca^{2+} permeability only in the presence of lipophilic cNMPs [66]. When *AtCNGC2* cRNA was injected into *Xenopus* oocytes, inward rectified currents carried by K^+, Rb^+, Cs^+ and Ca^{2+} (but not by Na^+) could be elicited by increasing cytoplasmic cNMP concentrations [66,67]. Inward (monovalent) cation currents through AtCNGC2 were reduced by increasing $[Ca^{2+}]_{ext}$. Interestingly, the substitution of two amino acids (ND for ET) in the hairpin loop of the selectivity filter in the pore of AtCNGC2 allowed Na^+ to permeate. The activation of cNMP-dependent currents could be prevented by increasing cytoplasmic Ca^{2+}/calmodulin concentrations [68]. Both AtCNGC1 and NtCBP4 also catalyse cAMP-dependent cation currents when expressed in *Xenopus* oocytes [67].

Mutants lacking or overexpressing *CNGC* also indicate their involvement in cation transport. Since it has been suggested that Pb^{2+} enters plant cells through Ca^{2+}-permeable channels [69], it is interesting that an *Arabidopsis* mutant lacking AtCNGC1 (*cngc1*) showed improved Pb tolerance [70]. Likewise, transgenic tobacco plants expressing a truncated version of *NtCBP4* lacking the C-terminal regulatory domains showed reduced Pb accumulation and improved Pb tolerance [70]. Overexpression of *NtCBP4* in transgenic tobacco increased Pb uptake and Pb hypersensitivity [60].

Since many defence responses in plants are mediated through $[Ca^{2+}]_{cyt}$ signals, it is significant that the *Arabidopsis* mutant (*dnd1*), in which the AtCNGC2 gene is interrupted by an early stop codon, failed to show a hypersensitive response to an avirulent strain of the pathogen *Pseudomonas syringae* and exhibited constitutive systemic disease resistance [71]. Köhler et al. [64] have speculated that AtCNGC2 is also involved in the initiation of developmentally regulated cell death programs based on the

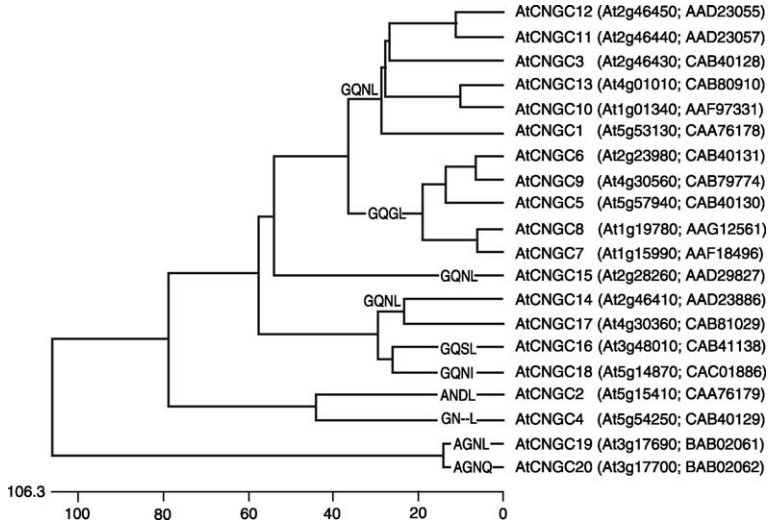

Fig. 2. Dendrogram showing sequence relationships between the 20 CNGC proteins identified in *A. thaliana*. The GenBank accession numbers (gene; protein) are given in parentheses. Protein alignments were performed using the programme ClustalV [101] using the DNAstar default parameters (MegAlign™ 4.05). The quartet of amino acids in the pore loop implicated in cation selectivity is indicated.

expression profile of AtCNGC2 in coleoptiles following illumination, in leaves and flowers upon senescence, and in the dehiscence zone of siliques. A role for CNGC in polar growth may also emerge in future studies given the demonstration that cAMP can modulate pollen tube growth and reorientation [72].

9. The low-affinity cation transporter, LCT1

The cDNA *LCT1*, which is expressed in wheat roots, was cloned by functional complementation of a yeast mutant lacking high-affinity K^+ transporters [73]. Subsequently, it was shown that heterologous expression of *LCT1* in *S. cerevisiae* stimulated the uptake of many cations including K^+, Na^+, Li^+, Cd^{2+} and Ca^{2+} [6,73,74]. The transport of Ca^{2+} by LCT1 was prevented by 10 μM La^{3+}, 10 μM Pb^{2+} or 100 μM Zn^{2+}, both Mn^{2+} (100 μM) and Cd^{2+} (100 μM) reduced Ca^{2+} uptake, but Co^{2+} and Ni^{2+} had no effect [6]. *LCT1* encodes a protein with 8–10 putative membrane-spanning domains, which has little homology with any known transport protein [73]. Thus, it is not known whether LCT1 encodes a Ca^{2+}-permeable channel or another transport mechanism.

10. Ionotropic glutamate receptor homologues

Ionotropic glutamate receptors (iGluR) form nonselective (Ca^{2+}-permeable) cation channels in animals [31]. There are two functional subtypes. One subtype is activated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) or kainate, whilst the other subtype is activated by *N*-methyl-D-aspartate (NMDA).

The application of L-glutamate (but not AMPA or NMDA) triggers both membrane depolarisation and a large, transient elevation of $[Ca^{2+}]_{cyt}$ in roots of *Arabidopsis* seedlings [75]. A half-maximal response is obtained at a glutamate concentration of approximately 1 mM. Both phenomena can be prevented by pretreatment of seedlings with 3 mM La^{3+} or EGTA, suggesting that Ca^{2+} influx from the apoplast contributes to each [75]. These results are consistent with the observations of Demidchik et al. [56] that glutamate activates voltage-independent Ca^{2+} currents in protoplasts from root cells with a K_m of 0.2–0.5 mM. The channel(s) mediating these currents are also permeable to monovalent cations (Na^+, Cs^+, K^+) and sensitive to quinine, La^{3+} and Gd^{3+}.

Homologues of animal *iGluR* genes are present in many plant species [9,76,77]. In *Arabidopsis*, 20 genomic sequences (*AtGLR*) homologous to animal *iGluR* have been identified. The *Arabidopsis* GLR have been divided into three protein families (Fig. 3; [9]). The GLR proteins have four predicted transmembrane regions (M1–M4) and possess two potential glutamate-binding domains and a long (putatively extracellular) N terminus with similarity to both extracellular calcium sensors, and glutamate and γ-aminobutyric acid receptors [76–78]. The amino acids lining the pore of plant GLR proteins (the M2 segment) differ from their animal counterparts [76,78]. Significantly, a key site for ionic selectivity in animal iGuRs (the 'QRN site'), which determines Ca^{2+} permeability and block by Mg^{2+}, is absent from plant GLR. Thus it is difficult to predict the selectivity of plant GLR simply from protein structure. When expressed in *Xenopus* oocytes AtGLR3.4 mediated Ca^{2+} influx, but AtGLR2.8 apparently did not (B. Lacombe et al., cited in Ref. [56]). When *AtGLR3.7* was expressed in *Xenopus* oocytes, voltage-independent, nonspecific cation

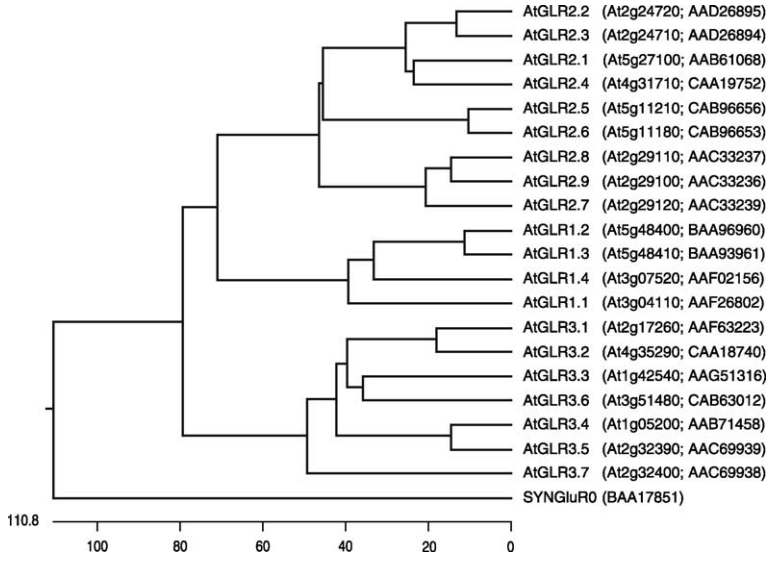

Fig. 3. Dendrogram showing sequence relationships between the 20 GLR proteins identified in *A. thaliana*. The bacterial amino acid binding protein SYNGluR0 was used as an outgroup [78]. The GenBank accession numbers (gene; protein) are given in parentheses. Protein alignments were performed using the programme ClustalV [101] using the DNAstar default parameters (MegAlign™ 4.05).

currents (Ca^{2+}, K^+, Na^+) were recorded [79]. Extracellular La^{3+} or Gd^{3+}, but not verapamil, reduced these cation currents, and extracellular Na^+ blocked Ca^{2+} influx (C. Cheffings, personal communication). Constitutive currents were observed even in the absence of glutamate. This is consistent with the suggestion of Chiu et al. [78], based on protein sequence homologies between plant GLR and a mutant rat δ2 iGluR, that some plant GLR might be unable to bind glutamate and be constitutively open under certain cellular conditions.

Members of the *AtGLR* gene family are expressed throughout the plant [77,80,81]. *AtGLR2.7* is preferentially expressed in the shoot [81]. *AtGLR3.1* is present in cDNA libraries from both shoot and root tissues (TIGR), *AtGLR3.2* (*AtGluR2*) is expressed in both shoot and root, where its promoter directs GUS expression in stelar tissues [81] and *AtGLR3.4* and *AtGLR3.5* are expressed in shoot and root [81]. *AtGLR3.6* is present in a root cDNA library (TIGR) and *AtGLR3.7* is expressed predominantly in the shoot [81].

When the expression of *AtGLR3.2* in *Arabidopsis* was reduced by antisense, plants became more sensitive to Ca toxicity than wildtype plants [81]. Conversely, *Arabidopsis* overexpressing *AtGLR3.2* had a critical Ca content (at which growth rate was 90% maximal) three-fold higher than wildtype plants [77]. However, the shoot Ca content of plants overexpressing *AtGLR3.2* was similar to that of wildtype plants when grown at low $[Ca^{2+}]_{ext}$. This suggests that the Ca deficiency symptoms observed in plants overexpressing *AtGLR3.2* at low $[Ca^{2+}]_{ext}$ was not simply a consequence of reduced Ca uptake, but of altered Ca homeostasis. Consistent with the notion that *AtGLR* encode NSCs, *Arabidopsis* overexpressing *AtGLR3.2* also exhibited hypersensitivity to Na^+ and K^+ ionic stresses, which was ameliorated by supplemental Ca^{2+} [77].

Indirect, pharmacological evidence suggests that AtGLRs have a role in photomorphogenesis: *Arabidopsis* grown in the presence of 6,7-dinotropuinoxaline-2,3(1*H*,4*H*)dione (DNQX) [76] or *S*(+)-β-methyl-α,β-diaminoproprionic acid (BMAA) [82], both antagonists of animal iGluRs, phenocopy long-hypocotyl (*hy*) mutants impaired in light-signal transduction.

11. Annexins

Annexins are a multigene family of Ca^{2+}-, phospholipid- and cytoskeleton-binding proteins with representatives in animals, plants and fungi [83–86]. They may exist in a soluble or membrane-bound state and, in plant cells, they may contribute up to 0.1% of the total protein. The identity of annexins is derived from a conserved C terminal domain, the 'annexin core'. This is composed of four similar sequences (or eight in the case of mammalian annexin VI) of approximately 70 amino acids that each form a compact α-helical domain comprising a region termed the 'endonexin fold', which contains the G-X-G-T-{38}-(D/E) motif that binds calcium. This motif is often conserved only in the first quarter of plant annexins. The α-helical domains create a curved planar structure with a central pore lined with conserved hydrophilic residues, thus allowing ion channel formation [85–87]. Annexins are atypical membrane channel proteins as they do not possess transmembrane domains. The predicted α-helices are too small to span the membrane.

Several annexins from animal sources have been shown to form voltage-dependent (hyperpolarisation-activated) cation-channels in PLB and liposomes enriched in acidic phospholipids such as phosphatidylserine [85,86]. The cationic selectivity of annexins from animal sources varies, but nevertheless all are permeable to Ca^{2+}, often with an unitary conductance of about 30 pS with 25–50 mM Ca^{2+} as the charge carrier [85,86]. Inhibitors include La^{3+}, Cd^{2+} and nifedipine. It has been proposed that annexins increase membrane permeability to calcium by two distinct mechanisms depending on the concentration of annexin in the membrane and their aggregation state. At high annexin concentration, an intermolecular pore is formed by annexin hexamers, at lower concentration permeation is through the monomer.

In all plants, annexins appear to comprise a small gene family. For example, in *Arabidopsis*, seven annexin genes have been identified (*AnnAt1-7*; Fig. 4; [10]), with predicted molecular masses of between 36.0 and 36.6 kDa. Isoforms of annexins with apparent molecular masses between 33 and 35 kDa (designated p33, p34 and p35) have been purified from many plant species [10,83,88–90]. Immunolocalisation of these within the root places them at the periphery of highly secretory cell types, such as the developing vascular tissue, epidermal cells including root hairs, and the outer rootcap [88,89]. Partial or complete cDNA clones encoding annexins have also been obtained [10,83,84,91,92].

Most annexin genes are expressed throughout the plant [10,83,93–97]. All *Arabidopsis* annexins are expressed in roots, with the possible exception of *AnnAt6* [10,94], but only the expression of *AnnAt1* and *AnnAt2* has been studied

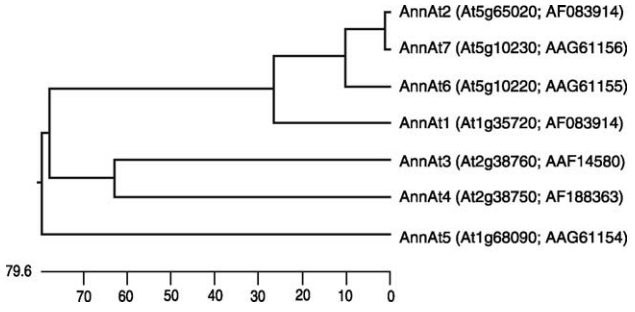

Fig. 4. Dendrogram showing sequence relationships between the seven annexin proteins identified in *A. thaliana*. The GenBank accession numbers (gene; protein) are given in parentheses. Protein alignments were performed using the programme ClustalV [101] using the DNAstar default parameters (MegAlign™ 4.05).

in detail. *AnnAt1* is expressed throughout the root (including the root hairs) except at the root tip, where its expression is limited to outer cells of the rootcap [10], in a pattern similar to a maize annexin (p35; [90]). Its expression is upregulated by the presence of H_2O_2 or salicylic acid [94]. *AnnAt2* is expressed in endodermal cells within the collet, throughout initiating lateral roots and in the epidermal cells of the root tip [10]. By analogy with their occurrence in plasma membrane fractions from leaves [98], it is expected that *Arabidopsis* annexins, with the possible exception of AnnAt3, which has a putative nuclear targeting sequence, will be present in the plasma membrane of root cells [10].

Based on their location within the root, and the known involvement of some animal annexins in exocytosis, a role for annexins in membrane trafficking and/or secretion has been proposed [10]. Certainly, annexins have been clearly implicated in maize root cap exocytosis [90]. Significantly, annexin 24 (Ca32) from bell pepper (*Capsicum annuum*) has been shown to support Ca^{2+} flux [89]. The increased expression of certain annexins upon specific developmental or environmental challenges has linked them with Ca^{2+} signalling during, for example, nodulation [95], ABA responses [99,100] or cold acclimation [97]. The possibility that annexins form Ca^{2+} channels, coupled with their presence in root hairs and the REZ, suggests a role in generating the elevated $[Ca^{2+}]_{cyt}$ required for tip or extension growth.

12. Conclusions and perspective

Several Ca^{2+}-permeable channels in the plasma membrane of root cells have been classified according to their electrophysiological characteristics (Table 1). These include depolarisation-activated 'Ca^{2+} channels', hyperpolarisation-activated 'Ca^{2+} channels', VIC channels and the outward-rectifying K^+ channels KORC and NORC. By comparing the electrophysiological characteristics of these channels recorded in planta with those resulting from the expression of genes encoding candidate root plasma-membrane Ca^{2+} channels in heterologous expression systems, some insight into the genetic counterparts of the physiological activities has been obtained.

It is evident from both their electrophysiology and tissue expression patterns that *AtSKOR* encodes a KORC in stelar cells and that *AtGORK* is likely to encode a KORC in the plasma membrane of other root cells. However, several distinct KORC activities in the plasma membrane of root cells can be differentiated electrophysiologically and it is not clear whether these represent oligomeric proteins, splice variants or modifications of AtSKOR and AtGORK, or are encoded by other genes. The NORC have, as yet, no obvious genetic correlates.

Two candidate gene families (*CNGC* and *GLR*) have been proposed as the genetic correlates of VIC channels. Notwithstanding the ambiguous consequences of ligand binding on channel activities, the limited functional characterisation of AtCNGC2, AtGLR3.4 and AtGLR3.7 indicates that these proteins form channels permeable to both monovalent and divalent cations including Ca^{2+}. Further evidence that *AtCNGC* and *AtGLR* are the genetic correlates of VIC will require electrophysiological confirmation of the voltage dependence, selectivity and pharmacology of these channel proteins.

Three observations suggest that hyperpolarisation-activated Ca^{2+} channels in the plasma membrane of root cells might be encoded by members of the annexin gene family. First, annexins from animal sources form voltage-dependent cation channels that are inhibited by La^{3+} and nifedipine. These properties are shared by the hyperpolarisation-activated Ca^{2+} channels in the plasma membrane of root cells. Second, the expression of annexins in highly secretory cell types, and also in cells undergoing extension growth, parallels the occurrence of hyperpolarisation-activated Ca^{2+} channels. Third, insertion of annexins into a membrane is dependent on their binding to cytosolic Ca^{2+}, which may explain the upregulation of hyperpolarisation-activated channels as $[Ca^{2+}]_{cyt}$ is increased in root hairs [16]. However, despite these intriguing correlations, it has yet to be confirmed by experimentation that annexin genes encode hyperpolarisation-activated Ca^{2+} channels.

It has been argued that *AtTPC1* encodes a depolarisation-activated Ca^{2+} channel, based on sequence homology to depolarisation-activated Ca^{2+} channels in animal cell membranes, and the effects on $[Ca^{2+}]_{cyt}$ transients induced by sucrose in transgenic *Arabidopsis* with reduced expression of *AtTPC1* and/or H^+/sucrose symporter genes. Since several distinct depolarisation-activated Ca^{2+} channels have been identified using electrophysiological techniques, further characterisation of AtTPC1 will be required to determine which (if any) is its physiological counterpart.

In conclusion, future studies should endeavour to determine the electrophysiological characteristics of the putative Ca^{2+}-permeable channels encoded by *AtTPC1* and the *CNGC*, *GLR* and annexin gene families, and to identify their physiological counterparts. In parallel, the expression of these genes in different cell types within the root, the membrane location of their gene products, and the physiological consequences of downregulation or overexpression of these genes should all be examined to elucidate their physiological roles.

Acknowledgements

We thank everyone who allowed us to cite information from their abstracts at the 12th International Workshop on Plant Membrane Biology and Martin Broadley for his comments on an early manuscript. This work was supported

by the Biotechnology and Biological Sciences Research Council (UK).

References

[1] P.J. White, Biochim. Biophys. Acta 1465 (2000) 171–189.
[2] H. Miedema, J.H.F. Bothwell, C. Brownlee, J.M. Davies, Trends Plant Sci. 6 (2001) 514–519.
[3] A.-A. Véry, H. Sentenac, Trends Plant Sci. 7 (2002) 168–175.
[4] F. Gaymard, G. Pilot, B. Lacombe, D. Bouchez, D. Bruneau, J. Boucherez, N. Michaux-Ferrière, J.-B. Thibaud, H. Sentenac, Cell 94 (1998) 647–655.
[5] P. Ache, D. Becker, N. Ivashikina, P. Dietrich, M.R.G. Roelfsema, R. Hedrich, FEBS Lett. 486 (2000) 93–98.
[6] S. Clemens, D.M. Antiosiewicz, J.M. Ward, D.P. Schachtman, J.I. Schroeder, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12043–12048.
[7] T. Furuichi, K.W. Cunningham, S. Muto, Plant Cell Physiol. 42 (2001) 900–905.
[8] P. Mäser, S. Thomine, J.I. Schroeder, J.M. Ward, K. Hirschi, H. Sze, I.N. Talke, A. Amtmann, F.J.M. Maathuis, D. Sanders, J.F. Harper, J. Tchieu, M. Gribskov, M.W. Persans, D.E. Salt, S.A. Kim, M.L. Guerinot, Plant Physiol. 126 (2001) 1646–1667.
[9] B. Lacombe, D. Becker, R. Hedrich, R. DeSalle, M. Hollman, J.M. Kwak, J.I. Schroeder, N. Le Novère, H.G. Nam, E.P. Spalding, M. Tester, F.J. Turano, J. Chiu, G. Coruzzi, Science 292 (2001) 1486–1487.
[10] G.B. Clark, A. Sessions, D.J. Eastburn, S.J. Roux, Plant Physiol. 126 (2001) 1072–1084.
[11] K. Esau, Plant Anatomy, Wiley, New York, 1965.
[12] P.J. White, J. Exp. Bot. 52 (2001) 891–899.
[13] C.A. Moore, H.C. Bowen, S. Scrase-Field, M.R. Knight, P.J. White, Plant J. 30 (2002) 457–466.
[14] P.J. White, Ann. Bot. 81 (1998) 173–183.
[15] E. Kiegle, M. Gilliham, J. Haseloff, M. Tester, Plant J. 21 (2000) 225–229.
[16] A.A. Véry, J.M. Davies, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9801–9806.
[17] M. Gilliham, M. Tester, Abstracts of the 12th International Workshop on Plant Membrane Biology, University of Wisconsin, Madison, 2001, p. 103.
[18] R.J. Davenport, M. Tester, Plant Physiol. 122 (2000) 823–834.
[19] S.K. Roberts, M. Tester, J. Exp. Bot. 48 (1997) 839–846.
[20] L.H. Wegner, A.H. De Boer, Plant Physiol. 115 (1997) 1707–1719.
[21] P.J. White, J. Exp. Bot. 48 (1997) 499–514.
[22] A.H. De Boer, Plant Biol. 1 (1999) 36–45.
[23] L. Thion, C. Mazars, P. Nacry, D. Bouchez, M. Moreau, R. Ranjeva, P. Thuleau, Plant J. 13 (1998) 603–610.
[24] H. Miedema, J.H.F. Bothwell, A.A. Véry, C. Brownlee, J.M. Davies, Abstracts of the 12th International Workshop on Plant Membrane Biology, University of Wisconsin, Madison, 2001, p. 66.
[25] P. Thuleau, J.M. Ward, R. Ranjeva, J.I. Schroeder, EMBO J. 13 (1994) 2970–2975.
[26] P.J. White, M. Piñeros, M. Tester, M.S. Ridout, J. Membr. Biol. 174 (2000) 71–83.
[27] M. Piñeros, M. Tester, J. Exp. Bot. 48 (1997) 551–557.
[28] P.J. White, M.S. Ridout, J. Membr. Biol. 168 (1999) 63–75.
[29] C. Mazars, L. Thion, P. Thuleau, A. Graziana, M.R. Knight, M. Moreau, R. Ranjeva, Cell Calcium 22 (1997) 413–420.
[30] F.G.P. Lhuissier, N.C.A. de Ruijter, B.J. Sieberer, J.J. Esseling, A.M.C. Emons, Ann. Bot. 87 (2001) 289–302.
[31] B. Hille, Ionic Channels of Excitable Membranes, Sinauer Associates, Massachusetts, 2001.
[32] M. Fischer, N. Schnell, J. Chattaway, P. Davies, G. Dixon, D. Sanders, FEBS Lett. 419 (1997) 259–262.
[33] M. Paidhungat, S. Garrett, Mol. Cell. Biol. 17 (1997) 6339–6347.
[34] E.G. Locke, M. Bonilla, L. Liang, Y. Takita, K.W. Cunningham, Mol. Cell. Biol. 20 (2000) 6686–6694.
[35] D.W.A. Hamilton, A. Hills, B. Köhler, M.R. Blatt, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4967–4972.
[36] Z.M. Pei, Y. Murata, G. Benning, S. Thomine, B. Klusener, G.J. Allen, E. Grill, J.I. Schroeder, Nature 406 (2000) 731–734.
[37] D.W.A. Hamilton, A. Hills, M.R. Blatt, FEBS Lett. 491 (2001) 99–103.
[38] Y. Murata, Z.-M. Pei, I.C. Mori, J. Schroeder, Plant Cell 13 (2001) 2513–2523.
[39] J.W. Schiefelbein, A. Shipley, P. Rowse, Planta 87 (1992) 455–459.
[40] C.L. Wymer, T.N. Bibikova, S. Gilroy, Plant J. 12 (1997) 427–439.
[41] L.A. Romano, H. Miedema, S.M. Assmann, Plant Cell Physiol. 39 (1998) 1133–1144.
[42] Z.M. Pei, V.M. Baizabal-Aguirre, G.J. Allen, J.I. Schroeder, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6548–6553.
[43] S.K. Roberts, B.N. Snowman, J. Exp. Bot. 51 (2000) 1585–1594.
[44] A. Garrill, S.D. Tyerman, G.P. Findlay, P.R. Ryan, Aust. J. Plant Physiol. 23 (1996) 527–534.
[45] B. Lacombe, G. Pilot, F. Gaymard, H. Sentenac, J.-B. Thibaud, FEBS Lett. 466 (2000) 351–354.
[46] F.J.M. Maathuis, S.T. May, N.S. Graham, H.C. Bowen, T.C. Jelitto, P. Trimmer, M.J. Bennett, D. Sanders, P.J. White, Plant J. 15 (1998) 843–851.
[47] N. Ivashikina, D. Becker, P. Ache, O. Meyerhoff, H.H. Felle, R. Hedrich, FEBS Lett. 508 (2001) 463–469.
[48] A. Amtmann, D. Sanders, Adv. Bot. Res. 29 (1999) 75–112.
[49] S.D. Tyerman, I.M. Skerrett, Sci. Hortic. 78 (1999) 175–235.
[50] P.H. Buschmann, R. Vaidyanathan, W. Gassmann, J.I. Schroeder, Plant Physiol. 122 (2000) 1387–1397.
[51] P.J. White, R.J. Davenport, Plant Physiol. (2002) in press.
[52] S.K. Roberts, M. Tester, J. Exp. Bot. 48 (1997) 431–440.
[53] V. Demidchik, M. Tester, Plant Physiol. 128 (2002) 379–387.
[54] P.J. White, M.A. Tester, Planta 186 (1992) 188–202.
[55] F.J.M. Maathuis, D. Sanders, Plant Physiol. 127 (2001) 1617–1625.
[56] V. Demidchik, R.J. Davenport, M. Tester, Annu. Rev. Plant Biol. 53 (2002) 67–107.
[57] W.N. Zagotta, S.A. Siegelbaum, Annu. Rev. Neurosci. 19 (1996) 235–263.
[58] P.J. White, M.R. Broadley, New Phytol. 147 (2000) 241–256.
[59] R.C. Schuurink, S.F. Shartzer, A. Fath, R.L. Jones, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 1944–1949.
[60] T. Arazi, R. Sunkar, B. Kaplan, H. Fromm, Plant J. 20 (1999) 171–182.
[61] F. Kurosaki, H. Kaburaki, A. Nishi, FEBS Lett. 340 (1994) 193–196.
[62] I.D. Volotovski, S.G. Sokolovsky, O.V. Molchan, M.R. Knight, Plant Physiol. 117 (1998) 1023–1030.
[63] I.N. Talke, A. Amtmann, B.S. Stanchev, D. Sanders, F.J.M. Maathuis, Abstracts of the Society of Experimental Biology Plant Transport Group Meeting, Hatfield, 2000.
[64] C. Köhler, T. Merkle, D. Roby, G. Neuhaus, Planta 213 (2001) 327–332.
[65] C. Köhler, T. Merkle, G. Neuhaus, Plant J. 18 (1999) 97–104.
[66] Q. Leng, R.W. Mercier, W. Yao, G.A. Berkowitz, Plant Physiol. 121 (1999) 753–761.
[67] Q. Leng, R.W. Mercier, B.-G. Hua, H. Fromm, G.A. Berkowitz, Plant Physiol. 128 (2002) 400–410.
[68] B.-G. Hua, R. Mercier, Q. Leng, E. Kastal, G.A. Berkowitz, Abstracts of the 12th International Workshop on Plant Membrane Biology, University of Wisconsin, Madison, 2001, p. 3.
[69] J.W. Huang, S.D. Cunningham, New Phytol. 134 (1996) 75–84.
[70] R. Sunkar, B. Kaplan, N. Bouché, T. Arazi, D. Dolev, I.N. Talke, F.J.M. Maathuis, D. Sanders, D. Bouchez, H. Fromm, Plant J. 24 (2000) 533–542.
[71] S.J. Clough, K.A. Fengler, I.-C. Yu, B. Lippock, R.K. Smith, A.F. Bent, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9323–9328.

[72] A. Moutinho, P.H. Hussey, A.J. Trewavas, R. Malhó, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10481–10486.
[73] D.P. Schachtman, R. Kumar, J.I. Schroeder, E.L. Marsh, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11079–11084.
[74] A. Amtmann, M. Fischer, E.L. Marsh, A. Stafanovic, D. Sanders, D.P. Schachtman, Plant Physiol. 126 (2001) 1061–1071.
[75] K.L. Dennison, E.P. Spalding, Plant Physiol. 124 (2000) 1511–1514.
[76] H.-M. Lam, J. Chiu, M.-H. Hsieh, L. Meisel, I.C. Oliveira, M. Shin, G. Coruzzi, Nature 396 (1998) 125–126.
[77] S.A. Kim, J.M. Kwak, S.-K. Jae, M.-H. Wang, H.G. Nam, Plant Cell Physiol. 42 (2001) 74–84.
[78] J. Chiu, R. DeSalle, H.-M. Lam, L. Meisel, G. Coruzzi, Mol. Biol. Evol. 16 (1999) 826–838.
[79] C. Cheffings, Abstracts of the 12th International Workshop on Plant Membrane Biology, University of Wisconsin, Madison, 2001, p. 28.
[80] R.J. Davenport, E.A. Kiegle, M. Tester, J. Exp. Bot. 51S (2000) 30.
[81] T. Zhu, P. Budworth, B. Han, D. Brown, H.-S. Chang, G. Zou, X. Wang, Plant Physiol. Biochem. 39 (2001) 221–242.
[82] E.D. Brenner, N. Martinez-Barboza, A.P. Clark, Q.S. Liang, D.W. Stevenson, G.M. Coruzzi, Plant Physiol. 124 (2000) 1615–1624.
[83] D.P. Delmer, T.S. Potikha, Cell. Mol. Life Sci. 53 (1997) 546–553.
[84] R.O. Morgan, M.P. Fernandez, J. Mol. Evol. 44 (1997) 178–188.
[85] J.I. Kourie, H.B. Wood, Prog. Biophys. Mol. Biol. 73 (2000) 91–134.
[86] V. Gerke, S.E. Moss, Physiol. Rev. 82 (2002) 331–371.
[87] A. Hofmann, J. Proust, A. Dorowski, R. Schantz, R. Huber, J. Biol. Chem. 275 (2000) 8072–8082.
[88] G.B. Clark, M. Dauwalder, S.J. Roux, Planta 187 (1992) 1–9.
[89] G.B. Clark, M. Dauwalder, S.J. Roux, Adv. Space Res. 14 (1994) 341–346.
[90] A.D. Carroll, C. Moyen, P. Van Kesteren, F. Tooke, N.H. Battey, C. Brownlee, Plant Cell 10 (1998) 1267–1276.
[91] H.D. Blackbourn, P.J. Barker, N.S. Huskisson, N.H. Battey, Plant Physiol. 99 (1992) 864–871.
[92] N.H. Battey, N.C. James, A.J. Greenland, Plant Physiol. 112 (1996) 1391–1396.
[93] M.F. Smallwood, S.J. Gurr, M.J. McPherson, K. Roberts, D.J. Bowles, Biochem. J. 281 (1992) 501–505.
[94] X. Gidrol, P.A. Sabelli, Y.P. Fern, A.K. Kush, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 11268–11273.
[95] F. de Carvalho Niebel, N. Lescure, J.V. Cullimore, P. Gamas, Mol. Plant-Microb. Interact. 11 (1998) 504–513.
[96] J. Proust, G. Houlné, M.-L. Schantz, R. Schantz, FEBS Lett. 383 (1996) 208–212.
[97] G. Breton, A. Vazquez-Tello, J. Danyluk, F. Sarhan, Plant Cell Physiol. 41 (2000) 177–184.
[98] V. Santoni, D. Rouquié, P. Doumas, M. Mansion, M. Boutry, H. Degand, P. Dupree, L. Packman, J. Sherrier, T. Prime, G. Bauw, E. Posada, P. Rouzé, P. Dehais, I. Sahnoun, I. Barlier, M. Rossignol, Plant J. 16 (1998) 633–641.
[99] I. Kovács, F. Ayaydin, A. Oberschall, I. Ipacs, S. Bottka, S. Pongor, D. Dudits, E.C. Tóth, Plant J. 15 (1998) 185–197.
[100] M.W. Bianchi, C. Damerval, N. Vartanian, Funct. Plant. Biol. 29 (2002) 55–61.
[101] D.G. Higgins, P.M. Sharp, Comput. Appl. Biosci. 5 (1989) 151–153.