Characterization of CIC transporter proteins

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Chapter 6

General discussion and conclusions

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Chapter 6

**Functional redundancy**

In the introduction we stated that we want to explore the position of the ClC proteins in the complex network of membrane transport and solute fluxes. This study shows that this is a complicated task. First of all, the most important problem seems to be the absence of obvious phenotypes for single mutants. It requires creativity of the scientist to generate conditions that evoke phenotypes. However, even the breeding of double and triple mutants is no easy road to success (Chapter 2). Apparently, the ClC channels exhibit high functional redundancy.

We started from the rational that the ClC proteins have a clear function in either Cl\(^-\) or NO\(_3\)\(^-\) homeostasis (Chapter 2 and 3). However, the absence of clear phenotypes when, for instance, seeds were germinated in the presence of high concentrations of these anions might indicate that their role in the homeostasis of those ions is limited. Plants apparently have developed other transporter systems for these functions.

Our results more clearly indicate that ClC proteins play an important role in energizing the membrane and pH homeostasis. Energizing the membrane and pH homeostasis depend protons fluxes and currents carried by other ions. For generating a large [H\(^+\)] gradient, necessary for a high capacity of secondary active transport of solutes, the electrical potential difference across the membrane has to be kept low. The electrical component of the proton pumping ATPase activity therefore has to be short-circuited. This shorting of the proton pump could be accomplished by any kind of current. It does not matter if it’s carried by Cl\(^-\) or NO\(_3\)\(^-\) or even K\(^+\) (but then in the opposite direction). Although the ClC proteins can have distinct functions, for instance being either a Cl\(^-\) or an NO\(_3\)\(^-\) transporter, their electro-physiological characteristics can still make them redundant as both can act as an electrical shunt in the generation of the proton-motive force (Chapter 4 and 5). The situation is probably even more complex as a shunt function can also be performed by a system not related to the ClC proteins, for instance, by a K\(^+\) channel. Hence, this suggests that in order to study the role of CIC proteins in energizing the membrane, the experiments should be highly defined and controlled. Composition of experimental media should be simple and the potential of alternative currents to occur should be limited. All electro-physiological tools should be used and, if possible, CIC-mediated fluxes should be studied in plants were alternative currents can be excluded.

The second role of CIC proteins for which we found indications, is in the accumulation in osmotically active solutes. Osmotically active solutes can be any
soluble ion or compound. A cell can accumulate other solutes if one of the normally used solutes (Cl\(^-\) or NO\(_3\)^-) is not available or can not be used (for instance in the absence of AtClC proteins). Hence, in this case complementation of the function can also be expected outside the group of CIC proteins: for example by malate or sugar transporters.

A nice example of this type of redundancy is shown in Chapter 5. In this chapter it is shown that Ca\(^{2+}\) can not alleviate Cd\(^{2+}\)-induced reduction of root growth if AtClCa and AtClCd are both absent. The currently favored model for explaining our observation is, that Cd\(^{2+}\) toxicity is countered in two ways: 1) Ca\(^{2+}\) protects the plant by competing with Cd\(^{2+}\) for essential sites in enzymes and transporters and 2) Cd\(^{2+}\) is sequestered in internal compartments (vacuole), rendering them harmless. We propose that in the latter mechanism CIC proteins play a role as shunts by allowing generation of a PMF across intracellular membranes and the transport of Cd\(^{2+}\) across these membranes (Chapter 5). In Chapter 1 (Table 1) it is suggested that AtClCa is a H\(^+\)/NO\(_3\)^- and AtClCd is a H\(^+\)/Cl\(^-\) antiporter. The fact that you need to remove both transporters demonstrates that both proteins can be used as a shunt and complement each other. If one of the two CIC proteins is missing, either the Cl\(^-\) or the NO\(_3\)^- ions can still be used to compensate the movement of positive charges (H\(^+\)) by the primary proton pumps. Thus, only in the case where both CIC proteins are removed, there are apparently no transporters (or their charged substrates) present that can take over the function as shunt. It would be interesting to study the effect of Ca\(^{2+}\) on Cd\(^{2+}\) toxicity when the various genotypes are grown either on low Cl\(^-\) or low NO\(_3\)^- media.

Root growth as studied in Chapters 2, 3 and 4 showed also that the main functions of the CIC proteins are electrical circuits, which allow the generation of a PMF to drive the uptake of osmotically active solutes or stimulate the capacity to acidify the apoplast and thus facilitate cell expansion.

**Intracellular localization of the CIC proteins**

Although we do not need the exact localization of the CIC proteins in the model proposed in Chapter 5. It remains an important issue for understanding the roles of these proteins. And, as proposed above, if CIC proteins can functionally be replaced by proteins that are not related to them, localization can become an important issue. As suggested by the articles of Moore and Murphy (2009) and Millar et al. (2009) localization studies are difficult and need a careful approach. Presently only the paper
of De Angeli et al. (2006) seem to meet some of the proper standards. In that study localization experiments on AtClCa are convincingly combined with physiological measurements aimed at elucidating the function (patch clamp measurements).

**Final conclusions**

The study of ClC proteins is a complicated one. They seem to play a role in different processes (osmo-regulation, detoxification, cell expansion, and maybe more). Roles that are so important in plant cells, that several protein systems exists which can fulfill these roles next to the ClC proteins. Functional studies on these proteins require, next to creativity of the researcher, very specific conditions in the experiments (for instance Ca\(^{2+}\) alleviation of Cd\(^{2+}\) effects) to observe phenotypes. Several important issues remain to be studied: the role of the two CBS domains and the role of ATP binding in the functioning of ClC proteins as shunts and osmo-regulators. Also important for plants is the strictness of the duality of ClC proteins as specific Cl\(^{-}\) or specific NO\(_3\)\(^{-}\) transporters and the strictness of the duality as channels or H\(^{+}\)-cotransporters. The study of the ClC protein family will remain a challenge for researchers in the coming years and, very likely, will yield unforeseen and surprising results.