OPINION PAPER

A molecular framework for coupling cellular volume and osmotic solute transport control

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Abstract

Eukaryotic cells expand using vesicle traffic to increase membrane surface area. Expansion in walled eukaryotes is driven by turgor pressure which depends fundamentally on the uptake and accumulation of inorganic ions. Thus, ion uptake and vesicle traffic must be controlled coordinately for growth. How this coordination is achieved is still poorly understood, yet is so elemental to life that resolving the underlying mechanisms will have profound implications for our understanding of cell proliferation, development, and pathogenesis, and will find applications in addressing the mineral and water use by plants in the face of global environmental change. Recent discoveries of interactions between trafficking and ion transport proteins now open the door to an entirely new approach to understanding this coordination. Some of the advances to date in identifying key protein partners in the model plant Arabidopsis and in yeast at membranes vital for cell volume and turgor control are outlined here. Additionally, new evidence is provided of a wider participation among Arabidopsis Kv-like K⁺ channels in selective interaction with the vesicle-trafficking protein SYP121. These advances suggest some common paradigms that will help guide further exploration of the underlying connection between ion transport and membrane traffic and should transform our understanding of cellular homeostasis in eukaryotes.

Key words: Ion channels, K⁺ nutrition, membrane vesicle trafficking, NHX transporters, Sec1/Munc18 proteins, SNAREs, split ubiquitin system.

Control of volume and osmolarity—and of turgor in plants and fungi—lies at the core of cellular homeostatic networks in eukaryotes. Animal cells maintain near iso-osmolarity with their surroundings by engaging ion exchange for osmotic balance and coordinating membrane traffic to ensure a balance of new membrane delivery and its removal (Orlowski and Grinstein, 1997, 2007). Plants and fungi accumulate solutes, powered by strongly electrogenic H⁺-ATPases, to generate substantial osmotic gradients which build turgor pressure against the walls of these cells and facilitate expansion. Control of volume and turgor is crucial for morphogenesis. Turgor pressure must be regulated and, thus, ion transport controlled in concert with membrane traffic (Blatt, 2000; Zonia and Munnik, 2007). Vesicle traffic in walled eukaryotes adds membrane surface area for expansion, much as it does in animal cells, and it contributes to wall remodelling as the cell grows (Zonia and Munnik, 2007; Walter et al., 2009). It is no surprise, then, that coordination of membrane traffic and ion transport is crucial for morphogenesis as it is for cell expansion: integrating volume, surface area, and osmolarity is fundamental to the life of walled cells, and its failure is associated with a number of physiopathologies, such as hypertrophy and cell death in plants (Zonia and Munnik, 2007), which reflect a loss in control of volume and osmotic homeostasis.

A question of coordination

Despite their fundamental importance, little is known of the mechanisms by which cells couple membrane traffic with osmotic solute transport, either at the plasma membrane or at endomembranes. For example, epidermal cells of roots, leaves, and shoots are key determinants of the morphology
of these plant organs (Zonia and Munnik, 2007; Walter et al., 2009). The expansion of epidermal cells depends on ion and water fluxes that form the drivers behind plant cell volume and turgor control (Zonia and Munnik, 2007). In epidermally derived root hairs (Feijo et al., 2004; Cole and Fowler, 2006; Campanoni et al., 2007; Zonia and Munnik, 2009) this expansion also facilitates the ‘mining’ of essential minerals and water from the soil (Schachtman and Shin, 2007). A number of signals promote K\(^+\) transport and root hair growth, including reactive oxygen species and cytosolic [Ca\(^{2+}\)], and are associated with an anisotropy in cytoskeleton and membrane traffic both to and from the growing root hair tip (Feijo et al., 2004; Campanoni et al., 2007; Zonia and Munnik, 2007, 2009), but the coordination of vesicle traffic with cell expansion is unresolved. Similarly, during stomatal movement the volume of guard cells changes by 20–40% and, necessarily, is accompanied by corresponding changes in membrane surface area (Shope et al., 2003; Meckel et al., 2007). Transport is coordinated with these events, but the relationships must be indirect to achieve quasi-steady-state changes in cell surface area (and volume) (Blatt, 2000; Sutter et al., 2007). Only during osmotically driven changes in protoplast volume is vesicular exo- and endocytosis known to maintain directly a parallel change in the density of transporters (Hurst et al., 2003; Meckel et al., 2004), but this situation differs fundamentally from the targeted membrane traffic and volume changes in vivo evoked by stimuli such as abscisic acid (ABA) (Sutter et al., 2007). Indeed, it is clear that plants and fungi control transport of osmotically active solutes, especially of K\(^+\), whether reversibly as in stomatal guard cells or during irreversible expansive growth as in root hairs and fungal hyphae (Blatt, 2000; Zonia and Munnik, 2009). How membrane traffic, changes in membrane surface area, and cell volume are coordinated with ion transport at the plasma membrane remains unknown, but it is clear that this coordination implies an additional layer of controls, the knowledge of which will be of great importance to understanding cellular homeostasis.

Equally puzzling is how cells coordinate endomembrane traffic in controlling organelle surfaces. In plants and fungi, the bulk of the cell volume is occupied by one or more vacuoles that are the single largest contributors to the osmotic content of the cells. For example, >90% of the volume increase during osmotic swelling of the guard cells and stomatal opening in Vicia can be ascribed to vacuolar volume (Shope et al., 2003; Meckel et al., 2007; Zonia and Munnik, 2009) and changes in vacuolar surface (Gao et al., 2005). Thus, ion transport across the vacuolar membrane, and across membranes targeted to the vacuole, must be coordinated with vacuolar membrane traffic. There is substantial evidence that cation exchange transporters are essential for membrane vesicle traffic; these ion exchangers—including the NHX family antiporters that exchange Na\(^+\) and K\(^+\) for H\(^+\) in plants and fungi, and the counterpart NHE Na\(^+\)/H\(^+\) exchangers in animals—have been implicated in the regulation of cellular volume and pH as well as resistance to salinity and acid stress (Pardo et al., 2006; Orlowski and Grinstein, 2007). The strongest evidence, in both plants and yeast, comes from genetic mutants of NHX transporters which exhibit lesions in endosomal traffic, as well as salt and osmotic sensitivities (Gaxiola et al., 1999; Nass et al., 1999; Bowers et al., 2000; Apse et al., 2003). NHX transporters of the plant and fungal endomembranes use the H\(^+\) electrochemical gradient, generated by the H\(^+\)-transporting vacuolar ATPases (or V-ATPases), to accumulate osmotically active cations in the vacuole/vesicle lumen. Much attention has focused on luminal pH, its acidification by V-ATPase activity, and the molecular mechanisms of pH dissipation by NHX transporters as factors determining membrane traffic (Muller, 1992; Nass and Rao, 1998; Ungermann et al., 1999; Brett et al., 2005; Mukherjee et al., 2006). Similarly, the sensitivity of vesicle traffic to the V-ATPase inhibitor bafilomycin A must be understood in the context of energizing K\(^+\)/H\(^+\) exchange to facilitate vesicle swelling and fusion (Cousin and Nicholls, 1997; Palokangas et al., 1998; Choi et al., 2007). Osmotic control has taken backstand although the literature provides unequivocal demonstrations that osmotic balance can facilitate membrane vesicle fusion (Finkelstein et al., 1986). Nonetheless, how membrane traffic and changes in membrane surface area and vacuolar volume are coordinated with cation exchange transport remains unknown.

### The mechanics of vesicle traffic and membrane fusion

Eukaryotic cells use vesicle traffic to shuttle membrane material, proteins, and soluble cargo between endomembrane compartments, the plasma membrane, and the extracellular space. Vesicles bud from the donor membrane surface, and their delivery is achieved by fusion and intercalation with the lipid bilayer of the target membrane. These processes are essential for diverse cellular functions, including neurotransmitter release and cytokinesis, and contribute to growth and development in yeast (Burgoyne and Morgan, 2007; Sudhof and Rothman, 2009) and in plants (Bassham and Blatt, 2008). Central to vesicle fusion is a family of membrane trafficking proteins, SNAREs [soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptors], that are conserved among all eukaryotes (Bassham and Blatt, 2008). Complementary SNAREs localize to different membrane compartments and bind selectively to form a tetrameric bundle of helices that draws the membrane surfaces together for fusion. Within the bundled \(\alpha\)-helices of the SNARE core complex, at least one each is contributed by a membrane-anchored protein associated with the target membrane (a t-SNARE) and with the vesicle (a v-SNARE); the complex is built of one element of each of four submotifs, designated QA, QB, QC, and R (Bock et al., 2001), the QA, QB, and QC domains centring about a glutamine residue within the submotif and the R domain centring about a complementary arginine residue. These submotifs confer an amphipathicity that strongly favours their coalescence through a semi-crystalline
Interweaving vesicle traffic with osmotic solute transport

In plants, SNAREs and their partners are essential for cell expansion and development, and they facilitate a number of homeostatic processes (Lipka et al., 2007; Bassham and Blatt, 2008). For example, the SNARE SYP121 drives vesicle fusion late in the secretory pathway to the plasma membrane (Geelen et al., 2002; Sutter et al., 2006b) as part of a canonical SNARE complex with SNAP33 and the functionally redundant v-SNAREs VAMP721 and VAMP722 (Kwon et al., 2008). It has been shown to affect controlled cell expansion (Geelen et al., 2002) and to function in penetration resistance to fungal pathogens (Collins et al., 2003), in the latter case probably through a targeted delivery of phenolic compounds to sites of penetration (Bhat et al., 2005).

SNAREs also form associations specialized to align vesicle fusion within other cellular processes. At least one SNARE, the mammalian syntaxin 1A, has been found to interact with several different ion channels in nerve and neuroendocrine tissues, an association that may play a role in fine-tuning of neurotransmitter release for long-distance signalling (Leung et al., 2007). Analogue interactions in plants, however, serve a very different function in coupling membrane ion transport with vesicle traffic during cell expansion. Key evidence has come from the discovery that the regulatory K⁺ channel subunit KC1 is a binding partner of the QA-SNARE SYP121 of Arabidopsis (Honsbein et al., 2009). These studies made use of a mating-based split-ubiquitin assay in yeast (Greffen et al., 2009), co-immunoprecipitation, and bimolecular fluorescence complementation to confirm protein interactions. They also used heterologous expression and electrophysiological recording to show that SYP121 is required for physiological gating within the tripartite SNARE-K⁺ channel complex of the SNARE, KC1, and its partner K⁺ channel subunit AKT1. Analysis in vivo showed that all three gene products are required to recover the inward-rectifying K⁺ current, K⁺ uptake at the root epidermis, and growth. In short, the SNARE appears to be a missing component essential for channel-mediated K⁺ nutrition.
Although incomplete, the available evidence shows that uncoupling membrane traffic and ion transport profoundly alters cell expansion, turgor, and growth (Geelen et al., 2002; Sokolovski et al., 2008). Therefore, it is likely that binding to the ion channel will also prove important in controlling vesicle fusion at the plasma membrane. Some clues to such a novel mechanism for SNARE regulation are now beginning to emerge. The KC1 binding site on the cytosolic N-terminus of SYP121 was recently isolated, demonstrating this site to be the principal determinant of interaction with functional consequences for channel-mediated K⁺ transport (Grefen et al., 2010). The binding site, defined by the core amino acid motif FxRF near the SNARE N-terminus, is significant because it overlaps with the consensus sequence in Qua-SNAREs required for mode 2 SM protein binding that is highly conserved between plants, animals, and yeast (Fig. 1). KC1 did not interact with the SYP121 H3 domain that assembles the SNARE core complex, so differentiating KC1 binding from ion channel interactions with the mammalian Qua-SNARE syntaxin 1A which can be promiscuous (Fletcher et al., 2003). These discoveries offer the first example in eukaryotes of an ion channel that interacts within this SM-binding motif, and they raise the possibility that KC1 may compete functionally with and/or substitute for the SM protein in vivo.

Is the SYP121–KC1 partnership unique? The short answer now is negative. Although a previous analysis suggested only a weak affinity of the SNARE for the AKT1 channel subunit, the interactions of SYP121 with other Kv-like K⁺ channels of Arabidopsis which, like AKT1, function as inward rectifiers have since been examined. Figures 2 and 3 show the results of yeast mating-based split-ubiquitin assays carried out in the same manner as before (Grefen et al., 2010), but using the KC1 and KAT1 coding sequences

Fig. 2. The SNARE SYP121, but not SYP122, interacts with the K⁺ channels KC1 and KAT1. Yeast mating-based split-ubiquitin assay for interaction of the SNARE chimeras with KAT1–Cub and KC1–Cub as the baits. Fusions were constructed as described previously (Honsbein et al., 2009). Channel–Cub fusions (Cub–PLV, where PLV comprised a fusion of protein A, LexA, and VP16) were placed under the Met25 methionine-repressible promoter; SNARE–Nub fusions of SYP121 and SYP122 were constructed as fusions with the N-terminal half of the mutated ubiquitin NubG to prevent spontaneous ubiquitin reassembly. Thus, only channel–SNARE interaction leads to reassembly of the two ubiquitin moieties and cleavage of the LexA-VP16 transactivator. The reporter genes ADE2 and HIS3 in the yeast strain are under the control of the LexA promoter, yielding growth on media lacking these supplements. (A) Yeast diploids created with NubG fusion constructs of SYP121, SYP122, and controls (negative, NubG; positive, wild-type Nub) spotted (left to right) on CSM medium without tryptophan, leucine, and uracil (CSM-L,W,Ura) to verify mating, CSM without tryptophan, leucine, uracil, adenine, histidine, and methionine (CSM-L,W,Ura,M,H,Ade) to verify adenine- and histidine-independent growth (second panel), and with the addition of 0.1 mM methionine (M) to verify interaction at low channel–Cub expression levels (Obrdlik et al., 2004; Grefen et al., 2009). Diploid yeasts were dropped at 1.0 and 0.1 OD₆₀₀ in each case. Growth at 30 °C on CSM-L,W,Ura was recorded after 24 h and provides a loading control; growth on CSM-L,W,Ura,M,H,Ade was recorded after 3 d at 30 °C. (B) Western blot analysis (5 μg of total protein per lane) of the diploid yeast clones with Ponceau loading controls (above), commercial VP16 antibody for the channels (centre), and both SYP121 and SYP122 polyclonal antibodies (below) for the SNAREs (Tyrrell et al., 2007).
in parallel in C-terminal (‘bait’) fusion constructs with the Cub-PLV-LexA transactivator that, on cleavage, translocates to the nucleus to activate reporter gene activity. In each case, it was found that the K⁺ channels were able to interact with SYP121, but not with its close homologue SYP122. This interaction was retained in the presence of methionine which suppresses the expression of the bait construct. Furthermore, tests of interaction with KAT1 using alanine-scanning mutagenesis of SYP121 (Fig. 3) showed that the interaction depends on the same N-terminal FxRF motif that determines SYP121 binding to KC1 (Grefen et al., 2010). Of the single-site mutants of SYP121, yeast growth was lost following alanine substitutions at Phe9, Arg11, and Phe12, indicating that replacing each of these residues interfered strongly with SNARE–K⁺ channel binding. Significantly, similar substitutions at the adjacent Arg13 and Ser14 residues had no effect on yeast growth, indicating that the effects were unique to the FxRF motif. In short, these results point to a broader scope for SYP121 interactions within the Kv-like subfamily of Arabidopsis K⁺ channels than was previously suspected. In addition, they suggest a role for these channels in regulating SYP121-mediated vesicle traffic through an entirely new, SM-associated mechanism.

Similar associations may also contribute to coordinating transport and membrane traffic control at the vacuole. For example, it is noteworthy that yeast mutants in both Nhx1p and Tlg2p are associated with so-called vps (vacuolar protein sorting) lesions that show closely related physiopathologies, including sensitivity to salt and osmotic stress (Abeliovich et al., 1998; Bowers and Stevens, 2005; Struthers et al., 2009). The significance of this parallel has yet to be addressed, but many of these proteins are already known to interact functionally. Tlg2p binds the SM protein Vps45p (Carpp et al., 2006) and assembles into complexes with its cognate SNAREs Tlg1p, Vti1p, and Snc2p (Bryant and James, 2001). Indeed, both Nhx1p and its Arabidopsis homologue NHX1 have a profound influence on endomembrane homeostasis and cell development (Nass and Rao, 1999; Bowers et al., 2000), and in the plant deletion of the cation exchanger leads to impaired cell expansion (Apse et al., 2003). These findings indicate genetic and biochemical associations between the endomembrane SNARE and cation exchanger. Together with the findings for SYP121 interaction with the Kv-like channels, they suggest a global paradigm of SNARE–transporter interactions to coordinate membrane traffic and ion transport for cellular volume and osmotic control. It is hypothesized that this coordination provides an underlying regulatory scaffold upon which additional levels of regulation may be built, including protein (de-)phosphorylation (Pardo et al., 2006).

**Conclusion**

There is now unequivocal evidence of non-canonical roles for SNAREs in controlling the activity of ion channels in plants through the direct binding of channel proteins and its consequences for mineral nutrition. This discovery and its seeming independence from signalling and vesicle traffic led previously to the proposal that its physiological function was as a ‘molecular governor’ coupling K⁺ transport with cell surface area in volume control (Grefen and Blatt, 2008). The findings of a critical site on SYP121 for this interaction and of a wider scope of partners for the SNARE add a new dimension to the molecular governor model: the potential
overlap of this interaction site with the consensus domain for binding of SM proteins that regulate the SNARE cycle suggests a complementary mechanism of molecular control that is widely distributed among Kv-like K⁺ channels and acts in regulating vesicle traffic. Therefore, the findings offer an entirely new mechanistic framework from which to explore the coordination of vesicle traffic and transmembrane ion transport, and they raise questions that will now require exploration. Among these, key issues are whether the K⁺ channels compete for SYP121 binding with *Arabidopsis* SM proteins or whether channel interaction with SYP121 might substitute in the role of an SM partner, and whether channel binding affects SYP121-mediated vesicle traffic to the plasma membrane (Fig. 4). Answers to these questions clearly will be essential to a further understanding of how coordination of membrane traffic with osmotically active solute transport is achieved during expansive cell growth.

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