Anion channels and hormone signalling in plant cells

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Abstract — Current evidences support a central role in signal transduction and turgor regulation for plasma membrane anion channels. The present review focuses on these channels as putative targets for plant hormones. Various approaches have been developed to investigate the contribution of anion channels to hormone responses at the level of integrated responses of intact cells or organs, or to study directly the hormonal regulation of anion channels at the membrane level. These approaches are mainly discussed for two biological models, stomatal guard cells and hypocotyl or coleoptile cells, both cell types being equipped with several types of anion channels. Membrane potential and anion flux measurements, together with pharmacological studies using anion channel inhibitors, reveal that anion permeabilities are involved in the responses of guard cells or hypocotyl cells to abscisic acid and/or auxin. In a few instances, a modulation of anion channel activity can be detected in voltage-clamp or patch-clamp experiments. From these data and other studies, anion channel activation seems to constitute a very early step in many transduction cascades within response pathways to endogenous hormonal signals, but also to abiotic and biotic environmental signals such as light or molecules involved in plant-pathogen interactions. This points to plasma membrane anion channels as major actors in plant signalling networks. © Elsevier, Paris

Anion channel / anion channel inhibitors / anion fluxes / membrane potential / plant hormones

ABA, abscisic acid / 9-AC, anthracene-9-carboxylic acid / CCCP, carbonyl cyanide m-chlorophenylhydrazone / DIDS, 4,4'-dithiobis(2-nitrobenzenesulfonic acid) / ETH, ethacrinic acid / GCA, guard cell anion channel 1 / IAA, indoleacetic acid / NAA, naphthaleneacetic acid / NPPB, 5-nitro-2,3-phenylpropylaminobenzoic acid / TSAC, tobacco suspension anion channel

1. INTRODUCTION

Membrane transport has long been recognised to play a central role in plant cell physiology, by either driving osmotic processes, participating in nutrient uptake or being involved in the transduction of signals or their transmission between cells. In many cases, the earliest detectable response of plant cells to a variety of endogenous and environmental signals is a modification in plasma membrane transport, detected through alterations of the electrical membrane potential. Early studies have detected such changes following hormonal treatment, for instance auxin application on pea internode segments [49], maize coleoptile [55] or oat coleoptiles [1]. Although in most cases the signal transduction pathways leading to these membrane responses are not yet clearly identified, ion transport proteins, in particular ion channels, appear as putative targets for hormonal signals [7]. Within such transduction cascades, ion channels may participate in the amplification and propagation of the signals by modulating the membrane potential, and also affect directly the intracellular ion content [72].

Anion channels are well documented in various tissues of algae and higher plants (reviewed in [33, 90]), and current evidence supports a central role in signal transduction and turgor regulation for plasma membrane anion channels [70, 93]. The present review focuses on these channels as putative targets for plant hormones. Two biological models are discussed, stomatal guard cells and hypocotyl or coleoptile cells. Both models provide powerful systems to dissect early signal transduction mechanisms because they respond to endogenous hormonal signals as well as to environmental stimuli, and some of their anion channels have already been characterised. Focusing on two hormones, abscisic acid (ABA) and auxin, we will describe the various approaches which have been developed to investigate the contribution of anion channels to hormone responses at the level of integrated responses of intact cells or organs, or to study directly hormone regulation of anion channels at the membrane level.
2. STOMATAL MOVEMENTS AND COLEOPTILE/HYPOCOTYL ELONGATION: TWO MODELS TO INVESTIGATE THE INVOLVEMENT OF ANION CHANNELS IN ABA AND AUXIN RESPONSES

The stomatal complex constitutes a simple system which responds dynamically to multiple stimuli by means of ion transport. Stomatal movements rely on changes in the turgor and volume of the pair of guard cells which control the size of the diffusion pore. An increase in guard cell volume driven by the accumulation of potassium salts and water leads to stomatal opening, whereas these salts are released and guard cell volume decreases during stomatal closing. Ion transport in these cells participates in both the initiation of signal transduction pathways and the occurrence of net ion flows [90, 93]. Stomatal guard cells have been studied extensively over the past decades and their responses to hormones such as auxins and abscisic acid are well documented. Natural and synthetic auxins were shown to elicit stomatal opening when applied exogenously to epidermal peels from Vicia faba leaves [51], with a dose-response curve of stomatal opening to 1-NAA showing an optimum around 5 μM, and a decrease in the aperture for higher concentrations and even an inhibition for 300 μM 1-NAA. Abscisic acid triggers stomatal closure with a monotonous dose-response curve, the maximal effect being observed for instance at a concentration of 1 μM in Commelina communis [53].

The regulation of cell elongation is another situation in which ion channels could play important roles because it also involves ionic fluxes implicated in turgor control and orchestrated by various signals. Coleoptile represents the most widely used model for studying the regulation of cell elongation by auxin. On coleoptile segments from maize or oat, exogenous application of 1 μM IAA stimulates the growth rate by a factor of 7 to 10 [13]. The hypocotyl from dicot plants is also an attractive system to study elongation processes, and auxin is known to promote a transient increase in the elongation rate of excised hypocotyl segments [14]. However, in intact plants, exogenous auxins exert in the long term (several days) an inhibitory effect on hypocotyl elongation. In Arabidopsis thaliana, Thomine et al. [87] have shown that 7-d-old hypocotyls from plantlets cultured in the presence of 1 μM 1-NAA showed a reduced length by 60 to 90 % over control. This effect was associated with a thickening of the hypocotyl, a disintegration of the cortical cell layer, and the induction of adventitious root primordia on the hypocotyl. All these responses mimic the phenotypic traits observed in Arabidopsis mutants with increased auxin levels [9, 42], and probably reflect supraoptimal auxin effects.

Electrophysiological investigations using the patch-clamp technique have shown that stomatal guard cells and hypocotyl cells are equipped with several types of anion channels differing mainly by their regulation. There are at least three distinct anion channels co-residing at the plasma membrane of guard cells: a rapid anion channel (R-type or GCAC1 [40]) and a slow anion channel (S-type [71, 73]), both regulated by the transmembrane voltage, and a stretch-activated anion channel [15]. More recently, two distinct types of anion channels have been characterised in hypocotyls cells of Arabidopsis, a voltage-dependent rapid-type channel [88] and a blue light-activated anion channel [12]. A third hypocotyl anion channel, resembling the S-type channel from guard cells, is being currently investigated by our group (Frachisse et al., unpubl. results). All these anion channels are tightly regulated in multiple ways, from both the external (apoplastic) and the internal (cytosolic) sides of the plasma membrane. For instance, extracellular anions act in different ways on the activity of the R-type guard cell channel GCAC1. On one hand, malate causes a shift of the channel voltage-gate toward hyperpolarised potentials [34, 36], which provides a mechanism to adapt stomatal aperture to the mode of CO₂ fixation and current photosynthetic capacity. On the other hand, a rise in extracellular chloride concentration increases the unit conductance of GCAC1 [18, 34]. Modulation of channel activity by intracellular ATP appears to be a common feature of R- and S-type anion channels from stomatal and hypocotyl cells, but proceeds in each case through different mechanisms. Both GCAC1 from V. faba guard cells and the R-type anion channel from hypocotyl cells are modulated by a binding site for intracellular nucleotides [35, 75, 86]. However whereas in guard cells, ATP activates R-type anion channels, in hypocotyl cells, the main effect of nucleotides is a shift of the voltage-dependent gate of the R-type channel toward depolarised potentials leading to a strong reduction of current amplitude [86]. In contrast, in the case of S-type channel, the regulation by intracellular ATP reflects a control of channel opening by phosphorylation events [69]. Such regulations may couple the electrical properties of the membrane with the metabolic status of the cell in the case of R-type channels, as well as provide important mechanisms for signal transduction of hormonal or other stimuli in the case of S-type channels.
Knowledge of the pharmacology of anion channels is of importance to unravel their physiological functions. In guard cells, the pharmacological profiles of R- and S-type channels largely overlap, but the sensitivity to submicromolar concentrations of stilbene derivatives appears as a distinctive feature of GCAC1 [50, 52, 74, 76]. In hypocotyl cells, fast and slow anion channels also exhibit differential sensitivities to anion channel blockers ([12, 86] and Frachisse et al., unpubl. results).

3. INVOLVEMENT OF ANION PERMEABILITIES IN THE RESPONSES OF INTACT CELLS OR ORGANS TO ABA AND AUXIN

The opening of anion channels at the plasma membrane of plant cells results in plasma membrane depolarisation and anion efflux from the cell, two events that can be used to detect channel activation upon hormone application. The use of pharmacological agents able to block channel activity [31] is another way to test the possible involvement of anion channels in a given biological response. These different approaches have been used in stomata, excised coleoptile segments and hypocotyls from intact plantlets to investigate the implication of anion channels in ABA- and auxin-induced responses.

3.1. Membrane depolarisation

Abscisic acid, which induces stomatal closing through ion release from guard cells, also triggers plasma membrane depolarisation in these cells [6, 38]. According to the electrochemical gradient for Cl− in plant cells far above the membrane potential, anion channels are good candidates for the mediation of large chloride efflux and the generation of sustained membrane depolarisation. The depolarisation would serve subsequently to activate K+ efflux channels and then to drive K+ release [93]. Such a mechanism, as suggested by Schroeder and Hagiwara [71], gives a central role to anion channels in the ABA-induced stomatal closure.

The modification of membrane potential is one of the most rapid response induced in cells from elongating organs by exogenous application of natural, as well as synthetic auxins [1, 24, 65, 82]. Such an event is likely to be triggered by early steps in the auxin signal transduction pathway and may itself be critical to the growth response. The typical response shows different phases, an immediate depolarisation followed by a delayed hyperpolarisation and sometimes oscillations. While auxin-induced hyperpolarisation is usually interpreted in terms of variations in the activity of the proton ATPase [24, 78], the origin of the depolarisation phase is still a matter of debate. Keller and Van Volkenburgh [41] focused their interest on the possible involvement of a Cl− conductance in the early response of Avena sativa coleoptiles to exogenous auxin application. They showed that both active and inactive auxins transiently depolarise the plasma membrane of cortex cells but a subsequent hyperpolarisation, presumably involving the H+-ATPase, occurred only with active auxins. The amplitude of the initial depolarisation induced by 10 μM 1-NAA and 100 μM IAA varied with the concentration of external Cl−, suggesting that it follows from a chloride efflux. In addition, the presence of the anion channel inhibitor 9-AC at 1 μM in the medium prevented the Cl−-sensitive depolarisation induced by 1-NAA. These results suggest that the auxin-induced depolarisation is the result of the opening of a population of Cl−-permeable channels. However, as the same 9-AC concentration failed to block the elongation of coleoptile fragments induced by 10 μM 1-NAA on one hand, and the maximal Cl−-sensitive depolarisation was obtained with 100 μM 1-NAA which does not induce growth on the other hand, the authors conclude that the activation of these Cl− channels is not required for the auxin-induced growth of Avena coleoptiles.

3.2. Anion fluxes

In stomatal guard cells, potassium, malate and chloride constitute the dominating osmotica [62]. Since plasmodesmata are virtually absent from functional guard cells, these cells must obtain K+ and Cl− from the apoplast while malate can be produced within the cells. The ion exchange capacity of the apoplast suffices to supply the guard cells with K+, but Cl− cannot be stored in the apoplast in large amounts because of the negative fixed charges of epidermal cell walls. Species with rapidly responding stomata, such as Zea mays, possess subsidiary cells that evolved as effective ion reservoirs in close vicinity to the guard cells [57, 63]. Chloride can account from 6 to 73 % of the K+ charge balance depending on the environmental conditions [62, 64]. It has been shown by chloride microprobe analysis combined with malate measurements that these two anions complement each other in balancing potassium in guard cells of isolated epidermal strips of V. faba [64].

Very few studies concern a direct effect of hormones upon anion efflux. Bentrup et al. [5] showed that the
apparent influx of $^{36}$Cl into suspension cells from *Petroselinum sativum* is reduced by $10^{-5}$ M IAA, and interpreted this effect as resulting from the enhancement of chloride efflux by auxin. The same type of measurements performed on *Avena* coleoptile segments led Rubinstein and Light [66] to a different conclusion. Indeed, these authors observed a promotion of Cl$^-$ uptake by growth-active auxins that they attributed to an enhancement of the primary uptake system. The effects of ABA on anion effluxes have also been rarely investigated. Beffa et al. [2] showed in the water plant *Elodea densa* (a plant without stomata) that ABA strongly inhibits Cl$^-$ efflux while no significant effect of the hormone on the membrane potential could be detected. Conversely, the chloride efflux strongly correlated with the value of cytosolic pH, the highest release occurring at the lower pH values. The authors reported that cytoplasmic acidification induced by ABA cannot be the cause of the declined chloride efflux and they hypothesise that ABA might have a primary role in regulating Cl$^-$ efflux, presumably by directly affecting a class of Cl$^-$ permeable channels. Similar conclusions were reached from investigations by the same group on *Arabidopsis* plantlets [3].

Most studies on hormonal regulation of anion fluxes were focused on chloride effuxes. It should be taken into account that other anions such as nitrate are highly accumulated in plant cells [16], and that plant anion channels described so far show a higher permeability for nitrate compared to chloride [18, 19, 20, 34, 68, 80, 84]. Nitrate and other anions should be considered for further investigation of hormone effects on membrane permeability.

### 3.3. Effects of anion channel blockers

Another set of evidence for the involvement of anion channels in the control of stomatal aperture by ABA is provided by the use of pharmacological tools. Schwartz et al. [76] have shown in *V. faba* and *C. communis* that stomatal responses induced by ABA can be inhibited by anion-channel blockers. These authors showed that the inhibition of stomatal opening by ABA was alleviated when 9-AC, probenecid or niflumic acid were added to *Commmelina* epidermal peels. The concentrations inducing 50% reversal of ABA effect were 120, 400 and 18 µM for the three anion channel blockers, respectively [76]. These inhibitors were also effective in reversing ABA inhibition of stomatal opening in *V. faba* (figure 1 A). It was then suggested that abscisic acid-induced stomatal closing requires anion channel activation. This hypothesis is consistent with previous data from Schroeder et al. [74] who showed that exogenous application of another anion channel blocker, NPPB, completely abolishes stomatal closing triggered by a combined exposure of guard cells to ABA and malate (figure 1 A). In this case, DIDS does not modify significantly the ABA response. This pharmacological profile, in particular the insensitivity to DIDS, is similar to that of the slow anion channel present in the guard cell plasma membrane, thus providing evidence for the involvement of this channel in ABA-induced stomatal closure. In *A. thaliana* guard cells, only 9-AC was able to inhibit slow anion currents, to promote stomatal opening in the dark and to reverse the effect of abscisic acid in the light [27].

Thomine et al. [87] also used a pharmacological approach to investigate the contribution of anion

![Figure 1](image.png)
channels in the regulation of the development of hypocotyls by auxins. Several anion-channel blockers which produced little or no stimulation of the hypocotyl elongation by themselves, were able to counteract the inhibition induced by auxins (figure 1 B) with the following efficiency sequence: 9-AC = DIDS > SITS > IAA94. This interference appeared to be specific for auxins since it did not occur when hypocotyl elongation was inhibited by other growth regulators such as ethylene or cytokinins. Two other blockers, niflumic acid and NPPB, strongly inhibit hypocotyl growth by themselves and do not affect the auxin response (figure 1 B). These data argue for a possible contribution of anion channels in the regulation of the development of *A. thaliana* hypocotyls by auxins. The fact that the voltage-dependent rapid-type anion channel [86, 88] and the blue-light-stimulated anion channel [12], already characterised at the plasma membrane of *Arabidopsis* hypocotyl cells, have a pharmacological signature distinct from what could be expected from the effects described at the whole-plant level emphasises the necessity of a more extensive description of the diversity of anion channels in hypocotyl cells. The slow-type anion channel recently identified by our group (Frachisse et al., unpubl. results) may be an interesting candidate, but the possibility that the anion channel involved in the auxin-regulated growth responses resides at the vacuolar membrane should also be considered.

Although ion channel blockers constitute powerful tools to reveal the contribution of a channel in a biological response, their effects should be interpreted with caution as non-specific interactions of plant ion channels with molecules developed to block animal channels have been described. For instance, antagonists of animal L-type Ca\(^{2+}\) channels (nifedipine and verapamil) as well as anion channel blockers (NPPB and niflumic acid) are potent inhibitors of plant K\(^{+}\) outward-rectifying channels [28, 85, 89].

4. ANION CHANNEL REGULATION BY ABSCISIC ACID AND AUXIN AT THE MEMBRANE LEVEL

All the studies performed at the level of intact cells, organs or whole plants provide evidence for the functional role of anion channels in a hormonal context, but do not allow to establish if the channel is the primary target for the hormonal signal or an intermediary element of the transduction cascade. A means of answering this question is to test the direct effects of hormones on channel activity at the membrane level, in patch-clamp or voltage-clamp experiments (table I).

### 4.1. Regulation of slow-type anion channels by abscisic acid

Although it could be inferred from a whole set of biophysical, biological and pharmacological data that the activation of slow anion channels represents a limiting step in ABA-induced stomatal closure, direct evidence for ABA activation of these channels had been lacking until recently. Application of the patch-clamp technique to the previously inaccessible *Arabi-

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**Table I.** Hormonal regulation of plasma membrane anion channels.

<table>
<thead>
<tr>
<th>Channel type</th>
<th>Cell type</th>
<th>Plant species</th>
<th>Conditions of measurement</th>
<th>Hormone and mode of application</th>
<th>Hormone effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow anion channel</td>
<td>Guard cell protoplasts</td>
<td><em>A. thaliana</em></td>
<td>Patch-clamp whole-cell</td>
<td>ABA 1 to 50 μM (ext + int) during 1.5 h</td>
<td>Activation of the anion current</td>
<td>[59]</td>
</tr>
<tr>
<td>Slow anion channel</td>
<td>Guard cell protoplasts</td>
<td><em>V. faba</em></td>
<td>Patch-clamp whole-cell</td>
<td>ABA 100 μM (ext) or 50 μM (int), direct application in absence of internal ATP</td>
<td>Stabilisation of current activity</td>
<td>[77]</td>
</tr>
<tr>
<td>Anion current</td>
<td>Intact guard cells in epidermal peels</td>
<td><em>V. faba</em></td>
<td>Voltage-clamp</td>
<td>ABA 20 μM (ext)</td>
<td>Increase in channel conductance, alteration of channel kinetics</td>
<td>[30]</td>
</tr>
<tr>
<td>GCAC1 (rapid channel)</td>
<td>Guard cell protoplasts</td>
<td><em>V. faba</em></td>
<td>Patch-clamp, whole-cell and single channel</td>
<td>1-ANA, 1 to 20 μM (ext), direct application</td>
<td>Shift of the activation potential and modulation of the current amplitude</td>
<td>[51]</td>
</tr>
<tr>
<td>TSAC (rapid channel)</td>
<td>Cell suspension protoplasts</td>
<td><em>N. tabacum</em></td>
<td>Patch-clamp whole-cell</td>
<td>1-ANA, 1 to 50 μM (ext), direct application</td>
<td>Shift of the activation potential</td>
<td>[98]</td>
</tr>
</tbody>
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dipsis guard cells opened the way for the electrophysiological analysis of ABA signalling mutants and provided new hints of the position of anion channels in the early ABA transduction cascade. Pei et al. [59] analysed the effects of ABA on the slow anion channel in both the wild-type and the ABA-insensitive mutants ab1 and ab2 [25, 44]. The AB11 and AB22 proteins both encode a 2C-type protein phosphatase [45, 46, 54]. In protoplasts from wild-type guard cells, no significant anion current could be detected in classical recording conditions. However, ABA evoked largewhole-cell currents analogous to those mediated by the slow anion channels in broad bean guard cells [59] (figure 2A, table 1). Protoplasts were treated with1–50 μM ABA for 1.5 h before recordings and the hormone was applied in both bath and pipette solutions, which precludes any conclusion to be drawn on the location of the primary perception sites for ABA and the time course of the pathway leading to channel activation. Interestingly, in ab1 and ab2 mutants which are unable to achieve ABA-induced stomatal closing, the activation of slow anion channels by ABA was strongly impaired [59]. These findings underline the role of these anion channels as a central control in the ABA signalling cascade. Very recently, Pei et al. [60] demonstrated that deletion of the farnesyltransferase gene ERA1 or application of farnesyltransferase inhibitors result in ABA hypersensitivity of guard cell anion channel activation and of stomatal closing, thus uncovering a new step in this ABA response pathway in Arabidopsis.

Using a voltage-clamp approach, Grabov et al. [30] have examined the influence of ABA on anion channel activity in stomatal guard cells of wild-type Nicotiana benthamiana plants and of transgenic plants expressing the dominant-negative mutant protein AB11-1 from Arabidopsis. In wild-type guard cells, an anion current showing characteristics similar to the slow anion current in V. faba [73] was observed. Adding ABA (20 μM) in the external medium evoked a rapid increase in the anion channel conductance, and altered its kinetics (delay and attenuation of channel deactivation at negative voltages) (table 1). The resulting effect was to enhance the steady-state anion current by up to four-fold. Expression of the ab1-1 mutant protein in N. benthamiana plants confers a wilty phenotype and aberrant control of stomatal closure as observed in the ab1-1 Arabidopsis mutant. However, in contrast with the results of Pei et al. [59], no effect of the mutant protein could be detected on the background anion current or on its response to ABA. Besides the fact that the use of acetate-filled electrodes in tobacco may lead to an inhibition of slow anion channel activity [59] and thus mask possible ABA effects, this apparent discrepancy may be due to species-specific differences between A. thaliana and N. benthamiana concerning the availability of the substrate or the intracellular targeting of the phosphatase. Alternatively, it may reflect a fundamental difference between patch-clamp conditions where the cell content is dialysed against the pipette solution, and intracellular microelectrodes which leave the cytosolic content intact. Such distinction may be of importance when analysing signalling cascades possibly involving soluble second messengers or transduction proteins.
In *V. faba* guard cells, slow anion channels were shown to be activated by phosphorylation events, and depletion of intracellular ATP leads to a strong down-regulation of slow anion currents [69]. When analysing anion currents under ATP-free pipette solutions, the current declines over time with an average half-maximal deactivation time of 9.4 min [77], due to ATP removal from the cytosol. In the absence of cytosolic ATP, but in the presence of either external or internal ABA, slow anion currents were maintained even 15 min after gaining access to the whole-cell configuration, at levels comparable to control cells supplied with ATP (table I). These data, together with those showing that the phosphatase inhibitor okadaic acid also maintains the slow anion current in the absence of ATP [69], support a hypothesis in which ABA down-regulates protein dephosphorylation events during stomatal closure in *V. faba* guard cells, thereby maintaining the activity of the slow anion channel. The ABA regulation of channel activity thus appears as an indirect process, but the fact that ABA effects could be observed in the whole-cell configuration, and that slow anion currents could be reactivated after re reintroduction of ATP into the cytosol suggests that the involved kinase(s)/phosphatase(s) would be associated with the plasma membrane.

4.2. Regulation of rapid-type anion channels by auxins

When externally applied to isolated guard cell protoplasts from *V. faba*, auxins alter in a dose-dependent manner the activation potential and current amplitude of the rapid anion channel GCAC1 [51]. The auxin-induced shift towards negative voltages brings the activation potential closer to the resting potential, thus allowing the channel to open and cause membrane depolarisation (figure 2 B, table I). Auxin effects were observed at the level of single channels in isolated membrane patches, and correlated with the action of several structurally related anion channel blockers [50, 52], which favours the idea of channel gating through a direct interaction with extracellular hormone. The auxin-induced shift of the activation potential to hyperpolarised potentials was reduced in the presence of extracellular chloride or malate, suggesting that auxins and anions compete for common sites [47]. In addition, the fact that not only the active auxin 1-NAA, but also 2-NAA, an inactive auxin with respect to stomatal opening, modulate the activation threshold and kinetics of GCAC1, reinforces the idea that auxin interacts directly with the channel in a similar manner to other gating modifiers [34, 36, 50, 52].

In protoplasts from tobacco suspension cells which exhibit an anion channel sharing similarities with GCAC1 (TSAC for tobacco suspension anion channel), auxin also induced a time- and concentration-dependent modulation of the whole-cell anion current, similar to the one observed in guard cell protoplasts (table I) [98]. However, in contrast with GCAC1, TSAC is insensitive to the inactive auxin 2-NAA. In addition, the antibody D16, raised against a domain of the maize auxin-binding protein covering part of the auxin binding site [91], was able to mimic the auxin effects on the tobacco channel, suggesting that auxin and D16 recognise the same binding site [98]. Whether this binding site is present at the channel protein itself, or is located at an independent auxin-binding protein has not been established. Short-term auxin effects on anion channel activity such as those observed in *Vicia* guard cells or tobacco cells could not be detected on the rapid-type anion channel from *Arabidopsis* hypocotyl cells [88].

5. ANION CHANNELS ARE INVOLVED IN THE RESPONSES OF PLANT CELLS TO ENVIRONMENTAL SIGNALS

Plant anion channels, besides their involvement in hormonal signalling cascades, likely contribute to the transduction of several biotic and abiotic environmental stimuli, as illustrated by the few examples below.

5.1. Elicitors of defence reactions and hypo-osmotic stress

Several examples show that chloride effluxes are triggered in plant cells by elicitors of defence reactions and constitute an early step in the transduction chain leading to a modulation of gene expression. In tobacco cell suspensions, the proteinaceous elicitor cryptogein from *Phytophthora cryptogea* induces a complex transduction pathway including extracellular alkalisation and K⁺ efflux [8], production of active oxygen species [10] and NADPH oxidase activation [61]. An early step of this pathway involves a calcium-dependent Cl⁻ efflux as well as a membrane depolarisation [61], suggesting that anion channels play a crucial role in the cryptogein responses. Zimmermann et al. [97] recently provided further evidence for the involvement of anion channels in elicitor-induced chloride efflux in tobacco cell suspensions. The pars-
ley cells/Phytophthora megasperma system is another well-studied model [39, 56] where a fungal glycoprotein or its active 13-amino acid oligopeptide (Pep13) trigger a set of reactions similar to those observed during tobacco/cryptogein interaction. In this case too, changes in ion permeabilities of the plasma membrane (H+, Ca2+, K+ and Cl−) were among the earliest events detectable after treatment of parsley cells by Pep13, and within 2–5 min Ca2+ uptake was observed together with a rapid increase in extracellular Cl− concentration. Furthermore, several ion channel blockers belonging to the classes of 9-AC and IAA-94 derivatives were able to inhibit not only Cl− efflux, but also K+ efflux, H2O2 production and phytoalexin accumulation [39], suggesting that anion channel activation represents an upstream step in the response pathway. Patch-clamp analysis of parsley protoplasts recently identified an ion channel which was transiently activated by the Pep13 elicitor [96] and mediated Ca2+ influx, but the putative elicitor-responsive anion channel in the plasma membrane has still to be identified.

Interestingly, plant cell suspensions submitted to a hypo-osmotic shock react by a set of membrane events very similar to those triggered by fungal elicitors, including modifications of ion fluxes and oxidative burst [11, 83, 95]. In tobacco cells, one of the earliest response to the hypo-osmotic stress is a chloride efflux. Anion channel inhibitors block this anion efflux and the oxidative burst to the same extent, further illustrating the upstream position of anion channels in the signalling pathway [11].

5.2. Light

Early events in the transduction of light signals in plant cells are often associated with transient depolarisation of the plasma membrane [21, 22, 81]. In caulonemal filaments of the moss Physcomitrella patens, red light triggers a phytochrome-mediated calcium-dependent depolarisation [22]. Ion flux measurements and current-voltage analyses revealed that Ca2+-, Cl−- and K+-permeable channels were open during the membrane potential transient [23]. In mesophyll cells of pea, white light induces a transient depolarisation to a role of this channel in transducing blue light into membrane depolarisation and growth inhibition. Recently, the same group [58] using a mutant lacking the HY4 blue light receptor demonstrated that membrane depolarisation resulting from the activation of anion channels was indeed initiated by the perception of blue light by the receptor. In both mesophyll and hypocotyl protoplasts, light regulation of anion channels could be detected only in the cell-attached patch-clamp mode, indicating that cell integrity has to be maintained to ensure proper functioning on the signalling pathway from the receptors for light signals to plasma membrane channels.

6. CONCLUSION

From the few examples discussed here, it appears that higher plant cells are equipped with a variety of anion channels co-residing at the plasma membrane and controlled by various signalling pathways and their messengers, including Ca2+, pH, nucleotides, and protein kinases and phosphatases. Interestingly, such a degree of redundancy is not restricted to specialised guard cells for a tight control of stomatal movements, but also concerns elongating organs such as the hypocotyl. Within response pathways to either endogenous hormonal signals or environmental signals such as light or molecules involved in plant-pathogen interactions, anion channel activation seems to constitute a very early step in all transduction cascades, which points to plasma membrane anion channels as major actors in plant signalling networks. Another role devoted to plasma membrane anion channels which has not been discussed in the present paper concerns the generation of action potentials at the origin of long distance electrical signalling, leading in some instances to gene activation [92, 94]. Studies of action potentials in characean cells have shown that the depolarisation phase involves the activation of a chloride conductance [43, 79]. In higher plants for which action potentials have been described [4, 17], Cl− efflux has also been identified in a few cases [67].
await further exploration. Abscisic acid and auxin induce various and complex effects on channel activity. The slow-type anion current of guard cells is globally amplified by ABA, which induces channel activation (Arabidopsis), or maintains channel activity (Vicia), or increases anion current amplitude and slows down deactivation kinetics (Nicotiana). These effects all contribute to trigger and maintain a sustained anion efflux in response to ABA, emphasising the physiological role of the slow anion channel in osmoregulation processes leading to stomatal closure. On the rapid-type anion channel, auxins induce a shift of the voltage gate (Vicia and tobacco) which in turn allows channel activation in the resting potential range, but at the same time, in Vicia protoplasts, elevated auxin levels reduce the anion permeability over the whole voltage range. Such contrasting effects may account, at least partly, for the characteristic bell-shaped dose-response curve of stomatal guard cells to auxin. According to its properties, it has been suggested that the rapid anion channel takes part in the initial electrical signalling in response to the hormone rather than in long-term osmotic processes. Although in some cases a direct channel-hormone interaction has been suggested [51], hormonal regulation of anion channel activity most likely results from the activation of reception-transduction cascades. If the involved pathway is not entirely membrane-associated, cytosolic integrity has to be maintained to allow soluble second messengers to be released and reach their targets. This cannot be achieved in patch-clamp experiments on isolated protoplasts when looking for direct hormone effects in whole-cell or excised patch configurations, and may explain why long term incubation of intact protoplasts in the presence of hormone is required before patch-clamping to detect a modulation of channel activity [59]. In these respects, voltage-clamp studies such as those described on intact guard cells from various species [26, 27, 30] provide more physiological conditions for the study of ion channel regulation by hormones.

Little is known as yet on the molecular structure of plant anion channels and a major challenge is to identify the genes encoding for the various anion channels described so far. Several cDNAs homologous to the voltage-dependent chloride channels genes of the CLC family have been isolated from N. tabacum (CLC-N1 and CLC-N2, [48]) and A. thaliana (AtCLC-a to -d, [32]). The functional characterisation of plant CLCs in their native membranes has still to be achieved, and the question whether any of the channels already functionally characterised might belong to the CLC family is entirely opened. Reverse genetic strategies are being developed to investigate the function of CLC proteins, as recently described for potassium channels [29, 37]. The ongoing study by our group of a T-DNA insertion mutant within the AtCLC-a gene has already revealed that this channel might play a general role in the control of nitrate balance (Geelen et al., unpubl. results). Further physiological analysis of this mutant, especially in terms of hormonal responses, and isolation and study of other CLC mutants will provide new insights in the functions of these channels in plant cells and their possible involvement in hormone signalling pathways.

REFERENCES


[34] Hedrich R., Marten I., Malate-induced feedback regulation of plasma membrane anion channels could provide a CO₂ sensor to guard cells, EMBO J. 12 (1993) 897–901.


[47] Lohse G., Hedrich R., Anions modify the response of guard cell anion channels to auxin, Planta 197 (1994)


[61] Pugin A., Frachisse J.M., Tavernier E., Bligny R., Gout F., Douce R., Guern J., Early events induced by the


