The central lytic vacuole fulfills a multitude of different functions in plant cells, among which turgor-driven cell expansion, storage (of water, nutrients, waste products, pigments, etc), ion and pH homeostasis, macromolecule degradation and autophagy. In these last aspects, the vacuole represents the terminal destination on the endocytic pathway and can therefore be considered as the plant counterpart to the animal lysosome. But differently to the lysosome, the vacuole can reach tens of micrometers in diameter and occupy up to 90% of the cellular volume. To fulfill its diverse functions, the membrane enclosing the vacuole (termed tonoplast) contains a rich set of ion channels and transporters. Monopolar transport proteins participate in the reversible accumulation and release of osmotically active ions in guard cells, specialized cells controlling stomatal aperture in response to environmental stimuli, which makes them a widely used model for studies on cellular signaling and ion transport regulation.

The membranes of both vacuoles and lysosomes contain phosphatidylinositol-3,5-bisphosphate (PI(3,5)P2), a low-abundance derivative of the membrane lipid phosphatidylinositol regulating conserved aspects of membrane dynamics and cellular signaling in eukaryotic cells. PI(3,5)P2 levels increase in plants and yeast under hyperosmotic conditions and in animal cells upon hormonal and growth factor stimulation. In lysosomes and in budding yeast vacuoles, PI(3,5)P2 modulates organelle morphology and acidification, but the mechanistic relationships between these processes are at present unclear. In guard cells, genetic or pharmacological interference with PI(3,5)P2 synthesis depressed both hyper-acidification and structural remodeling of the vacuole which are observed during stomatal closure in response to the drought stress hormone abscisic acid (ABA). Tonoplast H+ pumps likely contribute to this acidification, since it was reduced in Arabidopsis null mutants of the 2 major vacuolar H+ pumps, V-ATPase and H+-pyrophosphatase (V-PPase). Similarly, vacuolar fission in budding yeast exposed to hyperosmotic stress requires V-ATPase, and PI(3,5)P2 has stabilizing effects on the pump complex. In our recent work, we tested the hypothesis that PI(3,5)P2-evoked luminal acidification may be due to increased tonoplast H+ pumping. Whole-vacuole currents carried either by V-ATPase or V-PPase were not affected by PI(3,5)P2 applied to isolated Arabidopsis vacuoles, but rather currents mediated by the anion/H+ exchanger CLC-a were inhibited by PI(3,5)P2 with high affinity and specificity.

Based on these results, the origin of the PI(3,5)P2-evoked hyper-acidification is still an open question. The function of CLC-a is principally related to vacuolar anion accumulation consuming the trans-tonoplast pH gradient, also during stomatal opening. In this light, shut-down of CLC-a activity by PI(3,5)P2 during stomatal closure appears to be a useful mechanism, to avoid futile anion cycling. On the other hand, clc-a knockout plants are impaired, apart from stomatal opening, also in stomatal closure. A similar phenotype has also been observed in clc-c knockout plants, suggesting functional redundancy between the 2 CLC homologues. In principle, anion release by CLCs would be linked to vacuolar H+ uptake, providing a possible source for luminal acidification during...
the closing of stomata. However, as noted by others,\(^2\) it seems debatable that vacuolar anion release (down their electrochemical gradient) during fast stomatal closure should be driven by a secondary active carrier having a relatively slow turnover rate, instead of using an anion channel. A look at tonoplastic K\(^+\) transport may be instructive. Vacuolar K\(^+\) uptake during stomatal opening is performed by the K\(^+\)/H\(^+\) exchangers NHX1 and NHX2, while during stomatal closure K\(^+\) is released, at least in part, through selective TPK1 (two-pore potassium) channels.\(^2\) Notably, also nhx1/nhx2 plants show an impairment of both stomatal opening and closing. Here and in the clc mutants, the closing phenotype likely results from altered ion gradients and pH status in guard cells at the onset of stomatal closure. Probing ABA-induced vacuolar pH changes in guard cells of clc-a (and possibly clc-a/clc-c) knockout plants may shed more light on this question.

Taken together, luminal hyper-acidi\(fi\)cation during stomatal closure may be achieved by the combined action of i) direct H\(^+\) pumping and ii) PI(3,5)P\(_2\)-mediated inhibition of secondary active carriers consuming the H\(^+\) gradient created by H\(^+\) pumps. Indeed, PI(3,5)P\(_2\) may act as a common messenger in the guard cell tonoplast serving to shut down different carrier types at the same time. Consequently, we may expect also NHX1/NHX2 activity to be PI (3,5)P\(_2\)-sensitive, as recently suggested by others,\(^6\) albeit based on different considerations.

Finally, considering the similar functions performed by PI(3,5)P\(_2\) in eukaryotic cells, the identification of CLC-a as a vacuolar PI(3,5)P\(_2\) target raises the question of a conserved responsiveness of endolysosomal CLC proteins in animal cells. This may not necessarily be the case, since homologous proteins evolve to respond differently to cellular signals according to their function in the organism. For example, it has been shown that PI(3,5)P\(_2\) activates endolysosomal two-pore channels, but not their vacuolar homologue in plants.\(^7\) Notably, the Cl\(^-\)/H\(^+\) exchanger CLC-7 is a candidate protein for the proposed counterion pathway dissipating the membrane voltage created by H\(^+\) pumping, thereby contributing to the efficient acidification of the lysosomal lumen.\(^8\) However, there are several open questions regarding this role, and a possible modulation of CLC-7 activity in response to the physiological stimuli leading to increased PI(3,5)P\(_2\) levels is currently unexplored.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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