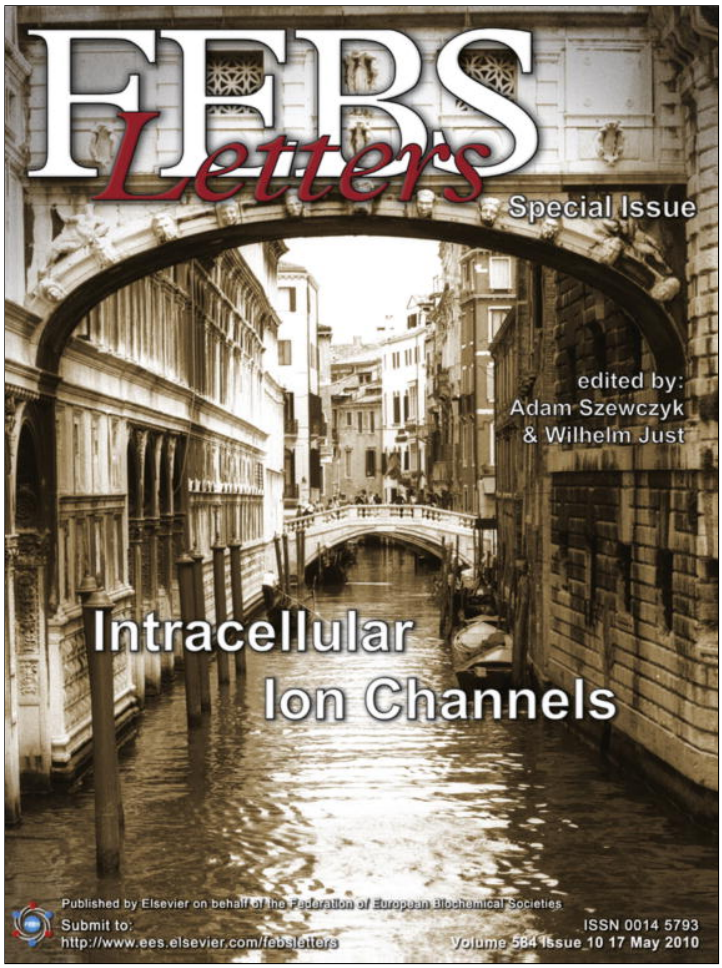

FEBS Letters 584 (2010) 2122–2127

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

CLC transport proteins in plants

G. Zifarelli *, M. Pusch

Istituto di Biofisica, CNR, Via De Marini 6, I-16149 Genova, Italy

ARTICLE INFO

Article history:
Received 9 October 2009
Accepted 21 December 2009
Available online 28 December 2009

Edited by Wilhelm Just

Keywords:
Nitrate
Chloride
Homeostasis
CLC
Channel
Transporter

ABSTRACT

Nitrate compartmentalization in intracellular organelles has been long recognized as critical for plant physiology but the molecular identity of the proteins involved remained unclear for a long time. In *Arabidopsis thaliana*, AtClC-a has been recently shown to be a NO_3^-/H^+ antiporter critical for nitrate transport into the vacuoles. AtClC-a is a member of the CLC protein family, whose animal and bacterial members, comprising both channels and H^+-coupled antiporters, have been previously implicated exclusively in Cl^- transport. Despite the different NO_3^- over Cl^- selectivity of AtClC-a compared to the other CLC antiporters, it has similar transport properties.

Other CLC homologues have been cloned in *Arabidopsis*, tobacco, rice and soybean.

1. Introduction

Anion channels and transporters are central for several plant functions, such as regulation of turgor, stomatal movement, nutrient transport, metal tolerance and generation of action potentials, exerting their role at the plasma membrane and in membranes of intracellular compartments in a highly coordinated manner [1–3].

Among the different anions, NO_3^- has a prominent role in plant physiology. Nitrogen is the mineral nutrient needed in greatest abundance by plants and it is mostly taken up as NO_3^- [4]. The NO_3^- uptake system must be versatile and robust because it has to cope with external NO_3^- concentrations that can vary by five orders of magnitude [4]. Furthermore, the diversified metabolic functions of NO_3^- require a tight and specific regulation of its concentration in different tissues and cellular compartments [5,6].

After entry into the cell through H^+-symporters of the NNP (nitrate–nitrite transporter) and PTR (peptide transporter) protein families [7–9], NO_3^- accumulates into the vacuoles where its concentration can be as high as 50 mM, i.e. 25 times higher than in the cytoplasm [10,11]. Vacuolar NO_3^- contributes to the homeostasis of cytosolic NO_3^-, which has been reported to be relatively constant under a wide range of conditions [10,12]. It was early recognized that such a large accumulation of NO_3^- in vacuoles would require the presence of an active transport system and it was suggested that it would function as a H^+ antiporter, in which the efflux of H^+ from the acidic vacuoles is coupled to inward NO_3^- transport [11]. However, so far only two proteins have been unambiguously shown to be involved in vacuolar NO_3^- transport, a nitrate transporter of the NNP family, AtNRT2.7 (almost exclusively expressed in seeds), and the anion/proton exchangers of the CLC protein family AtClC-a [13,14].

Cl^- ions are also critical for several cellular processes like membrane depolarization, regulation of cell volume, resistance to salinity stress, tolerance to metals and pathogen response that have been characterized in quite some detail. Identification of the proteins responsible for Cl^- movement through intracellular compartments has not been achieved yet [3], but it has been speculated that CLC proteins might be involved [1,2].

The first member of the CLC protein family to be characterized was ClC-0, a voltage-gated chloride channel from the *Torpedo* electric organ [15]. Following expression cloning [16], CLC proteins were ubiquitously found in eukaryotes and prokaryotes [17]. Mammalian CLCs comprise both Cl^- channels expressed at the plasma membrane level (ClC-1, -2 -Ka and -Kb) and Cl^-/H^+ antiporters localized to intracellular compartments (ClC-4, -5 and -7 and probably also ClC-3 and -6). The fact that in spite of these different transport mechanisms CLCs proteins share a high degree of structural similarity represents a biophysical puzzle [18,19].

The first CLC genes in plants were cloned independently in tobacco [20] and *Arabidopsis* [21] that is by far the plant system for which most information on CLC proteins has been accumulated.

In *Arabidopsis*, seven homologues have been identified, named AtClC-a to AtClC-g. Based on sequence analysis, AtClC-a to -d and -g define a separate phylogenetic branch [22] with the highest homology with the subfamily of mammalian CLCs comprising

* Corresponding author. Fax: +39 0106475 500.
E-mail address: zifarelli@ge.ibf.cnr.it (G. Zifarelli).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.12.042

ClC-6 and ClC-7 (around 30% identity). AtClC-e and -f have in general very low homology with the other CLCs and are more closely related to bacterial homologues (20% identity).

AtCLCs are ubiquitously expressed in plant organs [13,22], however, it has been recently highlighted that AtClC-a to -g are predominantly expressed in vascular tissues, suggesting a possible role in long-distance ion transport [22].

The localization in intracellular compartments and the lack of a suitable expression system amenable to direct electrophysiological analysis has limited our understanding of the properties of plant CLCs so that the functional role of most of them has been inferred only indirectly, from the phenotype of plants harboring inactivating mutations that disrupt the function of single CLC proteins [13] or from their ability to complement growth phenotypes in yeast strains in which the only endogenous CLC protein, ScClC (or GEF1) was inactivated [23]. A notable exception is represented by AtClC-a.

2. Characteristic features of bacterial and animal CLCs

The X-ray structure of the bacterial homologue ClC-ec1 [24,25] confirmed a number of features elucidated in previous functional studies [18,19]; CLC proteins express as dimers with each subunit harboring an independent ion conduction pathway. Each monomer possesses three Cl^- binding sites. When the permeation pathway is shut, the more external binding site is occupied by the side chain of a conserved glutamate residue (Fig. 1), which therefore controls the conduction state of the protein and is also named the "gating" glutamate (E148 in ClC-ec1) [25]. Mutations of this Glu severely affect or abolish gating in CLC channels and turn the antiporters into pure anionic conductances that are not sensitive to pH_{ext} [19].

Another important glutamate residue, the "proton" glutamate (E203 in ClC-ec1), is located on the cytoplasmic side of the protein (Fig. 1). It is conserved in all CLC antiporters where it is critical for anion/H^+ coupling, whereas the corresponding amino acid is a valine in CLC channels [26]. Mutations of this glutamate into several non-titratable residues ablate transport activity of mammalian CLC antiporters [27], whereas they uncouple anion and proton fluxes in ClC-ec1 [26,28].

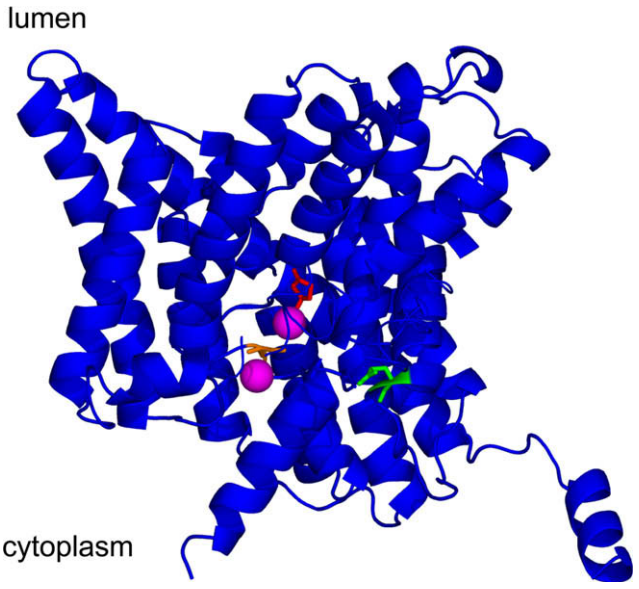

Fig. 1. Location of critical residues for anion selectivity and transport mechanism. The figure shows a lateral view of the structure of a single subunit of the bacterial ClC-ec1 (PDB entry 1OTS). The residues E148, S107 and E203 are colored in red, orange and green, respectively. Chloride ions are shown in pink.

Recently, the transport stoichiometry for hClC-5 [29] and hClC-7 [30] has been elucidated and conforms to the 2:1 Cl^-/H^+ coupling ratio already found for ClC-ec1 [31], suggesting a common coupling mechanism in all CLC transporters.

Another characteristic element of eukaryotic CLC proteins is the presence of two CBS domains (cystathionine β-synthetase) in the cytoplasmic C-terminal portion, structural motifs present in different proteins but whose function is in general poorly understood. Recently, it was shown that the isolated C-terminal part of hClC-5 could bind adenine nucleotides in a cleft formed at the interface between CBS1 and CBS2 [32]. AMP, ADP and ATP produce an increase of hClC-5 currents [33] and in agreement with structural and biochemical results they act with similar affinities [32].

3. AtClC-a is the most thoroughly investigated plant CLC

A first hint of AtClC-a physiological function came from a mutational analysis: Plants homozygous for a T-DNA insertion that disrupted AtClC-a function, displayed, in spite of a similar morphology, a 50% reduction of the overall NO_3^- content compared to WT with no alteration of the concentrations of Cl^- [34]. This phenotype was surprising because it pointed to a role of AtClC-a in NO_3^- and not Cl^- homeostasis when all CLC proteins known at that time were thought to be Cl^- channels. The specific feature that confers NO_3^- selectivity to AtClC-a has been identified only recently (see below).

This work also suggested a link between AtClC-a and vacuolar transport because only defective accumulation in vacuoles that account for up to 99% of the NO_3^- storage capacity of plant cells [35] could explain the massive reduction of NO_3^-. The tonoplast localization of AtClC-a has been recently confirmed [36]. In the same study, the transport activity of AtClC-a was directly investigated, a major breakthrough allowed by application of the patch-clamp technique to vacuoles. AtClC-a mediated slightly outwardly rectifying and strongly NO_3^- selective currents [36]. In AtClC-a, the two critical glutamate residues (Fig. 1), the "gating" and the "proton" glutamates, are conserved (Table 1). In particular, the presence of the "proton" glutamate immediately suggests that AtClC-a might function as a NO_3^-/H^+ antiporter. This was confirmed by measurements of the reversal potential of the currents at different NO_3^- and H^+ cytoplasmic concentrations, showing a NO_3^-/H^+ coupling ratio of 2:1 as also found for ClC-ec1, hClC-5 and -7, indicating a conserved transport mechanism. In a physiological setting, the observed transport stoichiometry would lead to a vacuolar accumulation of nitrate by a factor of 50 [36], in agreement with previous estimates [10].

Table 1
Overview of the localization, transport properties and conservation of important residues of plant CLC proteins. No data are available for the anion selectivity of AtClC-b and -g. In the last two columns the residue in parenthesis indicates the corresponding residue in ClC-ec1.

Protein	Intracellular localization	Suggested selectivity	Residues in the selectivity filter	Gating Glu (E148)	Proton Glu (E203)
AtClC-a	Vacuole	NO_3^-	GPGIP	E	E
AtClC-b	Vacuole	?	GPGIP	E	E
AtClC-c	Vacuole	NO_3^-/Cl^-	GSGIP	E	E
AtClC-d	Golgi	Cl^-	GSGIP	E	E
AtClC-e	Chloroplast	NO_3^-	ESAGK	E	S
AtClC-f	Golgi	Cl^-	EILDQ	E	T
AtClC-g	Vacuole	?	GSGIP	A	E
OsClC-1	Vacuole	Cl^-	GSGIP	E	E
OsClC-2	Vacuole	Cl^-	GSGIP	E	E
GmClC-1	Vacuole	Cl^-	GPGIP	E	E

The question of anion selectivity is particularly interesting in both physiological and biophysical terms because NO_3^- (and other polyatomic anions) are transported also by hClC-5 and ClC-ec1, but with much weaker coupling to H^+ compared to Cl^-, such that it has been suggested that NO_3^- permeation occurs through slippage [27,37]. Sequence comparison reveals that a Ser residue, conserved in animal and bacterial CLC channels and antiporters (S168 in hClC-5), is substituted in AtClC-a by a Pro (P160) (Table 1). In the crystal structure of ClC-ec1 this Ser contributes to the central binding site (Fig. 1) and is comprised in the highly conserved GSGIPE sequence stretch (Table 1). Its critical role was already indicated by the fact that its substitution drastically altered gating and selectivity of ClC-0 [38].

In hClC-5, substitution of S168 with a Pro, inverted the Cl^- over NO_3^- selectivity typical of the WT, such that the anion selectivity of the mutant is similar to AtClC-a [29]. Importantly, in this mutant NO_3^- and H^+ transport were coupled as for AtClC-a and not uncoupled as in WT hClC-5.

These experiments proved that the hClC-5 residue at position 168 is the major determinant of anion selectivity and coupling efficiency. Similar results were obtained also for the Cl^- channel ClC-0 [39,40] and the bacterial Cl^-/H^+ antiporter ClC-ec1 [40], indicating a general property of CLC proteins.

The critical role for the corresponding residue of AtClC-a (Pro160) was proved by Bergsdorf et al., who succeeded for the first time in expressing AtClC-a in *Xenopus* oocytes [39]. Importantly, even though the transport properties of AtClC-a measured in oocytes were in general similar to those found upon measurement of isolated vacuoles [36], there is a quite important difference; in patch-clamp measurements from vacuoles, WT AtClC-a strongly selects NO_3^- over Cl^- [36], whereas upon expression in oocytes, this selectivity, although retained, was strongly reduced [39]. In any case, even in the oocyte expression system, AtClC-a showed stronger NO_3^-/H^+ than Cl^-/H^+ coupling. However, mutating P160 into Ser, as found in hClC-5, basically abolished the difference in Cl^- and NO_3^- permeability. Moreover, the mutant showed the same efficiency of H^+ transport in both Cl^- and NO_3^- solutions, a feature that distinguishes it from both WT AtClC-a and hClC-5.

In summary, two independent and complementary studies on hClC-5 and AtClC-a, proved that the homologous residue in the selectivity filter (S168 in hClC-5 and P160 in AtClC-a) is critical for the anion selectivity and coupling efficiency in both proteins [29,39]. Interestingly, introduction of a Pro in hClC-5 does not exactly reproduce the behavior of AtClC-a, as this mutant, unlike AtClC-a, shows the same NO_3^-/H^+ and Cl^-/H^+ transport efficiency; nor does introduction of a Ser in AtClC-a exactly reproduce the behavior of hClC-5 (no nitrate-induced slippage). Moreover, substitution of the P160 with Gly in AtClC-a did not alter the preference for NO_3^- over Cl^- typical of the WT, neither did it affect NO_3^-/H^+ coupling [39], whereas introduction of a Gly at the equivalent position in hClC-5 maintained the Cl^- over NO_3^- selectivity and a much stronger Cl^- over NO_3^-/H^+ coupling typical of WT ClC-5 [29]. These observations suggest that selectivity and coupling in CLC proteins are also influenced by other, still elusive elements. However, on the basis of the present limited knowledge, it can be suggested that CLCs carrying a Ser in the selectivity filter (Table 1) are characterized by Cl^-/H^+ antiport activity, whereas the ones carrying a Pro are NO_3^-/H^+ antiporters.

In AtClC-a the roles of the "gating" and "proton" glutamates appear to be conserved compared to ClC-ec1 and hClC-5. Mutating the gating glutamate E203 to Ala induced uncoupling of the anion and H^+ transport and the resulting anion flux became pH-independent [39] as found for hClC-4 and -5 [27]. Mutation of the proton glutamate E270 abolished transport activity, whereas simultaneous mutations of both glutamate residues produced currents similar to those of the single mutant E203A [39], in analogy with the behavior observed for ClC-4 and -5 [27,41].

Very recently, it was reported that like hClC-5, also the activity of AtClC-a is regulated by intracellular nucleotides [42]. However, whereas for hClC-5 ATP, ADP and AMP where found to bind with similar affinities [32] and produced the same current potentiation [33], for AtClC-a it was reported that ATP had an inhibitory effect, with 5 mM ATP producing 60% current reduction [42]. AMP and ADP did not have any direct effect, however, at difference with ADP, AMP could effectively compete ATP inhibition. The potential physiological implications of AtClC-a regulation by nucleotides are still unclear. However, major differences seem to exist between AtClC-a and hClC-5 regarding nucleotide binding and/or subsequent conformational changes that affect transport activity.

4. The other six AtClCs

It has been recently suggested by YFP fusion proteins and co-localization with specific vacuolar markers that like AtClC-a, also AtClC-b, -c and -g are expressed in the tonoplast [22]. Although for AtClC-b no functional data are available, the high similarity with AtClC-a (87% identity) and the conservation of critical residues (Table 1), potentially suggest similar properties.

Quantitative trait loci analysis and subsequent mutational analysis by transposon insertion identified AtClC-c as another major component for NO_3^- accumulation [43]. In shoots, mutant plants showed a reduced NO_3^- content compared to WT. However, at difference with AtClC-a mutants, the plants had also altered concentrations of Cl^-, potentially indicating a broader anion specificity of AtClC-c compared to AtClC-a.

AtClC-d is expressed in the trans-Golgi network and co-localizes with a V-type ATPase [44]. Plants harboring a loss of function mutation of this protein displayed normal Cl^- and NO_3^- content and normal morphology; however, root growth and cell elongation were impaired [44]. These defects were also reported upon H^+-ATPase knock-down by RNAi [45]. The co-localization at the Golgi level and the similar phenotypes produced by mutations in AtClC-d and H^+-ATPase suggest a functional link between this CLC and acidification of this compartment. This is particularly interesting because the interdependence of CLC activity and acidification of intracellular compartments was already established in mammals [46,47].

AtClC-e and -f have in general a modest homology with the other plant and animal CLCs, such that they seem to form a distinct family branch [22,48]. It is therefore very difficult to extrapolate their functional properties from the features of other CLCs, especially considering the lack of conservation of residues in the selectivity filter (Table 1). In any case, it can be speculated that substitution of the conserved proton Glu with a Ser (in AtClC-e) and a Thr (in AtClC-f) residue is indicative of channel-like rather than antiporter behavior.

For AtClC-e, co-localization of GFP-fusion protein with chlorophyll and Western blot analysis indicated localization in the thylakoid membrane [22,48]. It has been shown that plants with inactivating mutations of AtClC-e have an altered photosynthetic activity [48] and a reduced level of NO_3^- compared to WT without significant alterations of other anions, but a slight over-accumulation of malate [49] similar to the results obtained for AtClC-a [34]. However, the interpretation of this result is also complicated by the finding that inactivating mutations of AtClC-a and -e differentially affected the expression level of other AtClCs and proteins involved in NO_3^- uptake [49]. The reduced NO_3^- content displayed by AtClC-e inactivating mutations and the fact that AtClC-e is not able to compensate the GEF1 yeast phenotype (see below), potentially indicates that it is selective for NO_3^-.

AtClC-f and -g, have been suggested to have, respectively, Golgi and vacuole localization [22,48], but their functional properties are still unknown.

5. Rice

The rice genome has been completely sequenced and comprises five CLC homologues [1], but only two members from *Oryza sativa* L., OsClC-1 and OsClC-2 have been characterized [50,51]. They show the highest homology with the plant subgroup comprising AtClC-a to -g (about 76% identity with AtClC-c). OsClC-1 is expressed in most tissues, whereas OsClC-2 is expressed only in roots, nodes, internodes and leaf sheaths, but for both of them vacuolar localization has been suggested [50]. Plants with inactivating mutations in either genes displayed growth impairment [50]. Treatment with NaCl increased the transcription of OsClC-1 but not OsClC-2 suggesting that OsClC-1 is somehow involved in Cl^- transport [50,51].

6. Soybean

Li et al. reported the cloning of a CLC homologue in soybean, GmClC-1 [52], with 78% identity with AtClC-a. It is localized in the tonoplast and is induced by the presence of NaCl and water stress. Expression of this protein increased Cl^- transport from the cytoplasm into the vacuoles and conferred NaCl tolerance to tobacco BY-2 cells [52]. Interestingly, GmClC-1 has a Pro in the selectivity filter (Table 1), a feature that in AtClC-a is critical to select NO_3^- over Cl^- in H^+ coupled transport.

In principle, based on the properties of other CLCs, the presence of a Pro seems to conflict with the involvement in Cl^- transport suggested for GmClC-1 and therefore further experimental evidence is warranted to draw conclusions about its anion selectivity. However, GmClC-1 presents the conserved gating and proton glutamates (Table 1) suggesting that it is an H^+-coupled antiporter.

7. Yeast complementation as a means to study plant CLCs

In the yeast *Saccharomyces cerevisiae*, inactivating mutations of the only CLC homologue present in its genome, ScClC (or GEF1) [23], produce an iron limited and pH dependent growth and hypersensitivity to several extracellular cations [53]. It has been suggested that this phenotype is due to impaired acidification of Golgi compartments [21,54,55] but the precise molecular function of GEF1 is still unclear. Based on the presence of conserved residues (the Ser in the selectivity filter, the gating and the proton glutamates), it can be hypothesized that GEF1 functions as a Cl^-/H^+ antiporter.

Complementation of the GEF1 yeast phenotype by expression of *Arabidopsis* CLCs has been the first tool to gain insight into their possible physiological role, but produced conflicting results also due to the specific phenotypic traits analyzed. Hechenberger et al. found that the iron-limited growth could be complemented by expression of AtClC-d (which in *Arabidopsis* showed the same subcellular localization of GEF1) but not of AtClC-a, -b nor -c [21]. Marmagne et al. reported that AtClC-f but not AtClC-e is also able to complement the growth defect [48]. Gaxiola et al. found that AtClC-c and -d suppressed the pH induced phenotype and salt sensitivity [53]. A very recent work indicated that, AtClC-c and -d were the only AtClCs that could rescue the Mn^{2+}- hypersensitive growth defect [22]. Two rice homologues (OsClC-1 and -2) are also able to compensate the growth defect of GEF1 null mutant at high pH but not the iron-limited growth defect [50].

However, it must be kept in mind that the functional insight provided by the yeast complementation experiments is limited because of the intrinsically indirect nature of the assay and because the functional link between loss of function mutations in GEF1 and the resulting yeast phenotypes is still elusive. For example it is still controversial whether ClC-0 is able to rescue the iron-limited growth phenotype [53] (Schwappach, B., personal communication).

8. Conclusions

The quest for plant intracellular anion/H^+ antiporters, hypothesized on the basis of extensively characterized plant physiological processes, has been a long one, due to difficulties in their molecular identification and the inaccessibility of most plant endomembranes to direct electrophysiological analysis.

Recently AtClC-a, a member of the CLC protein family, has been unambiguously identified as the first plant NO_3^-/H^+ antiporter in *Arabidopsis thaliana* with transport properties similar to the mammalian and bacterial CLC antiporters, like the anion/H^+ coupling with a 2:1 stoichiometry and the role of the gating and proton glutamates, but with the important difference of a preference for NO_3^- over Cl^- in the coupled transport with H^+ [36]. This property is mostly determined by the presence of a Pro residue in the selectivity filter that substitutes a Ser conserved in all CLCs antiporters studied [29,39,40] (Table 1).

However, our understanding of the role of other plant CLCs in anion transport through vacuoles and other intracellular compartments is still very limited. On the one hand, their transport properties are known only indirectly, from the consequences of inactivating mutations, the ability to compensate the yeast GEF1 defect or to confer specific properties in transgenic plants. Furthermore, the specific expression in organelles, cells and tissues of these proteins is also critical to understand their function, but the analysis of this factor is still incomplete. However, taking as a starting point the characteristics of known CLC antiporters, we can discuss the features of these proteins in relation to the amino acids that they present in critical positions and, at least provocatively, indicate some of the open questions regarding their function. No speculation can be made on AtClC-e and -f due to their very low similarity with other plant CLCs (Table 1).

An interesting regularity emerges considering the proteins suggested to participate in Cl^- transport, AtClC-c and -d, OsClC-1 and -2, and GmClC-1. All of them display both the gating and the proton glutamate, probably implying that they are antiporters and almost all of them have a Ser in the selectivity filter (Table 1 and Fig. 1) (except GmClC-1, already discussed). This is consistent with the results obtained in hClC-5 and AtClC-a suggesting that the presence of a Ser implies Cl^- over NO_3^- selectivity but also suggests an important question. In animal and bacterial antiporters with a Ser in the selectivity filter, NO_3^- produces uncoupling; if this is a characteristic also of plant CLCs, this would lead to dissipation of the NO_3^- gradient across cellular compartments. Is it possible that the nitrate-induced uncoupling has a physiological relevance in plants? If this is not the case, which is the distinctive feature of plant CLCs that prevents it?

A special case is represented by AtClC-c for which Cl^- transport activity would be consistent both with the presence of the conserved Ser and with its ability to compensate GEF1 yeast mutants. On the other hand, evidence from *atclc-c* knock-out plants suggests an involvement in both NO_3^- and Cl^- homeostasis. Thus, it seems that only direct measurements of AtClC-c transport properties, possibly in native membranes, can clarify its anion selectivity.

AtClC-a is endowed with the ideal features for a transporter devised to accumulate NO_3^- into the vacuoles. Importantly, in experiments in isolated vacuoles, it can also transport NO_3^- in the opposite direction. Do both of these roles have physiological

relevance? If NO_3^- efflux is coupled to H^+ entry in the vacuoles, is positive charge accumulation prevented by inward transport of other anions? Is it possible that other AtCLCs contribute to some of these functions?

Interestingly, AtClC-b, AtClC-c and AtClC-g have been suggested to have vacuolar localization, but no functional data are available for AtClC-b and -g. On the basis of the residues present in the selectivity filter it can be speculated that AtClC-b is selective for NO_3^-, whereas AtClC-c and -g are selective for Cl^-. Interestingly, whereas AtClC-b and -c are likely to function as antiporters due to the "gating" and "proton" glutamates, AtClC-g, in which the "gating" glutamate is substituted by an Ala (Table 1) is likely to function as a Cl^- channel. In this context, it is important to notice that in patches from vacuoles isolated from leaf cells of *atclc-a* knock-out plants, the currents were reduced to 60–85% compared to WT in the presence of either NO_3^- or Cl^- on the cytoplasmic side, suggesting a dominant or exclusive role of AtClC-a in vacuolar anion transport. However, subtle differences in localization with respect to cell and vacuole types have to be considered before such a conclusion can be reached.

The issue is very important because it has been recently suggested that AtClC-c is the plant CLC with the highest expression in guard cells [22], responsible for stomatal opening/closing by volume regulation. In these cells, Cl^- uptake into the vacuoles lowers the osmotic potential of this compartment and drives water influx that is responsible for volume increase [1]. Tonoplast Cl^-/H^+ antiporters were hypothesized to mediate this uptake [56,57] but so far they have not been identified [1,2]. Are AtClC-c transport properties compatible with this orphan role? Is it possible that AtClC-g serves as a previously unrecognized vacuolar Cl^- efflux/influx pathway? Do AtClC-c and -g function in a coordinated manner or are they expressed in different types of vacuoles and/or cells?

The biophysical characterization of AtClC-a has uncovered novel properties of the CLC protein family. Other plant CLCs pose new interesting questions, regarding both their individual properties and their concerted function, certainly difficult to tackle but that hold the premises for significant improvement of our understanding of CLC proteins biophysics and physiology.

Acknowledgments

We are grateful to S. Zimmermann, F. Gambale and A. De Angeli for critical reading of the manuscript and B. Schwappach for sharing results prior to publication. This work was supported by grants from Telethon Italy (grant GGP08064) and Compagnia di San Paolo.

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