Vacuolar calcium channels

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Abstract

The central vacuole is the largest Ca\textsuperscript{2+} store in a mature plant cell. Ca\textsuperscript{2+} release from this store contributes to Ca\textsuperscript{2+}-mediated intracellular signalling in a variety of physiological responses. However, the routes for vacuolar Ca\textsuperscript{2+} release are not well characterized. To date, at least two voltage-dependent and two ligand-gated Ca\textsuperscript{2+}-permeable channels have been reported in plant vacuoles. However, the so-called VVCa (vacuolar voltage-gated Ca\textsuperscript{2+}) channel most probably is not a separate channel but is identical to another voltage-dependent channel—the so-called SV (slow vacuolar) channel. Studies in the last few years have added a new dimension to our knowledge of SV channel-mediated ion transport and the mechanisms of its regulation by multiple natural factors. Recently, the SV channel was identified as the product of the TPC1 gene in Arabidopsis. In contrast, the TPC1 channel from other species was thought to be localized in the plasma membrane. A re-evaluation of this work under the assumption that the TPC1 channel is generally a vacuolar channel provides interesting insights into the physiological function of the TPC1/SV channel. Considerably less is known about vacuolar Ca\textsuperscript{2+} channels that are supposed to be activated by inositol 1,4,5-trisphosphate or cADP ribose. The major problems are controversial reports about functional characteristics, and a remarkable lack of homologues of animal ligand-gated Ca\textsuperscript{2+} channels in higher plants. To help understand Ca\textsuperscript{2+}-mediated intracellular signalling in plant cells, a critical update of existing experimental evidence for vacuolar Ca\textsuperscript{2+} channels is presented.

Introduction

While calcium can make up to 5\% of the dry weight of a plant (Broadley et al., 2003), its cytosolic free concentration is extremely low, <1 \textmu M. A large portion of the total Ca\textsuperscript{2+} is bound to cell walls and anionic macromolecules inside the cell. The water-soluble Ca\textsuperscript{2+} in plant cells is compartmentalized into organelles functioning as Ca\textsuperscript{2+} stores, with the central vacuole containing most of the water-soluble Ca\textsuperscript{2+}. The huge Ca\textsuperscript{2+} concentration differences between Ca\textsuperscript{2+} stores and surrounding cytosol are the basis for the function of Ca\textsuperscript{2+} as second messenger in intracellular signal transduction. Since the vacuole is the largest Ca\textsuperscript{2+} pool in a typical plant cell, vacuolar Ca\textsuperscript{2+} channels play a critical role in Ca\textsuperscript{2+}-mediated signal transduction as well as in Ca\textsuperscript{2+} homeostasis (Bush, 1995; Hetherington and Brownlee, 2004). In this article, the evidence for different voltage-gated and ligand-gated vacuolar Ca\textsuperscript{2+} channels is reviewed, and—where information is available—their regulation, structure, and possible physiological functions are discussed.

Ca\textsuperscript{2+} transport across the vacuolar membrane

To understand the function of vacuolar Ca\textsuperscript{2+} channels, it is instructive to have a look at the driving forces for vacuolar Ca\textsuperscript{2+} transport. The free Ca\textsuperscript{2+} concentration inside vacuoles is typically ~1000-fold higher than in the surrounding cytosol (Evans et al., 1991; Bush, 1993). The electrical potential difference across the vacuolar membrane ranges from 0 mV to −30 mV (Bethmann et al., 1995; Walker et al., 1996). Both the Ca\textsuperscript{2+} concentration gradient and the membrane potential therefore drive Ca\textsuperscript{2+} efflux from the vacuole—via Ca\textsuperscript{2+} channels—while Ca\textsuperscript{2+} uptake into the vacuole requires energy.

Cytosolic free Ca\textsuperscript{2+} concentrations, as measured with ion-selective microelectrodes and fluorescent dyes, range from 100 nM to 350 nM at rest (Felle, 1989; Bethmann...
et al., 1995; Felle and Hepler, 1997; Plieth, 2001). During Ca$$^{2+}$$-mediated signal transduction processes, cytosolic free Ca$$^{2+}$$ concentrations may transiently reach 1 μM and more, but they always remain low compared with those in the vacuole. In higher plants, vacuolar free Ca$$^{2+}$$ concentrations of 1.5–2.3 mM have been measured with ion-selective microelectrodes (Felle, 1988). As in the cytosol, there can be a large difference between the total and the free vacuolar Ca$$^{2+}$$ concentration, due to Ca$$^{2+}$$ binding by proteins and organic acids.

Ca$$^{2+}$$ uptake into vacuoles, i.e. active transport against the electrochemical potential gradient, is mediated by P-type Ca$$^{2+}$$ pumps (Geisler et al., 2000; Sze et al., 2000) and H$$^{+}/$$Ca$$^{2+}$$ antiporters (Shigaki and Hirschi et al., 2006). Primary Ca$$^{2+}$$-pumps (Arabidopsis ACA-gene family) mediate high-affinity ($$K_m$$=0.2–1.0 μM) low-turnover Ca$$^{2+}$$ uptake, whereas H$$^{+}/$$Ca$$^{2+}$$ antiporters (Arabidopsis CAX-gene family) mediate low-affinity ($$K_m$$ ~10 μM) high-capacity Ca$$^{2+}$$ uptake. It was therefore speculated that the two vacuolar Ca$$^{2+}$$ uptake systems may be suited for operation at different levels of cytosolic Ca$$^{2+}$$ (Maeshima, 2001).

Because of the huge cytosol-directed electrochemical gradient for Ca$$^{2+}$$, the opening of any Ca$$^{2+}$$-permeable channel will result in Ca$$^{2+}$$ release from the vacuole that has to be very tightly regulated. At least four different vacuolar Ca$$^{2+}$$ channels have been described, two voltage-dependent Ca$$^{2+}$$ channels (VVCa and SV) and two ligand-gated channels (White, 2000; Sanders et al., 2002).

The VVCa channel

In contrast to the SV channel which is activated at positive tonoplast potentials, the VVCa (vacuolar voltage-gated Ca$$^{2+}$$) channel is gated open at negative tonoplast potentials. One might argue that the opening of a vacuolar Ca$$^{2+}$$-permeable channel at physiological conditions (negative membrane potentials, millimolar luminal Ca$$^{2+}$$) is hard to reconcile with the cytosolic Ca$$^{2+}$$ homeostasis. The VVCa Ca$$^{2+}$$ channel was characterized by single-channel recordings on isolated patches from vacuoles of Beta vulgaris tap roots (Johannes et al., 1992; Johannes and Sanders, 1995) and of Vicia faba guard cells (Allen and Sanders, 1994b). Amazingly, when comparing the properties of the SV channel (see below) and the VVCa channel, these show striking similarities, especially when data from the same species are taken. Table 1 and Fig. 1 summarize published data obtained with vacuoles of B. vulgaris tap roots. Obviously, the single-channel conductance for 50 mM K$$^+$$ or for 10 mM Ca$$^{2+}$$, or Mg$$^{2+}$$, or Ba$$^{2+}$$ is identical for the SV channel and the VVCa channel within error limits, and both channels show a high affinity (submillimolar $$K_m$$ value) for Ca$$^{2+}$$ and a low affinity for K$$^+$$ (Table 1). The SV channel is activated by Ca$$^{2+}$$ (Hedrich and Neher, 1987) and inhibited by H$$^+$$ (pK $$\approx$$ 6.8) from the cytosolic side (Schulz-Lessdorf and Hedrich, 1995), while the so-called VVCa channel is activated by Ca$$^{2+}$$ (Johannes et al., 1992) and inhibited by H$$^+$$ (pK $$\approx$$ 6.5) from the vacuolar side (Allen and Sanders, 1994b).

In other words, the VVCa channel has the properties of an SV channel that is inserted in the vacuolar membrane the ‘other way round’—or was measured in an isolated membrane patch that was oriented the ‘other way round’. The so-called VVCa channel has a high density—like the SV channel—and isolated patches always contained multiple channels, but whole-vacuole recordings from the VVCa channel do not seem to exist. To test further the possibility that the VVCa channel might be identical to the SV channel, channel activation by Ca$$^{2+}$$ was compared. As shown in Fig. 1, increasing Ca$$^{2+}$$ concentrations had the same effect on the voltage-dependent open probability of the SV channel and the VVCa channel. It seems unlikely that there exist two Ca$$^{2+}$$ channels with identical functional properties but opposite orientation in the vacuolar membrane, while there are no recordings documenting both channels at the same time—even though both have a rather high density. It is therefore postulated that the so-called VVCa channel is not a separate Ca$$^{2+}$$ channel of the vacuolar membrane but is identical to the SV channel—recorded the ‘other way round’. In the discussion of the SV channel following below, data from the so-called VVCa channel are included.

The SV channel

The SV (slow-activating vacuolar) channel is by far the best described vacuolar ion channel. Earlier reports of

<table>
<thead>
<tr>
<th>Unitary conductance (pS)</th>
<th>50 mM K$$^+$$</th>
<th>10 mM Ca$$^{2+}$$</th>
<th>10 mM Mg$$^{2+}$$</th>
<th>10 mM Ba$$^{2+}$$</th>
<th>$$K_m$$ values (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV</td>
<td>167abc</td>
<td>12.3c</td>
<td>18.4d</td>
<td>16.7b</td>
<td>103±14d</td>
</tr>
<tr>
<td>VVCa</td>
<td>188c</td>
<td>11.7±1.2c</td>
<td>17.4±1.2c</td>
<td>17.0±1.5c</td>
<td>143 (11.8)c</td>
</tr>
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</table>

a Data from Pottosin et al. (2001)
b Data from Pantoja et al. (1992)
c Data from Johannes and Sanders (1995)
d Data from Gradmann et al. (1997) were calculated assuming either a rigid pore model or a flexible pore model (data in parentheses).
vacuolar Ca\(^{2+}\) channels mediating Ca\(^{2+}\) uptake into the vacuole (Pantoja et al., 1992; Ping et al., 1992a, b) can probably be explained as recordings from SV channels at a time when the Ca\(^{2+}\) permeability of the SV channel was not yet understood (Ward and Schroeder, 1994). The SV channel is the most abundant tonoplast channel. Based on patch-clamp recordings, channel densities of ~1 SV channel per \(\text{mum}^2\) and higher have been calculated (Schulz-Lessdorf and Hedrich, 1995; Pottosin et al., 1997). Proteomic characterization of the vacuolar membrane proteins of Arabidopsis (Carter et al., 2004; Szponarski et al., 2005) revealed the SV channel (TPC1), but no other vacuolar ion channels. The SV channel seems to be ubiquitous among terrestrial plants (Embryophytes) including ferns and liverworts (Hedrich et al., 1988).

**Molecular identity**

Patch-clamp recordings on isolated vacuoles of Arabidopsis knock-out mutants lacking KCO1 (kco1) showed decreased slow-activating currents. This was interpreted as an involvement of KCO1 in the formation of SV channels (Schönknecht et al., 2001), while it might have been a pleiotropic effect. When KCO1 was expressed in yeast, it formed a voltage-independent, Ca\(^{2+}\)-activated, K\(^+\)-selective ion channel (Bihler et al., 2005). An Arabidopsis knock-out mutant lacking TPC1 (tpc1-2) does not show any SV channel activity, and TPC1-overexpressing lines have increased SV channel activity, demonstrating that the TPC1 gene of Arabidopsis encodes the SV channel (Peiter et al., 2005). TPC stands for two-pore channel, a family of voltage-gated cation channels consisting of two homologous domains with six transmembrane helices and one pore domain each (Fig. 2). Originally discovered in rat kidney, TPC channels can be understood as an evolutionary intermediate between single-domain, Shaker-type K\(^+\) channels and the family of voltage-dependent Ca\(^{2+}\) and/or Na\(^+\) channels from animals consisting of four homologous domains (Ishibashi et al., 2000). In higher plants, the TPC channel is highly conserved; especially the pore loops (White et al., 2002), and the membrane-spanning parts largely consist of identical or conserved amino acids (Fig. 2). In Arabidopsis, AtTPC1 (At4g03560) is the only member of the TPC family (Furuichi et al., 2001), indicating that the SV channel might be formed by a TPC1 homodimer. Only a single gene or mRNA homologous to AtTPC1 has been detected in rice (Kurusu et al., 2004), while in tobacco (Nicotiana tabacum) BY-2 cells two highly homologous (97.1% amino acid identity) NiTPCs were identified (Kadota et al., 2004). In this context, it is interesting that it had been observed that the single-channel conductance of SV channels in guard cells exceeds the single-channel conductance in other cell types (Schulz-Lessdorf et al., 1995). It now should be possible to determine whether the different unit conductance goes back to different gene products or is caused by post-translational or post-transcriptional modifications.

**Intracellular localization**

While SV channel activity in patch-clamp recordings only has been registered from vacuolar membranes, most of the published work about TPC1 in plants has been interpreted assuming that TPC1 is a plasma membrane channel. For AtTPC1, localization in the vacuolar membrane has been demonstrated by green fluorescent protein (GFP) constructs, antibody binding, a correlation between TPC1 expression level and SV channel activity (Peiter et al., 2005), and by proteomic analysis of vacuolar membranes (Carter et al., 2004; Szponarski et al., 2005). Even though, when first described, AtTPC1 was suggested to be a plasma membrane channel (Furuichi et al., 2001), its tonoplast localization now seems to be established (Peiter et al., 2005). The reported targeting of AtTPC1–GFP fusion proteins to the plasma membrane of BY-2 cells (Kawano et al., 2004) might be indicative of mistargeting, as has been observed.
with C-terminal GFP fusions of other integral membrane proteins (Tian et al., 2004). GFP fusion proteins of OsTPC1 from rice (Oryza sativa, GFP–OsTPC1) and TaTPC1 from wheat (Triticum aestivum, TaTPC1–GFP) were reported to localize in the plasma membrane of onion epidermal cells (Kurusu et al., 2005; Wang et al., 2005). Keeping in mind that it might be hard to distinguish between plasma membrane localization and vacuolar membrane localization of GFP in intact onion epidermal cells, and including the possibility of mistargeting of GFP fusion proteins (Tian et al., 2004), it is believed it might be worth reconsidering some of the experimental results obtained with NtTPC1 (Kadota et al., 2004), OsTPC1 (Hashimoto et al., 2004; Kurusu et al., 2004, 2005), and TaTPC1 (Wang et al., 2005) under the assumption that these might be SV channels of the vacuolar membrane (see below).

Not knowing the tertiary structure of the SV channel, useful information about its pore dimensions can be obtained by applying blocking cations of different size and length (Dobrovinskaya et al., 1999a). The outcome of such an approach is summarized in Fig. 3. It appears that substances with a diameter of \( \leq 7 \) Å (the size of a fully hydrated Mg\(^{2+}\) ion) can permeate the pore. In line with such a pore diameter, the SV channel has a high permeability for alkali cations (Amodeo et al., 1994; Paganetto et al., 2001) as well as alkali earth cations (Pantoja et al., 1992; Ward and Schroeder, 1994; Pottosin et al., 2001). Considering only physiologically abundant cations, the SV channel can mediate passive exchange of K\(^+\), Na\(^+\), NH\(_4\)\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) between the vacuole and cytosol. Early studies suggested a significant anion permeability of the SV channel (Hedrich et al., 1986; Hedrich and Kurkdjian, 1988; Schulz-Lessdorf et al., 1995). More recent analyses have shown, however, that anion (Cl\(^-\)) permeability of the SV channel is immeasurably low (Ward et al., 1994; Pottosin et al., 2001). Negative surface charges at the SV channel pore entrances probably contribute to the charge-selecting mechanism, attracting cations and rejecting anions (Pottosin et al., 1999, 2001, 2005).

Theoretical calculations based on physiologically relevant electrochemical ionic gradients across the tonoplast show that SV channel-mediated currents are dominated by K\(^+\), while Ca\(^{2+}\) currents are rather small. At zero voltage, 1 mM luminal and 1 µM cytosolic Ca\(^{2+}\), the single-channel Ca\(^{2+}\) current is \( \sim 100 \) fA and 400 fA for SV channels from Beta taproots and Vicia guard cells, respectively (Gradmann et al., 1997; Allen et al., 1998). Nevertheless, with only a few open SV channels per vacuole, Ca\(^{2+}\) release approaches the pA range, which is comparable with estimated maximum rates of Ca\(^{2+}\) uptake into the vacuole. The main route of vacuolar Ca\(^{2+}\) uptake is via proton motive force-driven Ca\(^{2+}/H^+\) exchange. The proton motive force is build up by tonoplast H\(^+\) pumps that generate whole-vacuole currents of 10–20 pA (\( \sim 30 \) µA cm\(^{-2}\)) (Hedrich and Kurkdjian, 1988; Hedrich et al., 1989). Obviously Ca\(^{2+}\) uptake into the vacuole cannot exceed H\(^+\) pump currents over an extended time period. This implies that active Ca\(^{2+}\) uptake into the vacuole can only compensate for passive Ca\(^{2+}\) release by a very small fraction of the thousands of SV channels per vacuole. Assuming just a 1 pA net Ca\(^{2+}\) release into a typical cytoplasmic volume of 1 pl, cytosolic free Ca\(^{2+}\) would reach 1 µM in \( \sim 1 \) min, even with only 1 out of 10 000 cytoplasmic Ca\(^{2+}\) ions being in a free form. Most vacuoles examined contain several thousand SV channel copies.
observed at increasing cytosolic Mg$^{2+}$ levels (Pei et al., 1999; Carpaneto et al., 2001). To explain the overlapping effects of Ca$^{2+}$ and Mg$^{2+}$, Pei and co-workers (1999) proposed two cytosolic binding sites, a Ca$^{2+}$-selective one, binding Ca$^{2+}$ with high affinity, and another site binding Ca$^{2+}$ and Mg$^{2+}$ with comparable affinity in the submillimolar to millimolar range. Binding of divalent cations to the latter site stabilizes the SV channel in its open state, shifting the activation threshold to less positive potentials. At physiological conditions (low cytosolic Ca$^{2+}$, submillimolar cytosolic Mg$^{2+}$), this site is preferentially occupied by Mg$^{2+}$. In an attempt to evaluate the impact of cytosolic Ca$^{2+}$ and Mg$^{2+}$ on vacuolar Ca$^{2+}$ release, the non-invasive MIFE technique was applied to isolated vacuoles (Wherrett, 2006). In the absence of divalent cations on the cytosolic side, vacuolar Ca$^{2+}$ release was <1 pA per vacuole (average diameter 40 μm), which is close to the detection limit. An increase of cytosolic Ca$^{2+}$ to 20–50 μM increased Ca$^{2+}$ release to a few pA per vacuole; Ca$^{2+}$ release was doubled upon addition of 1 mM Mg$^{2+}$. These Ca$^{2+}$ fluxes were inhibited by ~80% after addition of 0.1 mM Zn$^{2+}$, a known SV channel blocker (Hedrich and Kurkdjian, 1988), indicating that the measured Ca$^{2+}$ fluxes were largely mediated by the SV channel.

Reducing agents such as dithiothreitol (DTT) or glutathione increase the open probability of the SV channel (Carpaneto et al., 1999; Scholz-Starke et al., 2004). Further cytosolic factors affecting SV channel activity are reversible protein phosphorylation exerting either positive or negative control, depending on the phosphorylation site (Allen and Sanders, 1995; Bethke and Jones, 1997), and 14-3-3 proteins that reduce SV currents without affecting their voltage dependence (van den Wijngaard et al., 2001).

Several physiologically relevant cations, such as heavy metal ions (Zn$^{2+}$ and Ni$^{2+}$) and polyamines, inhibit the SV channel at micromolar concentrations (Hedrich and Kurkdjian, 1988; Dobrovinskaya et al., 1999a; Panagiotou et al., 2001; Carpaneto, 2003). Some of them, such as polyamines, act solely via binding within the channel pore, blocking the flow of permeable cations (Dobrovinskaya et al., 1999b), whereas others, such as Ni$^{2+}$, also modify channel gating (Carpaneto, 2003). An interesting example of such a dual action on the SV channel was recently demonstrated for the aminoglycoside antibiotic neomycin. Neomycin applied from the cytosolic side was shown to block the current through an open SV channel but at the same time activated SV channels by shifting their voltage dependence towards negative potentials (Scholz-Starke et al., 2006). This was interpreted as an indication that the SV channel can be activated at physiologically relevant (i.e. negative) tonoplast potentials by special regulatory molecules (Scholz-Starke et al., 2006). The stimulatory effect of neomycin on the SV channel may explain the observation that in the presence of divalent cations, such as Mg$^{2+}$, the SV channel is partially activated at negative potentials.
of neomycin, a voltage-evoked Ca\textsuperscript{2+} increase is followed by intracellular Ca\textsuperscript{2+} release in guard cells (Grabov and Blatt, 1999).

The composition of the vacuolar compartment is much more variable than the cytosolic composition (Leigh, 1997). Several vacuolar factors have been shown to control SV channel function. Lowering the vacuolar pH decreases SV channel activity (Schulz-Lessdorf and Hedrich, 1995; Pottosin et al., 1997). Even more efficient is the variation of vacuolar Ca\textsuperscript{2+} levels. Vacuolar Ca\textsuperscript{2+} competes with H\textsuperscript{+} and Mg\textsuperscript{2+} for the same binding sites. Removal of vacuolar Ca\textsuperscript{2+} at neutral pH results in a dramatic negative shift of the SV channel voltage dependence, and a threshold for activation as low as −100 mV is observed. Vacuolar Mg\textsuperscript{2+} is much less efficient compared with Ca\textsuperscript{2+}, in terms of both binding affinity (millimoles versus micromoles for Ca\textsuperscript{2+}) and voltage shift (Pottosin et al., 1997, 2004). Binding of vacuolar Ca\textsuperscript{2+} and Mg\textsuperscript{2+} causes stabilization of the channel’s closed states and shift of the activation threshold to unphysiological, positive potentials—the opposite effects compared with the action of these ions at the cytosolic side. An increase of vacuolar Ca\textsuperscript{2+}, therefore, albeit increasing the driving force for Ca\textsuperscript{2+} release, closes the Ca\textsuperscript{2+}-permeable SV channel. Extrapolation to physiologically relevant electrochemical gradients for Ca\textsuperscript{2+} across the tonoplast yielded an SV channel open probability of <0.03% (Pottosin et al., 1997). Other vacuolar cations potently shifting the SV channel voltage dependence to more positive potentials are Na\textsuperscript{+} (Ivashikina and Hedrich, 2005) and Al\textsuperscript{3+} (Wherrett et al., 2005). The inhibitory effects of multivalent cations (e.g. Ca\textsuperscript{2+}) on the SV channel at the vacuolar side are decreased at increasing ionic strength (Pottosin et al., 2005).

The SV channel is unquestionably the best characterized vacuolar ion channel, yet its physiological role is still unclear. Based on its voltage dependence, the SV channel could mediate uptake of cations into the vacuole. However, for most physiologically important cations, the direction of the electrochemical potential gradient only allows passive release from the vacuole. The SV channel, once gated open, will mediate the efflux of vacuolar Na\textsuperscript{+} (especially under salt stress), Mg\textsuperscript{2+}, and Ca\textsuperscript{2+}.

Initially the SV channel was postulated to allow the equilibration of K\textsuperscript{+} across the vacuolar membrane (Colombo et al., 1988; Amodeo et al., 1994; Pagano et al., 2001) and to be involved in turgor regulation (Hedrich and Schroeder, 1989). Under adequate K\textsuperscript{+} nutrition, cytosolic and vacuolar K\textsuperscript{+} concentrations are comparable (Bethmann et al., 1995; Walker et al., 1996), and a high K\textsuperscript{+} permeability of the tonoplast probably keeps the electrical potential low. The SV channel might contribute to this K\textsuperscript{+} permeability. Under K\textsuperscript{+}-replete and K\textsuperscript{+}-deficient conditions, in contrast, significant K\textsuperscript{+} gradients are established across the tonoplast to maintain a stable cytosolic K\textsuperscript{+} concentration while the vacuolar K\textsuperscript{+} concentration changes according to external availability (Walker et al., 1996). To establish significant trans-tonoplast K\textsuperscript{+} gradients, SV channel activity has to be regulated down to allow effective K\textsuperscript{+} compartmentalization. It is known that K\textsuperscript{+} starvation causes an increase in cellular putrescine content (Richards and Coleman, 1952; Smith, 1985), to levels which block the SV channel (Dobrovinskaya et al., 1999b). Moreover, low vacuolar K\textsuperscript{+} concentrations down-regulate SV channel activity (Pottosin et al., 2005). In non-halophytic plant cells, K\textsuperscript{+} is a major cellular osmoticum. Hence, control of SV channel activity by vacuolar K\textsuperscript{+} is likely to contribute to turgor regulation. It is known that \( ^{86}\text{Rb}\textsuperscript{+} \) (which is chemically similar to K\textsuperscript{+}) release during stomatal closure is controlled in a feedback manner by the remaining vacuolar cation content; this \( ^{86}\text{Rb}\textsuperscript{+} \) release depends, at least in part, on the elevation of cytosolic Ca\textsuperscript{2+} with a high threshold, suggesting an involvement of the SV channel (MacRobbie, 1995, 1998).

An important strategy of plants to adapt to salt stress is the effective compartmentalization of cytotoxic Na\textsuperscript{+} into the vacuole. Under these conditions, all routes allowing passive Na\textsuperscript{+} release from the vacuole—such as an open SV channel—have to be closed. Growth under salt stress resulted in reduced SV channel activity in two Plantago species, and only for the salt-tolerant species (\textit{P. maritima}) was a complete suppression of SV channel activity observed (Maathuis and Prins, 1990). Moreover, the SV channel is down-regulated by vacuolar Na\textsuperscript{+} (Ivashikina and Hedrich, 2005) and blocked by increasing levels of the polyamines spermidine and spermine, which are induced by salt stress (Smith, 1985; Erdei et al., 1990; Dobrovinskaya et al., 1999b).

Early on, the SV channel was postulated to be involved in Ca\textsuperscript{2+} uptake into the vacuole (Pantoja et al., 1992) or Ca\textsuperscript{2+}-mediated Ca\textsuperscript{2+} release from the vacuole (Ward and Schroeder, 1994). Meanwhile it seems to be clear that the huge trans-tonoplast Ca\textsuperscript{2+} gradient only allows channel-mediated Ca\textsuperscript{2+} release, and not uptake, at physiologically attainable tonoplast potentials (see above). Hence, the SV channel operates as a vacuolar Ca\textsuperscript{2+}-release channel. Under some circumstances, this Ca\textsuperscript{2+} release might be autoinducible because the SV channel is Ca\textsuperscript{2+} activated. As discussed above, even the opening of just a tiny fraction of the thousands of SV channels per vacuole inevitably results in a considerable increase of the cytosolic free Ca\textsuperscript{2+} concentration, pointing to an involvement in Ca\textsuperscript{2+}-mediated intracellular signal transduction. There are some indications from recent publications as to in which intracellular Ca\textsuperscript{2+} signalling pathways the SV channel might or might not be involved.

Al\textsuperscript{3+} stress causes a sustained elevation of cytosolic Ca\textsuperscript{2+} possibly via reactive oxygen species-dependent activation of the SV channel (Kawano et al., 2004). The Al\textsuperscript{3+}-induced Ca\textsuperscript{2+} increase is higher in Al\textsuperscript{3+}-sensitive
wheat plants compared with Al3+-resistant genotypes (Zhang and Rengel, 1999). At the same time, Al3+-resistant wheat plants, which show a smaller cytosolic Ca2+ increase in response to Al3+, show a higher degree of SV channel inhibition by Al3+ compared with an Al3+-sensitive wheat genotype (Wherrett et al., 2005). Disturbance of cytosolic Ca2+ homeostasis (Rengel, 1992) due to sustained SV channel activation may be an important part of Al3+ toxicity, and a more effective blockage of the SV channel by vacuolar Al3+ may contribute to increased Al3+ tolerance (Wherrett et al., 2005).

An H2O2-induced increase in cytosolic Ca2+ in tobacco BY2 cells was inhibited by co-suppression of NiTPC1A/B (NiTPC1A/B encode two highly homologous SV channels in tobacco), and enhanced by overexpression of AtTPC1 (Kawano et al., 2004; Kadota et al., 2005), suggesting that vacuolar Ca2+ release by the SV channel is an important part of oxidative stress-induced signal transduction. (As discussed above, in contrast to the original publications, a vacuolar localization of all TCP1 gene products is assumed here.) A sucrose-induced cytosolic Ca2+ increase was slightly enhanced by overexpression of AtTPC1 in Arabidopsis leaves, while suppression of TCP1 expression resulted in inhibition of the cytosolic Ca2+ increase in response to sucrose (Furuichi et al., 2001; Kadota et al., 2004). In contrast, the cytosolic Ca2+ increase in tobacco BY2 cells caused by a hypo-osmotic shock was not affected by co-suppression of NiTPC1A/B, whereas overexpression of AtTPC1 in the same cells resulted in an enhanced Ca2+ increase (Kadota et al., 2004; Kawano et al., 2004). While the SV channel seems to play a critical role in sucrose-induced Ca2+ increase, its contribution to the hypo-osmotic shock-induced Ca2+ increase is less clear.

There is evidence that the SV channel is an essential component of elicitor-induced signal transduction and programmed cell death in both monocotyledonous and dicotyledonous plants. In tobacco BY2 cells, co-suppression of NiTPC1A/B caused a reduced response to the fungal elicitor cryptogein, consisting of a smaller cytosolic Ca2+ increase and less defence-related gene expression and programmed cell death (Kadota et al., 2004). In suspension-cultured rice cells, an insertional knock-out mutant of OsTPC1 severely suppressed elicitor (Trichoderma viride xylanase)-induced activation of a mitogen-activated protein kinase (MAPK) and programmed cell death, while OsTPC1 overexpression caused enhanced elicitor sensitivity with an elevated oxidative burst, and increased activation of MAPK and programmed cell death (Kurusu et al., 2004).

Whereas Arabidopsis plants lacking or overexpressing AtTPC1 do not seem to display an obvious phenotype (Peiter et al., 2005), rice plants lacking OsTPC1 grew to slightly smaller size, and OsTPC-overexpressing plants showed reduced growth and greening of roots (Kurusu et al., 2004). In Arabidopsis knock-out lines lacking AtTPC1 (Attpc1), the plant hormone abscisic acid (ABA) is less effective at inhibiting germination, while ABA sensitivity of germination is increased in AtTPC1 overexpression lines (Peiter et al., 2005). In contrast to this, ABA-induced stomatal closure was affected neither by AtTPC1 overexpression nor by knock-out (Peiter et al., 2005). However, stomatal closure induced by high external Ca2+ (10 mM) could not be observed in Attpc1 knock-out mutants, while AtTPC1 overexpression lines showed Ca2+-induced stomatal closure comparable with that of the wild type (Peiter et al., 2005). Arabidopsis lines overexpressing TaTPC1 (T. aestivum) exhibited reduced stomatal apertures compared with wild-type plants at high external Ca2+ (Wang et al., 2005). Whereas the SV channel seems to be an essential component of ABA-induced inhibition of seed germination and stomatal closure under high external Ca2+, ABA-induced stomatal closure does not seem necessarily to require a functional SV channel. To explain the latter, it has been postulated that stomatal ABA signalling is robust, meaning that the loss of AtTPC1 is compensated by recruiting alternative cation release pathways (Peiter et al., 2005).

**Ligand-gated Ca2+ channels**

A variety of physiological responses in plants is mediated by intracellular ligands such as IP3 (inositol 1,4,5-trisphosphate, a product of phosphoinositol hydrolysis by phospholipase C) or cADPR (cADP ribose, an NAD metabolite), both known to activate distinct Ca2+ release channels in animal cells (Ehrlich et al., 1994; Guse et al., 1999). Photolysis of caged IP3 or microinjection of cADPR into guard cells produces stomatal closure (Blatt et al., 1990; Leckie et al., 1998). Internal levels of IP3 and cADPR in guard cells are increased upon ABA treatment (Lee et al., 1996; Wu et al., 1997). Voltage-evoked transient increases in cytosolic Ca2+ in guard cells are inhibited by high concentrations of ryanodine, implying an important contribution of cADPR-mediated signalling (Grabov and Blatt, 1999). At the same time, blocking of phospholipase C activity abolished ABA-induced cytosolic Ca2+ oscillations and stomatal closure (Staxen et al., 1999). Inhibitor analysis of vacuolar solute loss during ABA-induced stomatal closure revealed that cADPR- and IP3-linked pathways together make a significant or even dominant (at low ABA doses) contribution (MacRobbie, 2000). In Arabidopsis roots, hyperosmotic or NaCl treatment induced IP3 production and, simultaneously, an intracellular Ca2+ mobilization; both processes were blocked by the phospholipase C inhibitor U-73122 (DeWald et al., 2001). It appears that chilling also provokes a Ca2+ response including an IP3-mediated component (Knight et al., 1996). Therefore, both cADPR and IP3 are involved in intracellular Ca2+ release in plants.
The nature of plant intracellular ligand-gated Ca\textsuperscript{2+} release channels and their organelle location is less clear. Early studies on microsomes from Chenopodium album (Lommel and Felle, 1997) and B. vulgaris (Allen et al., 1995) indicated that cADPR- and IP\textsubscript{3}-induced Ca\textsuperscript{2+} release has a vacuolar origin. However, a more detailed study made on separated membrane fractions from cauliflower florets revealed that the contribution of IP\textsubscript{3}-induced Ca\textsuperscript{2+} release from vacuoles is minor compared with release from non-vacuolar stores (Muir et al., 1990; Alexandre and Lassalles, 1998) as the FV current is (Allen and Sanders, 1996). The non-invasive MIFE technique is a very sensitive method to measure Ca\textsuperscript{2+} fluxes from individual plant cells (Shabala et al., 2006). This technique was applied to the large central vacuoles from red beet taproots. An average Ca\textsuperscript{2+} current of \sim 1 \mu A per vacuole was measured at resting conditions, and no significant increase was observed upon application of 2 \mu M IP\textsubscript{3} (Wherrett, 2006).

Keeping in mind the strong evidence that IP\textsubscript{3} and ryanodine/cADPR-type receptor Ca\textsuperscript{2+} channels play an important role in Ca\textsuperscript{2+}-mediated signal transduction in plant cells, it is surprising that no corresponding genes have yet been identified in plants (Nagata et al., 2004). This may be explained by very weak homology between plant and animal Ca\textsuperscript{2+} release channels due to an early evolutionary divergence (Maathuis, 2004). Alternatively, homoplasys may be an explanation, since functionally similar Ca\textsuperscript{2+} signal transduction pathways may well have evolved from different molecular building blocks (Bothwell and Ng, 2005). In light of the importance of intracellular Ca\textsuperscript{2+} signalling, the identification of genes encoding the ion channels responsible for cADPR- or IP\textsubscript{3}-induced Ca\textsuperscript{2+} release in plants, along with the biochemical and physiological characterization of their products, is a priority task. In parallel, the search for corresponding single channels in different intracellular membranes (large central vacuole, small vacuoles, and different endoplasmic reticulum fractions) for a biophysical and pharmacological characterization should be reinforced.

Note added in proof

In a recent review article entitled ‘Inositol trisphosphate receptor in higher plants: is it real?’ Krinke et al. (Journal of Experimental Botany 58, 361–376, 2007) summarize the current knowledge about IP\textsubscript{3} receptor Ca\textsuperscript{2+} channels in plants. A recent publication by Bonaventura et al. (The Plant Journal 49, 889–898, 2007) indicates that a gain-of-function allele of TPC1 activates oxylipin biogenesis after leaf wounding in Arabidopsis.

Acknowledgements

Our research is supported by CONACyT (grant 38181 N to IP) and the National Science Foundation (MCB-0212663 to GS). We gratefully acknowledge the remarks of two anonymous reviewers who contributed to improving this review.

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