Minireview

Modulation of plant ion channels by oxidizing and reducing agents

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Abstract

Ion channels are proteins forming hydrophilic pathways through the membranes of all living organisms. They play important roles in the electrogenic transport of ions and metabolites. Because of biophysical properties such as high selectivity for the permeant ion, high turnover rate, and modulation by physico-chemical parameters (e.g., membrane potential, calcium concentration), they are involved in several physiological processes in plant cells (e.g., maintenance of the turgor pressure, stomatal movements, and nutrient absorption by the roots). As plants cannot move, plant metabolism must be flexible and dynamic, to cope with environmental changes, to compete with other living species and to prevent pathogen invasion. An example of this flexibility and dynamic behavior is represented by their handling of the so-called reactive oxygen species, inevitable by-products of aerobic metabolism. Plants cope with these species on one side avoiding their toxic effects, on the other utilizing them as signalling molecules and as a means of defence against pathogens. In this review, we present the state-of-the-art of the modulation of plant ion channels by oxidizing and reducing agents.

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Ion channels are proteins forming hydrophilic pathways through the membrane of all living organisms, from eukaryotes to prokaryotes [1,2]. They play important roles in the electrogenic transport of ions and metabolites. The main function of ion channels is to form a permeation pore for electrically charged molecules present in the aqueous solutions bathing the cellular membrane. The molecular structure of ion channels is highly specialized [3–7] as revealed by the high selectivity and high turnover rate for the permeant ion [8]. Moreover, the opening and closing of ion channels can be modulated by several physical (transmembrane potential, temperature) and chemical (calcium, pH) parameters. Because of the above biophysical properties ion channels are involved in several physiological processes both in animal cells [1] (e.g., maintenance of the membrane potential, generation/propagation of the action potential, and transduction of sensory stimuli) and in plant cells [9] (e.g., maintenance of the turgor pressure, opening and closing of stomata, and nutrient absorption by the roots). The importance of ion channels in plant cells has begun to emerge in the last decade. Increasing experimental evidence proves that ion channels play key roles in the transduction of environmental and internal signals [9]. In physiological processes which are essential for plant life and development such as light perception [10], plant defence reactions triggered by elicitors [11], hormones [12], and mechanical signals [13], a direct involvement of ion channels was demonstrated. Another emerging feature is the modulation of ion channel by a variety of parameters/elements that are coupled to cellular metabolism such as phosphorylation/dephosphorylation [14], G-protein interactions [15], nucleotides [16], cyclic nucleotides [17], cytoskeleton proteins [18], channel modulatory β subunits [19], 14-3-3 proteins [20], and glutamate [21]. However, the position and functions of ion channels in plant signal-transduction chains are

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largely unknown and this stresses the importance of the biophysical characterization of these proteins.

Unlike animals, plants cannot move. To cope with environmental changes, to compete with other living species, and to prevent pathogen invasions, plant metabolism must be flexible and dynamic. An example of this flexibility is represented by the use of the so-called reactive oxygen species (ROS). The term ROS is very general and includes substances such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen and free radicals like superoxide (O$_2^\cdot$) and hydroxyl radicals (OH$^\cdot$). The uncontrolled production of free radicals and related species is named oxidative stress [22]. The term antioxidant describes any compound capable of quenching reactive oxygen species without undergoing conversion to a destructive radical [23]. Among the various antioxidants (concerning the enzymes see [22,23]) glutathione and ascorbate are of paramount importance. They are low molecular-weight reducing agents present in many tissues at millimolar concentrations, which are able to operate rapidly and directly against oxidative stress.

In particular circumstances, the plant cell may favor the production of oxidizing agents like hydrogen peroxide, using its destructive power to reduce invasion by pathogens [24]. The above considerations reveal the role that molecules like glutathione, ascorbate, and hydrogen peroxide have in processes of rapid signal-transduction. To keep oxidative stress under control (to avoid its toxic effect and to utilize oxidative stress as a means of defense), changes in glutathione, ascorbate, and hydrogen peroxide concentrations are perceived by the plant as signals that activate a series of cellular reactions, e.g., the synthesis of appropriate enzymes that improve the plant’s response to stress [23,25,26]. These signal-transduction pathways may include increases in membrane permeability (an important role is certainly played by lipid peroxidation) and intracellular calcium [22]; this fact suggests a possible involvement of specific ion channels modulated by the redox state of the cell.

**Modulation of ion channel activity by ROS**

This part aims at summarizing direct and circumstantial evidence for ROS acting on plant ion channels. It should be noted that redox modulation of plant ion channels has also been reported without the involvement of ROS. As in these cases the interacting factor (which may be also ROS) has not been identified, we will summarize them using the general term “cellular redox environment”.

Common early events in many stress and developmental responses of plant cells are generation of ROS as well as increases in the cytoplasmic calcium concentration. Interestingly, it has become obvious that Ca$^{2+}$ and ROS interact at multiple levels forming an intricate network which influences the regulation of different cellular functions. Ca$^{2+}$ exerts both positive and negative regulatory roles on cellular ROS levels. ROS-producing NADPH oxidases contain a Ca$^{2+}$-binding EF-hand motif [27], and NAD kinase, providing the NADPH substrate, is dependent on calmodulin [28]. Furthermore, calmodulin was shown to activate catalase, the major H$_2$O$_2$ scavenging enzyme, of Arabidopsis thaliana [29]. On the other hand, ROS influence cytosolic Ca$^{2+}$ signaling in several ways. H$_2$O$_2$ was shown to inflict an oxidative methionine modification on calmodulin, thus inhibiting calmodulin-dependent activation of the plasma membrane Ca-ATPase [30]. Bovine calcineurin, a Ca$^{2+}$- and calmodulin-dependent protein phosphatase, was inhibited by H$_2$O$_2$ by oxidative formation of a disulfide bridge [31], while, contrary to O$_2^\cdot$ and NO, the human enzyme was insensitive to H$_2$O$_2$ [32].

Regulation of ion channels acts at different levels. Thus, in this context one has also to consider the possibility that they may be indirectly affected by these inhibitory effects of ROS. For example, it has been proposed that the activity of the slowly activating vacuolar channel (SV) [33], a channel that has been identified in the tonoplast of all plant tissues and species investigated so far, may be modulated by Ca$^{2+}$/calmodulin [34] and calcineurin [35].

Talking about immediate plant ion channel modulation, it should be emphasized that, in contrast to the animal field (for a review, see [36]), there are very few reports providing evidence for direct effects of ROS and, furthermore, in no case the molecular mechanism was revealed. After reconstitution into a black lipid membrane, Klüsener et al. [37] demonstrated that BCC1, a calcium channel from tendrils of Bryonia dioica, is inhibited by H$_2$O$_2$ applied on the cytoplasmic side. However, since H$_2$O$_2$ treatment triggered transient [Ca$^{2+}$]$_{cyt}$ elevations in Commelina guard cells [38] and tobacco [39,40], it is plausible to expect other calcium channels to be activated by ROS. These and further cases of ROS-mediated ion channel modulation will now be discussed in detail in their physiological context.

**Biotic and abiotic stress factors**

ROS generation has been reported for a wide range of biotic and abiotic stress factors: for pathogen attack [41], wounding [42], drought [43], high and low temperatures [44,45], ozone [46], metal ions [47], salinity [48], excess light [49], and UV-B radiation [50]. Therefore, it seems to be one common denominator in plant stress responses. The perturbation of the cellular redox envi-

**Abbreviations used:** ROS, reactive oxygen species; HR, hypersensitive response; SAR, systemic acquired resistance; AMAs, aromatic monoamine compounds; PCMBs, p-chloromercuribenzenesulfonic acid.
environment may explain response similarities and also the cross-tolerance phenomenon, i.e., the exposure to one stress confers greater resistance to further (the same or a different) stress [51].

A broad study reporting the involvement of the highly reactive hydroxyl radicals (OH•) in diverse stress conditions was presented by Demidchik et al. [52]. This type of ROS was shown to be produced by intact Arabidopsis roots in response to cold shock, high salt, and elicitor treatment. In root epidermal protoplasts, it activated both a nonselective cation channel mediating Ca2+ influx and an outward-rectifying K+ channel. By contrast, application of hydrogen peroxide did not affect cation currents. Furthermore, epidermal cells showed a greater sensitivity towards ROS than cells of the pericycle, indicating that channel activation may be involved in relaying environmental signals arriving from the rhizosphere.

**Defence responses to pathogen challenge**

Plants are able to respond to pathogen attack by sensing the presence of elicitors at their cell boundaries. Early steps in this receptor-mediated response are ion fluxes across the plasma membrane and ROS production. In this context, ROS play a dual role [53]: first, acting as a second messenger in the signal-transduction leading to defence responses, and second, being themselves part of these responses, i.e., the oxidative burst during the hypersensitive response (HR). Furthermore, salicylic acid-mediated induction of the systemic acquired resistance (SAR) mechanism in uninfected parts of the plant also involves ROS generation [54].

Kawano et al. [55,56] proposed aromatic monoamine compounds (AMAs) as potential defence inducers in plants. Addition of phenylethylamine or benzylamine to tobacco BY-2 cells triggered rapid generation of ROS with subsequent increases in [Ca2+]cyt [55].

Numerous studies demonstrated transient increases in cytosolic Ca2+ after elicitor treatment [57–60]. In some cases, Ca2+ influx was found to precede ROS production, e.g., in parsley cells [61] and in tobacco BY-2 cells [62]. On the other hand, H2O2 was shown to trigger Ca2+ influx in tobacco cells after salicylic acid [63] and elicitor treatment [60]. Supporting these results based on fluorescence measurements using the Ca2+-sensitive aequorin system, the activation of calcium-permeable channels by elicitors was demonstrated by means of the patch-clamp technique [11,64]. Guard cells respond to elicitor or salicylic acid treatment with ROS production and reduction of stomatal aperture [65,66]. Interestingly, Klüsener et al. [67] reported the elicitor- and H2O2-stimulated activation of a Ca2+ influx current, showing close similarities (i.e., NADPH-dependence, activation by hyperpolarization) to the one described for the ABA response of Arabidopsis guard cells ([43,68]; see below). Together with the fact that in both cases the NADPH oxidase catalytic subunit genes, AtRbohD and AtRbohF, were found necessary for ROS accumulation [69,70], these results point strongly towards shared pathways in pathogenic elicitor and ABA signalling in guard cells.

The identity of the involved calcium-permeable channels is not yet known. Recently, several cyclic nucleotide-activated channels (CNGCs) have been found to be involved in plant defence [71–73]. A possible modulation of their activity by ROS has not been examined, however expression of the PvCNGC-A gene from Phaseolus vulgaris was found to be induced upon both pathogen challenge and H2O2 treatment [72].

**ABA and guard cells**

Under drought conditions plants produce the phytohormone abscisic acid (ABA) which induces a signalling cascade in guard cells leading to stomatal closure, thereby reducing transpirational water loss. Stomatal closure is achieved by the concerted action of ion channels in both the vacuolar membrane and plasma membrane resulting in turgor loss by net efflux of potassium and anions (chloride and malate). Increases in cytoplasmic pH and [Ca2+]cyt play central roles in relaying the ABA signal [74] leading to inactivation of inward-rectifying K+ channels and activation of outward-rectifying K+ channels and Cl– channels in the plasma membrane. It has been shown that both Ca2+ release from internal stores (like vacuole and ER) and Ca2+ influx from the extracellular space are involved in the ABA-induced calcium increase [43,75,76].

Knowledge about the response of calcium channels in internal membranes to ABA is limited. Only recently a role for nitric oxide (NO) has emerged. NO is a free radical gas that plays a key role as signalling molecule in the immune, nervous, and vascular systems of animals. It seems likely that in plants NO can be synthesized and act together with H2O2 during cellular stress responses (for a review on NO as a signalling molecule in plants, see [77]). NO is generated by nitrate reductase after ABA treatment [78] and was found necessary for stomatal closure [78,79]. Providing first hints on the mode of NO action, Garcia-Mata et al. [80] demonstrated that nanomolar NO concentrations determine intracellular Ca2+ release, however leave plasma membrane calcium channels unaffected. NO-induced Ca2+ release is presumably mediated by activation of cADPR/ryanodine-sensitive Ca2+ channels via the cGMP pathway [80]. Indeed, cADPR has been identified as a signalling molecule in the ABA response [81,82], and cADPR-responsive Ca2+ release has been detected in guard cell vacuoles of Commelina communis [82] as well as in the endoplasmic reticulum of cauliflower inflorescences [83]. However, a direct modulation of the calcium channels by NO cannot be excluded. Indeed, in animals NO was shown to activate this channel type by S-nitrosylation of cysteine residues [84,85].
In contrast to internal Ca\textsuperscript{2+} release, a series of recent publications has allowed to build a model of ABA-induced activation of a hyperpolarization-activated Ca\textsuperscript{2+} channel in the plasma membrane of guard cells. The ABA signalling pathway involves the production of hydrogen peroxide which is dependent on the presence of cytosolic NADPH [68]. Inhibitor studies suggested a NADPH oxidase to be involved in H\textsubscript{2}O\textsubscript{2} generation [43]. This was confirmed by the analysis of Arabidopsis plants mutated in the NADPH oxidase catalytic subunit genes, AtrbohD and AtrbohF [70]. Acting as an essential second messenger, H\textsubscript{2}O\textsubscript{2} was shown to activate a calcium-permeable, nonselective cation channel leading to calcium influx and to an increase of [Ca\textsuperscript{2+}]\textsubscript{cyt} in Arabidopsis guard cells [43]. H\textsubscript{2}O\textsubscript{2} activation of calcium currents is disrupted in the Arabidopsis gca2 and abi2-1 mutants [43,68]. Whereas the function of the GCA2 gene is unknown, ABI2 represents a protein phosphatase 2C (PP2C), probably acting as a negative regulator of ABA signalling [86]. Adding a second probable function to ABA-induced ROS production, H\textsubscript{2}O\textsubscript{2} was shown to rapidly inactivate recombinant ABI2, presumably by oxidation of cysteine residues, thus relieving ABA signalling from negative regulation by ABI2 [87].

During stomatal closure Ca\textsuperscript{2+} serves as a second messenger in guard cells to initiate ion channel actions promoting efflux and preventing influx of ions (for review, see [88,89]). However, two studies on Vicia faba guard cell protoplasts suggest that inward-rectifying K\textsuperscript{+} channels, although inactivated by elevated [Ca\textsuperscript{2+}]\textsubscript{cyt}, may be subjected to the additional direct modulation by ROS. In whole-cell patch-clamp experiments, ABA-induced ROS production, H\textsubscript{2}O\textsubscript{2} was shown to rapidly inactivate recombinant ABI2, presumably by oxidation of cysteine residues, thus relieving ABA signalling from negative regulation by ABI2 [87].

Metal ions

Plants exposed to excess concentrations of iron and copper suffer from oxidative stress, since redox active heavy metal ions induce increased ROS production by autoxidation and Fenton reaction [47]. Although there is no report providing direct evidence for redox modulation of ion channels in response to copper, some findings may deserve further investigation in order to detect possible linkages. In wheat roots grown under Cu\textsuperscript{2+} excess conditions, superoxide-producing NADPH-dependent oxidase activity was found to be increased [92]. On the channel side, there are several examples of potential targets. First, roots of Arabidopsis seedlings respond to copper challenge with efflux of citrate, serving as a chelator, and concomitant K\textsuperscript{+} efflux to prevent membrane depolarization [93]. Second, a voltage-clamp study demonstrated a rapid general conductance increase accompanied by a decrease in Cl\textsuperscript{−} conductance in the plasma membrane of the alga Nitella flexilis treated with Cu\textsuperscript{2+} [94].

Aluminum (Al\textsuperscript{3+}) exerts its phytotoxicity mainly on root apex cells, thereby inhibiting root growth [95]. Although Al\textsuperscript{3+} is not able to catalyze redox reactions by itself, it induces oxidative stress in plant cells [96,97]. In tobacco cultured cells and pea roots, it was shown to impair mitochondrial function and to trigger ROS production, however after a long lag period [98]. By contrast, aluminum induced rapid production of superoxide involving NADPH oxidase in tobacco BY-2 cells. ROS accumulation was followed by a [Ca\textsuperscript{2+}]\textsubscript{cyt} increase, as shown by aequorin luminescence [99]. Despite the lack of direct evidence, these results may indicate that aluminum triggers ROS-dependent activation of Ca\textsuperscript{2+} channels involved in a signalling cascade eliciting tolerance mechanisms. Detoxification mechanisms conferring tolerance are considered to be mainly based on organic acids (like citrate, malate, and oxalate) forming stable Al complexes, both inside and outside the cell [100]. Recently, a series of patch-clamp studies on maize [101–103] and wheat [104] roots demonstrated the Al\textsuperscript{3+} triggered activation of anion channels, which are thought to be responsible for organic acid exudation. Experiments in excised patch configuration indicated that the activation mechanism locates either to the channel protein itself or to the close membrane environment [102]. Furthermore, concomitant K\textsuperscript{+} efflux in response to Al\textsuperscript{3+} may be regarded as secondary charge balance or regulatory mechanism or alternatively as an independent activation event [104,105]. Thus, several additional channels, in the first place anion channels, may be potential targets of Al\textsuperscript{3+} induced ROS.

Ozone

The air pollutant ozone (O\textsubscript{3}) shows two ways of exerting oxidative stress on plant cells. After its entry into the apoplastic space, it is spontaneously converted to ROS, reacting with extracellular components and affecting membrane integrity [106]. Additionally, it was found to act as an abiotic elictor of plant defence and antioxidant responses, thereby inducing additional endogenous ROS production [107]. In Vicia faba, O\textsubscript{3} applied to guard cell protoplasts was shown to inhibit inward K\textsuperscript{+} currents impairing stomatal opening, without affecting outward currents involved in stomatal closure [108]. Although the mechanism has not been studied yet, this inhibition might be mediated by ROS produced by the O\textsubscript{3}-triggered events mentioned above.
Osmotic and mechanical stress

Plant cells subjected to hyposmotic or mechanical stress produce an oxidative burst similar to the classical defence response, as was shown in suspension-cultured soybean [109] and tobacco [110] cells. Additionally, ROS appeared to contribute to K⁺ and Cl⁻ effluxes in tobacco [110]. However, the mechanism was not investigated further.

Embryonic cells of the alga Fucus serratus respond to hyperosmotic treatment with a signalling cascade involving localized ROS production at the plasma membrane and Ca²⁺ influx [111]. Indeed, cell-attached patch-clamp experiments demonstrated the activation of a nonselective cation channel upon application of H₂O₂ [111].

Cell growth and development

Only very recently a role of ROS in the control of cell growth and development has emerged. Root growth depends on Ca²⁺ influx in elongating cells [112]. Disruption of calcium uptake in the A. thaliana mutant rhd2 results in stunted roots and short root hairs [113–115]. Recently, the RHD2 gene was cloned and found to encode the NADPH oxidase AtRbohC [115]. Indeed, ROS accumulation is markedly decreased in the mutant plant, while external application of hydroxyl radicals restored elevation of cytosolic [Ca²⁺] and root hair growth. Both in elongating epidermal cells and in root hairs OH⁻ elicited an inwardly rectifying, hyperpolarization-activated calcium conductance [115]. Also Demidchik et al. [52] reported the activation of ion channels by hydroxyl radicals. Stimulation of a nonselective cation channel and a K⁺ channel mediating Ca²⁺ influx and K⁺ efflux, respectively, was higher in elongating compared to mature epidermal cells of Arabidopsis roots. Moreover, application of a hydroxyl radical scavenger led to a decrease in the root elongation rate. Thus, in addition to their probable responsiveness to stress signals (see above), a role of these hydroxyl radical-activated calcium channels in root growth was proposed. It is noteworthy that both studies on root growth found ROS activation of Ca²⁺-permeable channels to be mediated by hydroxyl radicals, but not by H₂O₂ [52,115].

Apart from AtRbohC [115], additional NADPH oxidase catalytic subunits, AtRbohD and AtRbohF, may be involved in developmental processes, since the Arabidopsis double mutant atrobohD/F shows impaired ABA-mediated seed germination and root elongation [70]. Both enzymes are also implicated in ROS production of the pathogenic elicitor and ABA signalling pathways ([69,70]; see above). Additionally, antisense studies in tomato plants suggest Rboh to act as a transducer in developmental and stress signalling [116].

Light is a key regulator of plant developmental processes. Specifically, radiation in the short range of the spectrum (near-UV and blue light) is involved in the control of cell extension, phototropism, and flowering time [117,118]. However, the signalling cascade leading to changes in gene expression patterns is largely unknown. ROS production has been identified as an early event following UV-B perception [50,119]. High UV-B flux elicits oxidative stress responses through unspecific damage of cell components and perturbation of chloroplast function, while at low “regulatory” dosage ROS may result from enzymatic activity [120]. Similarities in signal-transduction and gene expression also indicate shared pathways between the UV-B and defence response [121]. UV-B signalling involves both ROS and NO [50]. In maize seedlings, NO was found to act as a second messenger in UV-B-induced inhibition of mesocotyl elongation [122]. Perception of blue/UV-A and UV-B light initiated redox activity in the plasma membrane of Arabidopsis cells, which was inhibited by a NADPH oxidase antagonist [123]. Indeed, activity of this enzyme increases in response to UV-B [124] and blue light [125,126]. Long and Jenkins [123] proposed a model involving Ca²⁺ release from internal stores downstream of blue/UV light-induced redox activity. Furthermore, a voltage-dependent calcium-permeable channel in Arabidopsis mesophyll cells is activated by blue light perceived by phototropin receptors [127]. On the basis of these findings one could speculate that the Ca²⁺ channel in the plasma membrane may respond to light-triggered ROS production, thus allowing Ca²⁺ influx which in turn would initiate a Ca²⁺-induced Ca²⁺ release mechanism and finally calcium elevation leading to changes in gene expression. However, NADPH oxidase-derived ROS are presumably not involved, since application of the inhibitor DPI did not affect Ca²⁺ channel activation [127].

Programmed cell death and mitochondrial ion channels

The electron-transport chain of the inner mitochondrial membrane is a major source of cellular ROS [128]. Furthermore, a recent study on a tobacco mutant revealed a role for mitochondria in orchestrating whole cell redox balance [129]. Programmed cell death (PCD) is involved in both plant–pathogen interactions [130] and plant development [131], thus overlapping in part with previous topics. Here, we highlight the role of mitochondrial ion channels in PCD and hints to their redox modulation.

Thioredoxins (Trx) are small proteins with a redox-active disulfide group, present in the cytosol, chloroplasts, and mitochondria, exerting their regulatory function on target proteins by reducing specific S–S groups [132]. In a recent proteomics study, a VDAC (voltage-dependent anion channel) has been identified as Trx-linked and thus potentially redox-regulated protein in plant mitochondria [133]. This outer membrane
channel participates in the formation of the permeability transition pore (PTP) complex releasing death-promoting factors during PCD [134]. In Citrus sinensis, cell death following NO treatment was prevented by cyclosporin A (CsA), a specific inhibitor of PTP [135]. CsA acts through selective binding to cyclophilin D, a matrix protein also participating in the PTP complex [136]. On the other hand, CsA was found to activate a mitochondrial K_{ATP} channel [137] also implicated in plant PCD, since swelling due to channel opening caused partial rupture of the outer membrane and release of cytochrome c [138]. The channel was also activated by SH-group blocking agents and NO, while it was inhibited by \( \text{H}_2\text{O}_2 \) [138].

The impact of the cellular redox environment on ion channel activity

The importance of the cellular redox environment for the regulation of ion channels was shown by patch-clamp studies in the authors’ laboratory. The slowly activating (SV) channel in vacuoles of sugar beet root and leaves of the seagrass Posidonia oceanica responded to cytosolic application of sulfhydryl reducing agents like dithiothreitol (DTT) and glutathione (GSH) with an increase in activity [139]. By contrast, the oxidizing agent chloramine-T abolished channel activity. Single channel experiments demonstrated that channel activation was due to increased open probability or number of active channels and not to increased single channel conductance [139]. The immediate response of SV currents upon fast application and removal of DTT, both in whole-vacuole and excised-patch configuration, is inconsistent with the involvement of a cytosolic or tonoplast-associated factor, strongly suggesting a direct effect of reducing agents on the channel protein. These findings indicate that the status of the cellular antioxidant pool may have a major influence on SV activity, with significant negative implications of oxidative stress caused by the (biotic and abiotic) factors mentioned above.

Additionally, DTT had beneficial effects on SV current stability in patch-clamp experiments, preventing the so-called “run-down” phenomenon [139,140]. This observation demonstrates that the redox potential of the cytoplasm is very important in the regulation of some tonoplast channels; unfortunately, most studies on channels of internal membranes (especially vacuolar channels) do not consider this important parameter.

A recent study [141] shows that the vacuolar malate inward-rectifying channel from Mesembryanthemum crystallinum mesophyll cells was inhibited by the reducing agent mercaptoethanol, while it was activated by sulfhydryl modifying agents like PCMBS. In this case, all compounds were active on site(s) facing the luminar side of the vacuole. Accordingly, the authors assumed that an oxidizing vacuolar environment would favor channel activity, which was indeed supported by correlating the redox potential of the cell sap and channel activity [141]. However, the molecular nature of the redox system has not yet been identified.

Conclusions

It has recently become evident that oxidizing and reducing agents are important intermediates in plant signalling of stress and developmental responses. According to present knowledge, calcium-permeable channels are primary targets of these agents. At least plasma membrane calcium channels appear to be exclusively activated by ROS in response to diverse internal and external signals. As the intracellular calcium concentration in turn influences cellular ROS levels, both parameters cooperate to orchestrate cellular signal-transduction. While there are examples of calcium channels providing evidence for direct channel modulation, such evidence is missing for other channel types. Potassium channels, both inward and outward rectifiers, were found to be either activated or inhibited by ROS application. Finally, there is few and in some cases only weak evidence for redox modulation of plant anion channels.

There are still many questions concerning channel modulation by oxidizing or reducing agents to be addressed. For example, which is the molecular target of oxidation, does it directly affect the channel-protein, how does it influence the kinetics and gating of the channel, is it reversible? Electrophysiological characterization will help to clarify these points, moreover, cloning and mutations of specific sites within channel genes will provide useful information to understand the correlation between structure and function and the interactions with redox agents.

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References


