PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A New Scheme of Symbiosis: Ligand- and Voltage-Gated Anion Channels in Plants and Animals

Rainer Hedrich and Andreas Jeromin

Phil. Trans. R. Soc. Lond. B 1992 **338**, 31-38
doi: 10.1098/rstb.1992.0126

A new scheme of symbiosis: ligand- and voltage-gated anion channels in plants and animals

RAINER HEDRICH AND ANDREAS JEROMIN

Institut für Biophysik, Universität Hannover, Herrenhäuser Strasse 2, D-W-3000 Hannover 21, Germany

SUMMARY

Anion channels in the plasma membrane of both plant and animal cells participate in a number of important cellular functions such as volume regulation, trans-epithelial transport, stabilization of the membrane potential and excitability. Only very recently attention has turned to the presence of anion channels in higher plant cells. A dominant theme among recent discoveries is the role of Ca^{2+} in activating or modulating channel current involved in signal transduction.

The major anion channel of stomatal guard cell protoplasts is a 32–40 pS channel which is highly selective for anions, in particular NO_3^-, Cl^- and malate. These channels are characterized by a steep voltage dependence. Anion release is elicited upon depolarization and restricted to a narrow voltage span of -100 mV to the reversal potential of anions. During prolonged activation the current slowly inactivates. A rise in cytoplasmic calcium in the presence of nucleotides evokes activation of the anion channels. Following activation they catalyse anion currents 10–20 times higher than in the inactivated state thereby shifting the resting potential of the guard cell from a K^+-conducting to an anion-conducting state. Patch-clamp studies have also revealed that growth hormones directly affect voltage-dependent activity of the anion channel in a dose-dependent manner. Auxin binding resulted in a shift of the activation potential towards the resting potential. Auxin-dependent gating of the anion channel is side- and hormone-specific. Its action is also channel-specific as K^+ channels coexisting in the same membrane patch were insensitive to this ligand. It remains to be established whether the anion channel possesses an auxin binding site or consists of a hetero-complex with a member of the emerging family of auxin binding proteins.

Known inhibitors of anion channels from animal epithelial cells reversibly blocked the anion channel from the extracellular side. At the single-channel level, channel block is caused by a reduction of the long open transitions into flickering bursts. This plant anion channel as characterized by its steep voltage-dependence and interaction with intracellular and extracellular ligands shares interesting similarities with members of gene families of animal anion channels.

1. INTRODUCTION

Anion channels in the plasma membrane of living cells represent the key regulatory elements for volume control, stabilization of the resting potential and excitability. In animal cells, anion channels comprise a diverse class of channel proteins which can be divided into voltage-gated, agonist-gated and second messenger-gated sub-families (for review see Stühmer (1991) and Greger (1992)). In contrast to animal cells the attention has turned to anion channels of higher plant cells only very recently and studies on single-anion channels are rare (see Hedrich & Schroder (1989) and Blatt (1991) for review). The Ca^{2+}- and nucleotide-gated anion channel we will describe in detail has been identified in protoplasts of guard cells of the broad bean *Vicia faba*. Due to the detailed analysis of the voltage dependence, kinetics and modulator actions of the guard cell anion channel (GCAC1) in this specialized cell type we are now able to compare its properties to those of cloned Cl^- channel proteins from animal cells. Guard cells integrate a number of external and internal signals into temporally and spatially defined responses such as action potentials as well as transient and day-night-changes in volume and turgor (for review see Hedrich & Schroeder (1989) and Schroeder & Hedrich (1989)). Sudden changes of the membrane potential and cell volume are brought about by ion movement. Stomatal closure results from the release of K^+, Cl^- and malate through K^+ and anion channels in the plasma membrane of guard cells. Stomatal movement is also dependent on the extracellular Ca^{2+} concentration. Lowering external $[Ca^{2+}]$ causes stomatal opening whereas high external $[Ca^{2+}]$ favours stomatal closure. GCAC1 will be shown to be modulated by Ca^{2+}, nucleotides and growth hormones. In addition to GCAC1 another anion conductance has been described by Schroeder *et al.* (see the contribution by Schroeder in this volume). GCAC1 has initially been

Phil. Trans. R. Soc. Lond. B (1992) **338,** 31–38
Printed in Great Britain

resolved in the plasma membrane by the use of K^+ channel blockers and impermeant cations to block the co-existing K^+ channels (Keller *et al.* 1989). The opening of an anion efflux channel which possessed peak activity around the reversal potential of K^+ was further characterized by its voltage dependence, selectivity and pharmacology.

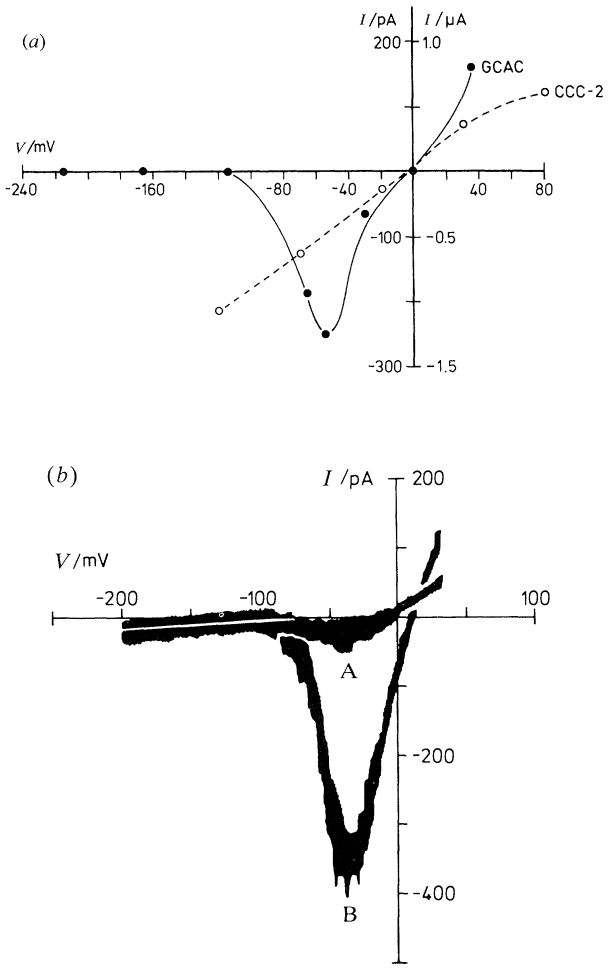

Figure 1. Voltage- and Ca^{2+}-dependence of GCAC1 in whole-cells compared with ClC-2 (see table 1). (*a*) Current–voltage relationship of GCAC1 in comparison to ClC-2 relative to the reversal potential (RP) of Cl^-. Currents of GCAC1 were elicited by voltage-ramps and steps from -200 to $+60$ mV in the presence of 2 mM MgATP in the pipette and 40 mM $CaCl_2$ in the bath. Cl^- concentrations were 154 mM in the pipette and 84 mM in the bath (from Hedrich *et al.* 1990). The instantaneous current–voltage relationship of ClC-2 is shown in the presence of 80 mM extracellular NaCl. Immediately after activation by hyperpolarization (-180 mV for 32 s), currents were evoked by fast voltage ramps (0.5 s) to minimize the probability of deactivation using the standard two-electrode voltage-clamp technique (from Thiemann *et al.* 1992). (*b*) Activation of GCAC1 channels by Ca^{2+} and ATP. Current–voltage relationship in the activated (A) and inactivated (B) state. (A) Scavange of ATP from the cytoplasm by adding hexokinase and glucose to the pipette solution. (B) Activation of anion currents by a rise in extracellular Ca^{2+} from 0.1 to 40 mM in the presence of ATP (from Hedrich *et al.* 1990).

2. VOLTAGE-DEPENDENCE AND SELECTIVITY

GCAC1 is activated within the physiological range of guard cell membrane potentials. The analysis of the currents evoked by depolarizing voltage pulses revealed that outward Cl^- currents are activated near -100 mV and peak at -30 to -40 mV (figure 1*a*). Inward currents (corresponding to Cl^--efflux) declined when decreasing the electrochemical gradient for Cl^- and reversed at $+15$–18 mV (figure 1*a*). At potentials 40–60 mV positive to E_{Cl} outward currents (corresponding to Cl^- influx) neither peaked nor saturated, but often increased in a sigmoidal manner. GCAC1 has a unique fingerprint of the kinetics of activation, de- and inactivation, reactivation from inactivation reminiscent to ion channels in excitable cells (Hille, 1992). Currents activate with a half-rise time of more than 30 ms at -70 mV and less than 10 ms at -10 mV (figure 2*b*). When the membrane potential was stepped from a holding potential of -160 mV to -30 mV and back to the holding potential, the instantaneous rise of the anion current was followed by a rapid and complete deactivation (within 20 ms) (Hedrich *et al.* 1990). The analysis of single-channel currents showed that under steady-state conditions the deactivation of anion currents at membrane potentials negative to -30 mV was caused by an increase of the closed times of the channel (figure 2*a* lower graph). Besides the voltage-dependent activation and deactivation, anion currents decreased during prolonged stimulation. Clamping the membrane potential to -40 mV for 1 min resulted in an exponentially declining anion current ($t_{\frac{1}{2}} = 10$–12 s) (figure 2*c*). Clamping the membrane potential to -100 mV for more than 1 min, however, restored depolarization-induced anion currents (reactivation). Replacement of K^+ by the channel blockers Ba^{2+}, Cs^+ or tetraethyl ammonium ions (TEA^+) on the cytoplasmic side did not basically alter the current–voltage relationship of the activated anion channel. Substitution of Cl^- by NO_3^- and malate and other halides revealed a permeability sequence of $NO_3^- > I^- > Br^- > Cl^- >$ malate (I. Marten & R. Hedrich, unpublished results). GCAC1 is, however, impermeable to glutamate and gluconate.

3. CALCIUM AND NUCLEOTIDES

Elevation of the extracellular $[Ca^{2+}]$ from 0.1 to 5–40 mM by replacing K^+ with Ca^{2+} reproducibly stimulated anion currents. External Ca^{2+} failed to activate the channel when intracellular $[Ca^{2+}]$ was buffered to nanomolar concentrations. Channel activation not only required high cytosolic Ca^{2+} but also the presence of nucleotides. When MgATP was excluded from the pipette solution in the whole-cell configuration or the intracellular ATP was scavenged by hexokinase and glucose only background currents were measured (figure 1*b*). To test the dependence of the activation on hydrolysable ATP and the nucleotide specificity, adenoside 5-0-(3-thiotriphosphate) and guanosine-5-(O-3-thiophosphate) were included

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

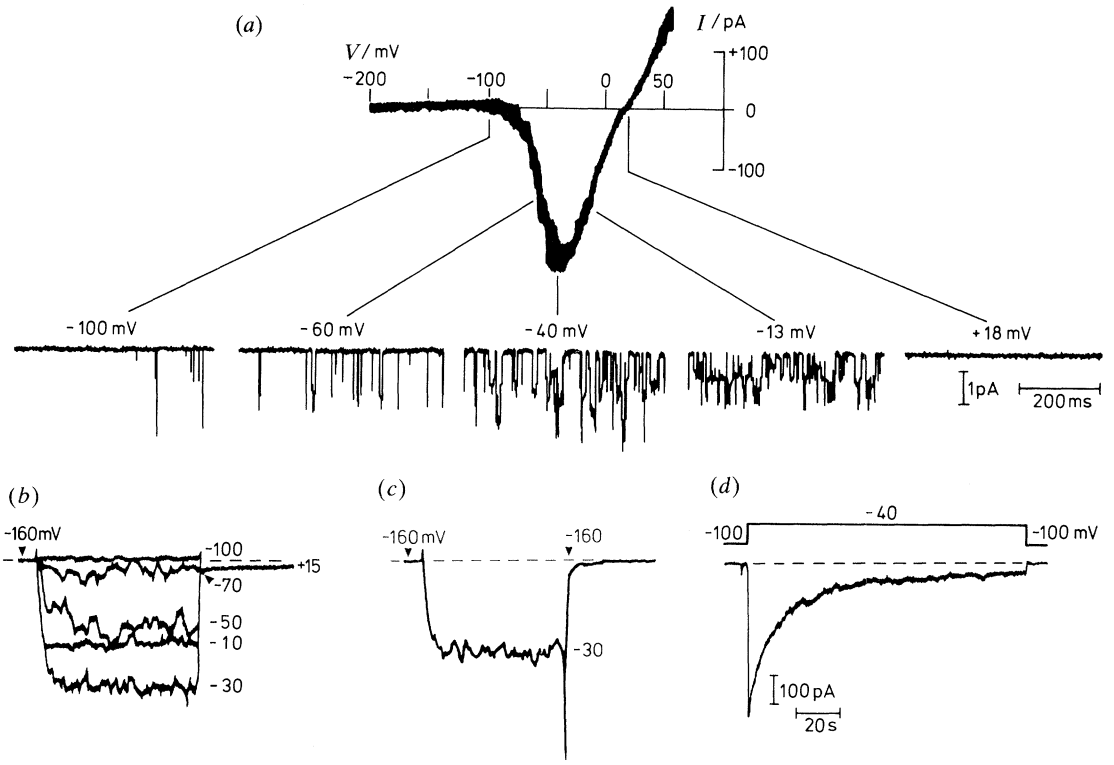

Figure 2. Voltage- and time-dependent activity of GCAC1 channels in whole-cells and outside-out patches. (*a*) *I–V* curve of GCAC1 in the whole-cell configuration. (Details are given in figure 1*a*.) Single-channel traces related to the whole-cell currents at the indicated voltages (from Hedrich *et al.* 1990). (*b*) Anion currents evoked by 200 ms voltage pulses from a holding potential of −160 mV to the −100, −70, −50, −30 and −10 mV each followed by a pulse to +15 mV (from Hedrich *et al.* 1990). (*c*) Deactivation of GCAC1; tail currents were evoked by a voltage pulse to −30 mV and followed by a pulse to −160 mV. (*d*) Inactivation of GCAC1 caused by prolonged activation; the voltage was stepped from −160 to −40 mV for the indicated duration (from Hedrich *et al.* 1990).

in the pipette solution instead of MgATP. Both non- and hydrolysable analogues supported the Ca^{2+}-dependent activation of anion channels under conditions where cytoplasmic ATP was not scavanged (Hedrich *et al.* 1990). We therefore postulate that nucleotides and Ca^{2+} cooperatively gate the channel at independent binding sites (see figure 6).

4. GROWTH HORMONES

Exposure of physiologically intact stomata to the auxins indole-3-acetic acid (IAA), 1-napthyl acetic acid (1-NAA) and 2,4, dichlorophenoxyacetic acid (2,4-D) caused stomatal opening. Dose-response studies revealed an optimum of efficiency at 0.5 to 10 μM (Lohse & Hedrich 1992). These observations indicate that guard cells possess binding sites for auxins and a functional signal transduction machinery converting the binding signal into ion and stomatal movement. The involvement of GCAC1 in auxin action was discovered and further investigated in patch-clamp studies on guard cell protoplasts isolated from auxin-sensitive stomata (Marten *et al.* 1991). When auxins were applied to the bathing solution the threshold potential of activation (activation potential) and corresponding current amplitudes were altered in a dose-dependent manner (Marten *et al.* 1991). Auxins and auxin analogues shifted the activation potential towards the resting potential (figure 3). This shift to the activation potential was dependent on the effector concentration in a logarithmic manner and fully reversible. Furthermore, auxin action was hormone-specific as neither the phytohormones abscisic acid and gibberellic acid nor the cytokinin, zeatin, did affect the activity of the anion channel. The anion channels were modulated only when the auxins were present on the extracellular surface of the channel. A comparison of the whole-cell activity indicated that the observed auxin-sensitive single-channel currents can fully account for the observed anion currents in guard cells. Cytoplasmic factors are therefore not required for mediating the hormone effect and for the targeting of auxins through the channel.

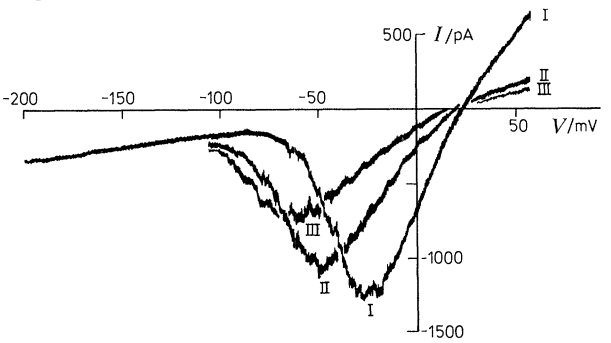

Figure 3. Shift of the activation potential of GCAC1 by the auxin 1-NAA. Current–voltage relation of whole-cell anion currents in the absence (I), 15 s (II) and 45 s (III) after application of 100 μM 1-NAA. Currents were evoked by voltage ramps (1 s) from −200 to +60 mV (from Marten *et al.* 1991).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A molecular picture of the family of auxin-binding proteins has just begun to emerge (Palme *et al.* 1991). They appear to reside in the endoplasmic reticulum and possess the H/KDEL signal sequence in their protein sequences, a sequence motif which is believed to be responsible for their retention in this compartment (Denecke *et al.* 1992). However, the so far identified putative guard cell chloride-channel protein shares no obvious similarities with any of the members of the family of auxin-binding proteins (Marten *et al.* 1992; Zettl *et al.* 1992). It remains to be shown whether the channel protein(s) itself possess an auxin-binding site or forms a hetero-complex with an auxin-binding protein.

5. GATING INHIBITORS AND MODIFIERS

A number of different anion channel blockers with different affinities and specificities for anion channels and transporters of animal tissues and cells and vesicles of intracellular origin have been identified (for a most recent review see Cabantchik & Greger 1992)). Although these inhibitors comprise a wide class of chemical structures with diverse biological effects, a common theme of inhibition has emerged. Most components are anionic at physiological pH, the anionic group is carboxylate. They all possess an amino group and an apolar side chain. For 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), it has been shown that the distances between the carboxylate and the amino group, between the nitro and the carboxylate group and between the phenyl ring and the amino group are very critical for function. For comparison, some of these compounds were thus tested for their ability to inhibit GCAC1 (Marten 1992; figure 5). When the channel blockers were applied to the extracellular face of GCAC1 it was generally observed that the activation potential and current amplitude were altered. Inhibition of outward current (corresponding to Cl^- influx) was observed before the peak inward current and the activation potential shifted to more hyperpolarized potentials (figure 4*a*). The channel modulation was fully reversible upon removal of the inhibitor (figure 4*b*). All channel blockers caused a concentration-dependent shift and block in the voltage-dependent activity of

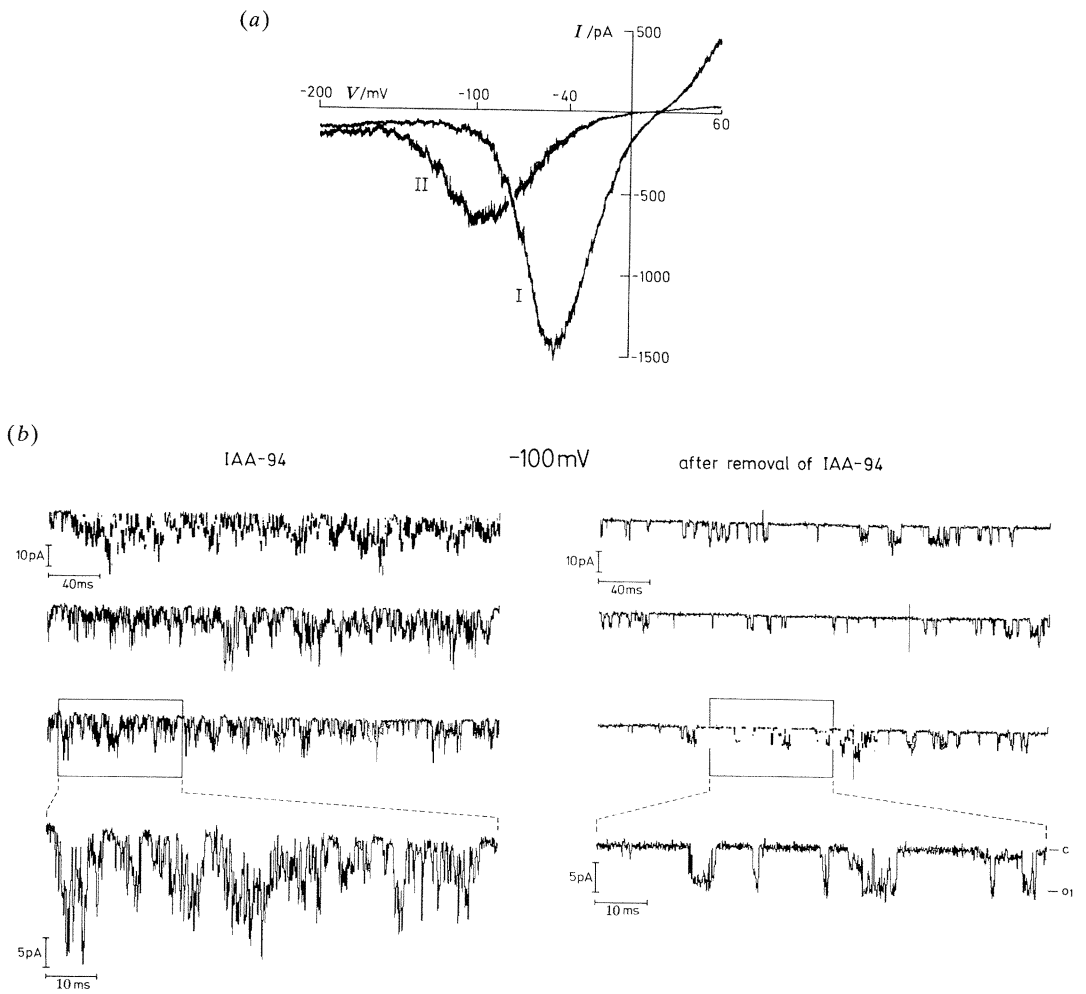

Figure 4. Modulation of GCAC1 properties by the anion channel blocker, IAA-94. (*a*) Current–voltage relationship of whole-cell anion fluxes in the absence (I) and presence (II) of 100 μM IAA-94 resulting from voltage ramps (1 s) from −200 mV to +60 mV. Conditions as detailed in figure 1*a* and Hedrich *et al.* (1990). (*b*) Single-channel activity of an outside-out patch excised from a whole-protoplast as shown (*a*). The left panel was recorded at −100 mV after the exposure to 100 μM IAA-94 and the right panel after its removal ((*a*) and (*b*) are from Marten *et al.* (1992). C denotes the closed-channel state and o_1 an open state.

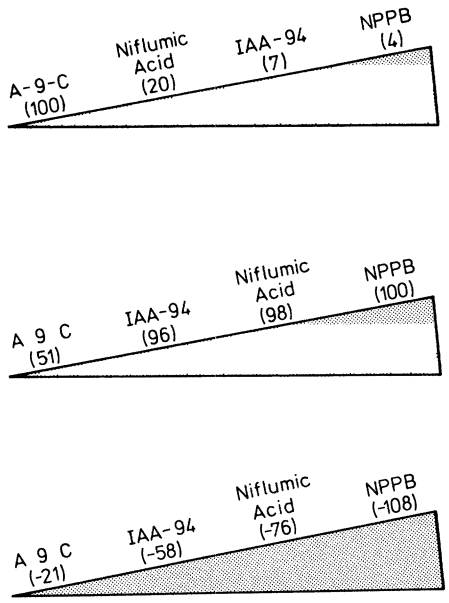

Figure 5. (*a*) Effectiveness of anion channel blockers in inhibition of GCAC1 (K_i values in μM are given in brackets). The given K_i values represent those concentrations of the effector which caused 50% inhibition of the anion current. Inhibition is expressed with respect to the peak current potential. (*b*) Relative inhibition of GCAC1 by anion channel blockers. (Relative efficiencies in % are in brackets in the presence of 100 μM gating modifier.) (*c*) Shift of the current peak. (The shift is expressed in absolute values relative to the peak current potential (in brackets); 100 μM of each inhibitor were used.) (*a*), (*b*) and (*c*) are from Marten *et al.* (1992). (Abbreviations for (*a*), (*b*) and (*c*): A-9-C, anthracene-9-carboxylic acid; IAA-94, [6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methy-1-oxo-1H-inden-5-yloxy] acetic acid; nifulmic acid, 2-(α,α,α,-trifluoro-m-toluidino)-pyridine-3-carboxylic acid; NPPB, 5-nitro-2-(phenylpropylamino) benzoic acid.)

GCAC1. At the single-channel level, these inhibitors typically transformed long open-intervals into short-lived conductance states ('flickering burst', figure 4*b*). A sequence of inhibition constants is outlined in figure 5. Within this group of blockers [6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-oxo-1H-inden-5-yl oxy] acetic acid (IAA-94), NPPB and 4,4′-diisothiocyanatostilbene-2,2′-disulphonate(DIDS) were the most effective showing half-maximal inhibitory concentrations of 6, 4 and 0.2 μM respectively (Marten *et al.* 1992). It should be noted that each inhibitor exhibited a specific saturable profile, the shape of the inhibition curve differed strongly, possibly indicating different binding sites. We favour an explanation which postulated the interaction of the inhibitor with the open pore of the channel to explain channel block. The shift of the activation potential, the 'gate-shifting', may result from tunnelling of the ligand into sites which directly alter the effective electric field and dipole moment of the channel. The effect of these gating inhibitors on the activation and inactivation kinetics of GCAC1 is the subject of current investigations. Further experiments will help to define a reaction cycle of molecular rate constants for the transitions between the individual states of GCAC1.

6. BIOCHEMICAL ISOLATION

In the survey of compounds of anion channel blockers IAA-94 and IAA-23 were included to test their capability as potential ligands for the affinity-purification of the channel subunits (Marten *et al.* 1992). These two ligands or inhibitors have been used successfully in the affinity-purification of anion channels from kidney and tracheal epithelial cells. IAA-23, resembling the structure of IAA-94 when bound to the affinity column was used to enrich ligand-binding

Table 1. *Comparison of GCAC1 to cloned animal anion channels*

(Abbreviations: depol.: depolarization; hyperpol.: hyperpolarization; G49.51.53A denotes the mutant in the nucleotide binding domain as described in Paulmichl *et al.* (1992); PKA: denotes phosphorylation by the cAMP-dependent protein kinase A. The abbreviations for the gating modifiers are the same as in the legend to figure 5.)

	GCAC1	CFTR	ClC-0[a]	H2B	Cl^- channel (MDCK cells)
channel conductance/p5	30–40	8–10	9 & 18	26–70 (?)	?
voltage-dependent activation	depol.	depol.	hyperpol.	?	depol.
selectivity	NO_3^{2-}	Br^-	SCN^-	?	?
	$> I^-$	$> Cl^-$	$> I^-$		
	$> Br^-$	$> I^-$	$> Br^-$		
	$> Cl^-$	$> F^-$	$> Cl^-$		
	$> malate^{2-}$				
modulation by calcium	+	–	–	?	–[b]
and nucleotides	+	PKA/MgATP	–	?	+[b]
inhibition by gating modifiers (only the three most effective)	NPPB > IAA-94 > nilfumic acid	?	DIDS: –	?	> DIDS

[a] Out of the ClC-X superfamily only ClC-0 has been included into this table as for this member single-channel measurements have been reported (Bauer *et al.* 1991).

[b] The wild-type protein is inhibited by nucleotides, whereas the G49.51.53A mutant has become sensitive to changes of the calcium concentration, but insensitive to nucleotides.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

polypeptides from the plasma membrane of guard cells (Marten *et al.* 1992). From these fractions a 60 kDa plasma membrane polypeptide (pm 60) cross-reacted with a polyclonal antiserum raised against the anion channel protein from kidney cells (H2B, see table 1). In contrast to guard cells mesophyll plasma membranes which lack functional voltage-dependent anion channels under guard cell-specific conditions failed to cross-react with the antiserum (Marten *et al.* 1992).

7. COMPARISON OF GCAC1 WITH CLONED CHANNEL PROTEINS OF ANIMAL CELLS

The cloning of anion channel proteins from animal cells has provided the molecular basis to further define their function and to define structure-function-relationships. After having outlined the properties and regulation of GCAC1, we will now focus on cloned chloride channel proteins from animal cells. Table 1 summarizes our current knowledge of these two classes of anion channels from plant and animal cells with respect to the single-channel conductance, voltage-dependence, selectivity sequence, regulation by Ca^{2+} and nucleotides and inhibition of gating.

(*a*) *The ClC-X superfamily*

Thomas Jentsch reported the cloning of a voltage-dependent chloride channel from the electric organ of Torpedo marmorata ClC-0 (Jentsch *et al.* 1990). Expression of the clone led to an outwardly rectifying anion current in *Xenopus laevis* oocytes activated by hyperpolarization. A related clone, ClC-1 has been identified in the skeletal muscle of mice. Expression of a mutated protein appears to be responsible for myotonia (Steinmeyer *et al.* 1991). A third clone, ClC-2 is expressed in rat brain and heart, but also in pancreas, lung and liver and in cell lines of fibroblastic, neuronal and epithelial origin (Thiemann *et al.* 1992). Expression of the ClC-2 derived cRNA in *Xenopus* oocytes led to Cl^- currents which increased slowly after hyperpolarization, but were not detectable under resting conditions. Activation began at -90 mV and did not saturate at even -180 mV (see figure 1*a* for comparison of its voltage-dependence to GCAC1). ClC-0 and ClC-1 are believed to participate in the stabilization of the resting potential and excitability, the physiological function of ClC-2 is unclear at present.

(*b*) *Anion channels in epithelial cells*

Mutations in a gene encoding a protein called the cystic fibrosis transmembrane conductance regulator (CFTR) are responsible for the disease cystic fibrosis (Riordan *et al.* 1989). The disease is characterized by the clinical and molecular symptoms of malfunction of salt transport in epithelia. When expressed in mammalian cells or *Xenopus* oocytes CFTR generated a cAMP-activated anion current of 5–10 pS (Bear *et al.* 1992; Kartner *et al.* 1991; Rich *et al.* 1990). Amino acid sequence analysis, similarities with other proteins and site-directed mutagenesis experiments suggested that CFTR consists of two repeats of a unit containing six membrane-spanning domains and a putative nucleotide-binding fold (Hyde *et al.* 1990; Gregory *et al.* 1991). Phosphorylation by protein kinase A (PKA) alone is not sufficient to open CFTR. Once phosphorylated by PKA, channels required hydrolysable ATP to open (Anderson *et al.* 1991). Paulmichl *et al.* (1992) described the cloning of a new type of Cl^- channel which when expressed in *Xenopus laevis* oocytes gave rise to an outwardly rectifying chloride current. The expressed current was Ca^{2+}-insensitive and could be blocked by nucleotides: the degree of reversible inhibition ranged from 0% from UMP and to about 70% by cGMP and cAMP. Mutation of the putative nucleotide binding site resulted in the loss of nucleotide block, but now conferred Ca^{2+} sensitivity on the current. DIDS and NPPB inhibited the current of the wild-type protein with moderate affinity from the extracellular side. The IC_{50} was 50 μM for DIDS and 1 μM for NPPB respectively. The Cl^- channel protein originally isolated from kidney cortex membranes is expected to be the most related to the anion channel protein from guard cells. The kidney channel protein, called H2B, exhibited measurable Cl^- channel activity when reconstituted (Redhead *et al.* 1992). Difficulties in expressing H2B have hampered the elucidation of its precise physiological function although it has been shown that suppression of H2B expression also reduced the amplitude of co-expressed currents carried by CFTR (Q. Al-Awqati, unpublished results). Immunoblots of a variety of species and cell types of both epithelial and non-epithelial cells have revealed that this protein is ubiquitous and highly conserved (Redhead *et al.* 1992). Its localization is not restricted to the plasma membrane, H2B can also be found in membranes of intracellular origin (Redhead *et al.* 1992).

8. PLANT ANION CHANNELS IN ENDOMEMBRANES

As in animal cells, anion-permeable channels have been found in plant endomembranes. The vacuolar membrane is characterized by a strongly voltage-dependent Ca^{2+}-activated inward rectifier (60–80 pS) (Hedrich & Neher 1987). Raising the extravacuolar $[Ca^{2+}]$ not only resulted in a dramatic change of the current activation kinetics, but also changed the voltage threshold of activation. The permeability ratio, P_K/P_{Cl} was calculated to be about 6 in the presence of Ca^{2+}. In the photosynthetic membrane of thylakoids anion-selective channels were activated by transient depolarization (Schönknecht *et al.* 1988). Increasing the Cl^- concentration from 30 to 110 mM led to a rise of the single-channel conductance from 65–110 pS. The approach followed for the characterization of GCAC1 should also be applicable to the anion channels in organelle membranes and should help to establish a picture of the coordination of ion fluxes between these endomembranes and the plasma membrane.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

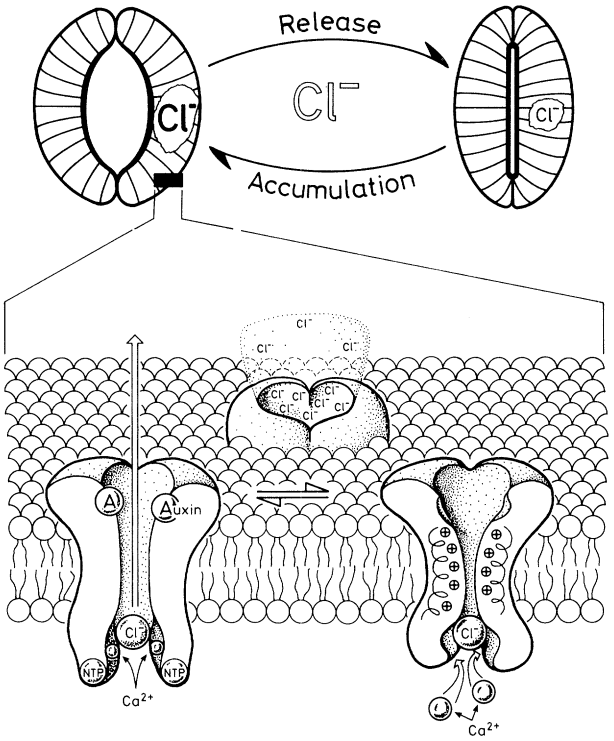

Figure 6. Schematic drawing of the regulation of GCAC1 by calcium and nucleotides. In the upper part a pair of guard cells and the dynamics of the intracellular [Cl^-] are shown. GCAC1 is assumed to be a homodimer embedded into the 2D-lipid matrix due to symmetry considerations. The front-view of a half of GCAC1-dimer represents the open state on the left and the closed state on the right side. Binding of Ca^{2+} and nucleotides (NTPs) to the sites on the cytoplasmic surface of GCAC1 open the channel leading to the release of anions represented by Cl^-. The binding pockets for gating modulators (A^-), such as auxins and inhibitors, are located on the external surface. For clarity, the putative selectivity filter and pore of the channel outlined by the positively charged α-helix has only been drawn in the right (closed) state.

9. CONCLUSION AND DISCUSSION

We have outlined the voltage-dependent activation and deactivation, inactivation, selectivity, gating by Ca^{2+}, nucleotides and growth hormones and pharmacology of GCAC1. By definition, this anion channel shares similarities with voltage- and ligand-gated channels. Its functional properties have been compared with those of cloned animal anion channels (table 1). The overall properties of the cloned animal Cl^- channels outlined above may seem to be unrelated to the type of anion channel found in guard cells. A sequence comparison of ClC-0-2 with H2B, CFTR and the channel protein isolated by Paulmichl *et al.* (1992) has revealed no significant sequence similarities between these proteins. It is therefore tempting to speculate that they define independent subgroups within the family of anion channels. Nevertheless, the primary sequences of animal anion channels have helped to further our understanding of the overall structure-function-relationship of anion channels. We believe that a new scheme of molecular symbiosis is just emerging. These sequences, especially H2B, will be instrumental in designing probes to identify related clones in plant tissues. The multifunctionality and multiple pattern of regulation of GCAC1 suggests that GCAC1 will be most related to if not the primordial type of anion channel.

We thank Irene Marten for stimulating discussions. The figures were made possible by the unique artwork of Bernd Raufeisen. R.H. was supported by the DFG.

REFERENCES

Anderson, M.A., Gregory, R.J., Thompson, S., Souza, D.W., Paul, Sucharita, Mulligan, R.C., Smith, A.E. & Welsh, M.J. 1991*a* Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science, Wash.* **253**, 202–205.

Anderson, M.A., Berger, H.A., Rich, D.P., Gregory, R.J., Smith, A.E. & Welsh, M.J. 1991*b* Nucleoside triphosphates are required to open the CFTR chloride channels. *Cell* **67**, 775–784.

Bauer, C.K., Steinmeyer, K., Schwarz, J.R. & Jentsch, T.J. 1991 Completely functional double-barreled chloride channel expressed from a single *Torpedo* cDNA. *Proc. natn. Acad. Sci. U.S.A.* **88**, 11052–11056.

Bear, C.E., Duguay, F., Naismith, A.L., Kartner, N., Hanrahan, J.W. & Riordan, J.R. 1991 Cl^- channel activity in *Xenopus* oocytes expressing the cystic fibrosis gene. *J. biol. Chem.* **266**, 19142–19145.

Blatt, M.R. 1991 Ion channel gating in plants: physiological implications and integration in stomatal movement. *J. Membr. Biol.* **124**, 95–112.

Cabantchik, Z.I. & Greger, R. 1992 Chemical Probes for anion transporters of mammalian cell membranes. *Am. J. Physiol.* **262**, C803–C827.

Denecke, J. De Rycke, R. & Botterman, J. 1992 Plant and mammalian sorting signals for protein retention in the endoplasmic reticulum contain a conserved epitope. *EMBO J.* **11**, 2345–2355.

Greger, R. 1992 Cl^- channels. In *New comprehensive biochemistry, molecular aspects of transport proteins* (ed. J. J. H. M. de Pont & E. M. Wright). Amsterdam: Elsevier. (In the press.)

Gregory, R.J., Rich, D.P., Cheng, S.H., Souza, D.W., Paul, S., Manavalan, P. Anderson, M.P., Welsh, M.J. & Smith, A.E. 1991 Maturation and function of cystic fibrosis transmembrane conductance regulator variants bearing mutations in putative nucleotide-binding domains 1 and 2. *Molec. Cell. Biol.* **11**, 3886–3893.

Hedrich, R. & Neher, E. 1987 Cytoplasmic calcium regulates voltage-dependent ion channels in plant vacuoles. *Nature, Lond.* **329**, 833–835.

Hedrich, R. & Schroeder, J.I. 1989 The physiology of ion channels and electrogenic pumps in higher plants. *A. Rev. Pl. Physiol. Molec. Biol.* **40**, 539–569.

Hedrich, R., Busch, H. & Raschke, K. 1990 Ca^{2+} and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. *EMBO J.* **9**, 3889–3892.

Hille, B. 1992 *Ionic channels of excitable cells*, 2nd edn. Massachusetts: Sinauer.

Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, S.R., Gallagher, M.P., Gill, D.R., Hubbard, R.E. & Higgins, C.F. 1990 Structural model of the ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature, Lond.* **346**, 362–365.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Jentsch, T.J., Steinmeyer, K. & Schwarz, G. 1990 Primary structure of *Torpedo marmorata* chloride channel isolated by expression cloning in *Xenopus* oocytes. *Nature*, *Lond.* **348**, 510–514.

Kartner, N., Hanrahan, J.W., Jensen, T.J., Naismith, A.L., Sun, S., Ackerley, C.A., Reyes, E.F., Tsui, L.-C., Rommens, J.M., Bear, C.E. & Riordan, J.R. 1991 Expression of the cyctic fibrosis gene in non-epithelial cells produce a regulated anion conductance. *Cell* **64**, 681–692.

Keller, B.U., Hedrich, R. & Raschke, K. 1989 Voltage-dependent anion channels in the plasma membrane of guard cells. *Nature*, *Lond.* **341**, 450–453.

Landry, D.W., Akabas, M.H., Redhead, C., Edelman, A., Cragoe, E.J. & Al-Awqati, Q. 1989 Purification and reconstitution of chloride channels from kidney and trachea. *Science*, *Wash.* **244**, 1469–1472.

Lohse, G. & Hedrich, R. 1992 Characterization of the plasma membrane H^+ ATPase from *Vicia faba* guard cells—modulation by extracellular factors and seasonal changes. *Planta* (In the press.)

Marten, I., Lohse, G. & Hedrich, R. 1991 Plant growth hormones control voltage-dependent activity of anion channels in plasma membrane of guard cells. *Nature*, *Lond.* **353**, 758–762.

Marten, I., Zeilinger, C., Redhead, C., Al-Awqati, Q. & Hedrich, R. 1992 Identification and modulation of a voltage-dependent anion channel in the plasma membrane of guard cells by-high-affinity ligands. *EMBO J.* (In the press.)

Palme, K., Hesse, T., Moore, I., Campos, N., Feldwisch, J., Garbers, C., Hesse, F. & Schell, J. 1991 Hormonal modulation of plant growth: the role of auxin perception. *Mechanisms Develop.* **33**, 97–106.

Paulmichl, M., Li, Y., Wickman, K., Ackerman, M., Peralta, E. & Clapham, D. 1992 New mammalian chloride channel identified by expression cloning. *Nature*, *Lond.* **356**, 238–241.

Redhead, C.R., Edelman, A.E., Brown, D., Landry, D.W., Al-Awqati, Q. 1992 A ubiquitous 64-kDa protein is a component of a chloride channel of plasma and intracellular membranes. *Proc. natn. Acad. Sci. U.S.A.* **89**, 3716–3720.

Rich, D.P., Anderson, M.P., Gregory, G.J., Cheng, S.H., Paul, S., Jefferson, D., McCann, J.D., Klinger, K.W., Smith, A.E. & Welsh, M.J. 1990 Expression of the cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. *Nature*, *Lond.* **347**, 358–363.

Riordan, J.R., Rommens, J.M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczack, Z., Zielenski, J., Lok, S., Plavisic, N., Chou, J.-L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S. & Tsui, L.-C. 1989 Identification of the cystic fibrosis gene: cloning and characterization of the complementary DNA. *Science*, *Wash.* **245**, 1066–1073.

Schonknecht, G., Hedrich, R., Jung, W. & Raschke, K. 1988 A voltage-dependent chloride channel in the photosynthetic membrane of a higher plant. *Nature*, *Lond.* **336**, 589–592.

Schroeder, J.I. & Hedrich, R. 1989 Involvement of ion channels and active transport in osmoregulation and signaling of higher plants. *Trends Biochem. Sci.* **14**, 187–192.

Steinmeyer, K., Ortland, C. & Jentsch, T.J. 1991 Primary structure and functional expression of a developmentally regulated skeletal muscle chloride channel. *Nature*, *Lond.* **354**, 301–304.

Stühmer, W. 1991 Structure-function studies of voltage-gated ion channels by site-directed mutagenesis. *A. Rev. Biophys. biophys. Chem.* **20**, 65–78.

Thiemann, A., Gründer, S., Pusch, M. & Jentsch, T.J. 1992 A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature*, *Lond.* **356**, 57–60.

Zettl, R., Feldwisch, J., Boland, W., Schell, J. & Palme, K. 1992 5′-azido-[3,6-$^3H_2$]-1-naphthylphthalamic acid, a photoactivatable probe for naphthylphthalamic acid receptor proteins from higher plants: identification of a 23 kDa protein from maize coleptile plasma membranes. *Proc. natn. Acad. Sci. U.S.A.* **89**, 480–484.

Note added in proof (9 September 1992): After submission of the manuscript we learned that J. I. Schroeder & B. U. Keller published (*Proc. natn. Acad. Sci. U.S.A.* **89**, 5025–5029 (1992)) the existence of two depolarization-activated anion currents in the plasma membrane of guard cell protoplasts of *Vicia faba*, terms 'R'- and 'S'-type.

B. Lindner & K. Raschke (*FEBS Lett.* Submitted) have detected a slowly activating anion current 'SLAC' in the plasma membrane of *Xanthium strumarium* guard cell protoplasts in addition to a 'quick anion channel' (QUAC).

Both the 'R'-type and 'SLAC' share functional similarities with GCAC1. Thus, we prefer the designation 'GCAC1' since it avoids ambiquities with the designations for optical enantiomers (R and S) and allows a systematic nomenclature to be developed for plant anion channels.

It remains to be shown whether 'R' and 'S' on the one hand and 'SLAC' and 'QUAC' on the other hand represent different gating modes of the same channel protein or different channel protein(s) in their hetero-oligomeric assembly.