Membrane trafficking and polar growth in root hairs and pollen tubes

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Abstract

Root hairs and pollen tubes extend by rapid elongation that occurs exclusively at the tip. Fundamental for such local, tip-focused growth (so-called ‘tip growth’) is the polarization of the cytoplasm that directs secretory events to the tip, and the presence of internal gradients and transmembrane flux of ions, notably Ca$^{2+}$, H$^+$, K$^+$, and Cl$^-$. Electrophysiological and imaging studies using fluorescent markers have sought to link ion gradients with growth and membrane trafficking. Current models recognize membrane trafficking as fundamental to tip growth, notably its role in supplying lipid and protein to the new plasma membrane and cell wall that extend the apex of the cell, and a complementary role for endocytosis in retrieving excess membrane and in recycling various protein fractions. The current state of knowledge is reviewed here in order to highlight the major gaps in the present understanding of trafficking as it contributes to polar growth in these cells and in recent results, that suggest a role for membrane trafficking in the active regulation of ion channel turnover and activity during polar tip growth, are discussed.

Key words: Cytosolic free calcium, cytosolic pH, endocytosis, exocytosis, ion channel, K$^+$ channel, root hair, SNAREs, tip growth, vesicle transport.

Introduction

Root hairs develop at the base of competent root epidermal cells (trichoblasts), and they serve to increase the total root surface area for water and nutrient acquisition, to anchor the plant to the soil, and to support symbiotic interactions with micro-organisms living in the soil (Gilroy and Jones, 2000). Pollen grains develop pollen tubes on the floral stigma and grow through the female tissues of the pistil to deliver their genetic material to the egg (Taylor and Hepler, 1997; Lord, 2000). To accomplish these tasks, both root hairs and pollen tubes display a highly specialized growth form in which the cell elongates unidirectionally and only at the very tip. This extreme form of polarized growth, or tip-growth, sustains prodigious rates of elongation: Root hairs grow at rates of 10–40 nm s$^{-1}$ (Galway et al., 1997; Wymer et al., 1997), comparable to that of animal neuron growth cones (Gomez and Spitzer, 1999), and lily pollen tubes (Lilium longiflorum L.) can achieve the astonishing growth rate of 250 nm s$^{-1}$ (Pierson et al., 1996; Messerli et al., 2000). It is no surprise, therefore, that to support these growth rates root hairs and pollen tubes must possess an appropriately amplified turnover of cytoskeleton, cytoplasmic structures and organelles, and an accelerated traffic of membrane vesicles that deliver membrane and cell wall material to the growing tip.

In root hairs and in pollen tubes growth is restricted to the very apex of the tip: in root hairs of Medicago truncatula, for example, maximal extension occurs in an annulus just behind the apex (Shaw et al., 2000) and, as a result, the tip is not perfectly hemispherical, but flattened at the very apex and tapered along the edges toward the cylindrical portion of the cell. Presumably these variations in extension rate across the apex dome reflect underlying variations in the cellular machinery for growth, notably in the delivery of secretory vesicles and release of cell wall constituents. Internally the cytoplasm exhibits a high degree of polarized zonation (Fig. 1): root hairs typically show a clear region of vesicle accumulation, the ‘cap’ in the extreme apical 2–3 μm of the tip and, behind this zone, a region of extremely densely packed cytoplasm containing endoplasmic reticulum (ER), Golgi apparatus...
and mitochondria (Fig. 1; endomembrane system). The nucleus follows the tip at a fixed distance (Galway et al., 1997; Geitmann and Emons, 2000). Pollen tubes similarly display a clear zone at the extreme apex that contains vesicles, while larger organelles and other structures are excluded from this zone and locate several microns behind the apex (Geitmann and Emons, 2000; Lennon and Lord, 2000). Further back from the tip, the bulk of both root hairs and pollen tubes is dominated by a large vacuole. The cytoplasm is restricted to a thin peripheral layer, with the exception of accumulation in the extreme apex.

Membrane trafficking in tip growing cells

Tip growth correlates strictly with the polarization of cytoplasm and secretion in root hairs. Once the pattern of trichoblasts is specified along the root epidermis, each cell establishes a characteristic apical–basal polarity, with the nucleus migrating to the centre of the trichoblast and then to its base, where the dome of the future hair first forms. It is at this stage that the polar tip growth is established: the cytoplasm beneath the dome polarizes to initiate a hyaline zone, and vesicle fusion at the plasma membrane must accelerate locally. Polarization of other organelles accompanies these changes, including a recruitment of the endoplasmic reticulum and Golgi behind the new growing tip. Figure 2A shows the accumulation of the ER at the base of the cell and beneath the dome, visualized here by expressing an ER-retained YFP–HDEL construct after transient transformation of root epidermal cells (P Campanoni, C Davis, J-U Sutter, MR Blatt, unpublished data). This organization of the cytoplasm is retained and must sustain tip growth during the development of the root hair (Fig. 2B). On maturation, the root hair stops growing and the endomembrane system redistributes along the hair, now largely vacuolarized (Dolan, 2001; Carol and Dolan, 2002). Remarkably, although membrane traffic is clearly central to these processes, very little is known of its mechanism, much less of its co-ordination. The following discussion serves to outline our current understanding of the membrane trafficking events involved in plant polar tip growth.

Exocytosis

In general, the endomembrane system involved in vesicle transport in plants is very similar to that described for other eukaryotic cells and, indeed, many regulatory and structural proteins involved in membrane traffic are well-conserved between plants and animals (reviewed in Battey et al., 1999; Sanderfoot and Raikhel, 1999; Vitali and Denecke, 1999; Pratelli et al., 2004; Sutter et al., 2006a). Knowing the function and localization of these proteins in tip growing cells will certainly add to an understanding of the relationship between vesicle transport and tip growth.

To date, several tip growth-impaired phenotypes have been correlated with mutations in different ras-like GTPases, such as the Rop-GTPases that contribute to cellular polarity by regulating the actin cytoskeleton, ARFs (ADP-ribosylation factors) that are essential for vesicle formation at the donor membrane, and Rab-GTPases that are responsible for vesicle docking to the target membrane (for review on small GTPase proteins in plants, see Molendijk et al., 2004).
Among these, Rop2 and RabA4b have been shown to localize at the tip during root hair elongation and appear to function as positive regulators of root hair growth (Molendijk et al., 2001; Jones et al., 2002; Preuss et al., 2004, 2006). Expression of constitutively active AtRop4 and AtRop6 GDPases abolished polarized growth (Molendijk et al., 2001).

By contrast, only two mutations have been reported so far that affect other elements of the machinery involved in mediating vesicle fusions to the plasma membrane and yield tip growth-defective phenotypes. In Zea mays the mutation sec3/rth1 (roothairless1) correlated with defects in root hair elongation (Wen et al., 2005), and in Arabidopsis thaliana sec8 mutants display defects in pollen tube initiation and growth (Cole et al., 2005). Rth1/Sec3 and AtSec8 are orthologues of two subunits of the exocyst complex that, in yeast and animal cells, guides polarized exocytosis. Intriguingly, in these cells Sec3 and Sec8 are part of the same multi-protein complex, composed of eight different subunits (Exo70, Exo84, Sec3, Sec5, Sec6, Sec8, Sec10, and Sec15; for review see Li and Chin, 2003). Although it is known that Arabidopsis and rice genomes contain genes orthologous to all the exocyst subunits (Elias et al., 2003), only recently have any of these subunits been shown to act as a complex (Zarsky et al., 2004). The fact that neither the sec3 nor the sec8 mutants were impaired concurrently both in root hair and in pollen tube growth, suggests that the two proteins may function within different pathways in each case. Alternatively, the contrasting phenotypes might be due to a redundancy in the genome, with specific isoforms differentially expressed into the two cell types.

Astonishingly, no clear connection has yet been made between tip growth and SNARE proteins. SNARE complexes mediate the final stages of vesicle fusion throughout the endomembrane system and at the plasma membrane (reviewed by Sanderfoot and Raikhel, 1999; Pratelli et al., 2004; Sutter et al., 2006a). Therefore it would be reasonable to expect that alterations in SNARE function might yield tip growth defects. At present, however, the only Arabidopsis SNARE mutants reported to display defects in pollen development are members of the Syp2 and Syp4 SNARE subfamilies of syntaxin-like proteins. These two subfamilies are thought to contribute to vesicle traffic between the vacuole and trans-Golgi network (Sanderfoot et al., 2001). No examples of mutations in SNARE proteins have yet been reported which impair root hair tip growth.

Endocytosis

Along with exocytosis, localized endocytosis is well-documented as a phenomenon associated with budding in yeast. For example, the endocytic protein Slh2p/End4p which links endocytosis with the actin cytoskeleton, is crucial for establishing zones of polarized growth in yeast (Castagnetti et al., 2005). In plants, recognition is fairly recent of endocytosis as a common cellular process, as for many years it was argued that endocytic events could not occur against the opposing force of cellular turgor pressure. Experiments demonstrating an internalization of impermeant fluorescent dyes provided a first direct confirmation of endocytosis in plants (O’Driscoll et al., 1993; Carroll et al., 1998). Subsequent studies showed that KAT1, an inward-rectifier K+ channel, was constitutively retrieved together with styryl dyes as putative membrane markers, and that these events proceeded from the plasma membrane against high turgor pressure, both in Vicia faba protoplasts and in the intact guard cells (Hurst et al., 2004; Meckel et al., 2004). For recent reviews on endocytosis, see Geldner (2004), Šamaj et al. (2005), Murphy et al. (2005), and also Sutter et al. (2006a, b).

Plant secretory vesicles are commonly 100–150 nm in diameter; they bud from the Golgi at a rate of 2–4 min⁻¹ per Golgi stack and, in hypersecretory cells such as growing pollen tubes, they arrive at the plasma membrane at rates of several thousands per minute within the region of the tip (Steer and Steer, 1989; Picton and Steer, 1983). Calculations of secretion rate in the growing tip of pollen tubes (Picton and Steer, 1983; Steer, 1988; Derksen et al., 1995) and root hairs (Morre and Van der Woude, 1974; Emons and Traas, 1986) have indicated that the total membrane surface area added to the plasma membrane exceeds its expansion by as much as 5-fold. Clearly, on the basis of these calculations, the excess membrane must be retrieved by endocytosis, but many details are still lacking. Whereas the vast bulk of exocytotic events occurs in the apical dome, because lateral displacements of membrane material are likely over the surface of the tip and behind, it is less obvious where endocytosis might occur or even whether such events are localized. In fast-growing tobacco pollen tubes, clathrin-coated endocytic vesicles are known to form in the subapical region 6–15 μm below the tip (Derksen et al., 1995) but, by contrast, endocytosis was found to take place at the apex in Arabidopsis pollen tubes even though their growth rates are generally much slower (Blackbourn and Jackson, 1996). Tip-localized internalization of the styryl dye FM4-64, a putative membrane marker, has also been reported in lily pollen (Parton et al., 2001).

In root hairs, these differences are probably related to maturation state. For example, Emons and Traas (1986), Galway et al. (1997), and Voigt et al. (2005) reported that the distribution of coated vesicles along the tip varied with both age and growth rate. Coated vesicles were observed to emerge from the subapical plasma membrane in the clear zone during active growth, but the frequency of these events dropped off once root hairs reached maturity. At least some of these endocytic vesicles were recycled for secretion in the tip or fused to larger bodies and proceeded to the base of the hair to fuse with the vacuole through a microfilament-driven ER-independent pathway (Ovečka et al., 2005; Voigt et al., 2005).
An important, but often overlooked point is that the exo- and endocytosis are almost certainly coupled. Obviously, it is essential for tip growth that a balance between the rates of secretion and endocytosis is preserved to ensure an appropriate flux of material for growth. It is also important that membrane components are recycled in a temporally and spatially consistent manner to support growth. Thus, it is no surprise that perturbing one process can result in changes in the other, or that inhibitors that affect membrane trafficking, such as brefeldin A (BFA), also rapidly suppressed cell growth (Schindler et al., 1994; Baskin and Bivens, 1995; Cho and Hong, 1995; Morris and Robinson, 1998). Remarkably, in pollen tubes treated with BFA, inhibition of exocytosis has been reported to accompany an increase in the rate of endocytosis (Wang et al., 2005; but also see Parton et al., 2001), suggesting that BFA-dependent growth inhibition is a direct consequence of both exocytosis inhibition and endocytosis stimulation.

Ion gradients

Both exocytosis and endocytosis are often sensitive to free cytosolic calcium levels (Taylor and Hepler, 1997; Bibikova et al., 1997; Sutter et al., 2000; Camacho and Malhó, 2003), and such observations correlate well with cytoplasmic tip-focused Ca2+ gradients that have been measured in tip-growing cells. The phenomenology of Ca2+ gradients is also closely coupled to internal gradients and transmembrane flux of several ions. In addition to Ca2+, a substantial body of electrophysiological and imaging data have associated K+, H+, and Cl− with tip growth.

Calcium

Several studies have shown an intracellular calcium gradient focused on the apex of both growing pollen tubes (Taylor and Hepler, 1997; Gilroy and Jones, 2000) and root hairs (Bibikova et al., 1997; Felle and Hepler, 1997; Wymer et al., 1997). This gradient is tightly coupled to polar tip growth and it corresponds closely with that of vesicle fusion and retrieval, as shown in Fig. 1 (Bibikova et al., 1997; Felle and Hepler, 1997; Taylor and Hepler, 1997; Wymer et al., 1997; Gilroy and Jones, 2000). In both root hairs and pollen tubes, the concentration of calcium decreases sharply, from very high levels near the inner surface of the plasma membrane at the tip, to basal levels some 20 μm behind the tip. Calcium gradients measured in the medium surrounding root hairs (Gilroy and Jones, 2000) and pollen tubes (Holdaway-Clarke et al., 1997; Messerli et al., 1999) also indicate a strong flux of calcium directed inward at the apex. Tip growth normally cannot be uncoupled either from these calcium gradients or from transmembrane flux of the divalent, although such a statement belies a complexity of detail behind their relationship, especially in relation to oscillations in growth and in free Ca2+ concentration (below).

Protons

Studies of lily pollen tubes with fluorescent pH-indicators first uncovered the presence of a constitutive alkaline band at the base of the clear zone and an acidic domain at the extreme apex, where active growth takes place (Feijó et al., 1999). In agreement with the intracellular proton gradient, measurements of the extracellular currents showed that growing pollen tubes maintained a circuit of protons with a net influx at the extreme apex and an efflux in the region corresponding to the alkaline zone (alkaline cytosolic pH). It has been postulated that this circuit of protons could be driven by plasma membrane H+-ATPases that are polarly localized along the tube (Feijó et al., 2004), but the balance of H+ flux implies that current return, presumably via H+-coupled transporters, is likely to be just as, if not more important for establishing any pH gradients.

Although the gradient of pH is generally recognized as fundamental for pollen tube tip growth, the presence of proton gradients in growing root hairs is still an issue of contention. A localized rise in cytosolic proton concentration in root hairs is detectable just at the very early stages of root hair development, at a time when the local cell wall acidification and loosening led to a bulging of the new hair from the trichoblast (Bibikova et al., 1997; Takahashi et al., 2003). This proton gradient is closely associated with the production of reactive oxygen species (Foreman et al., 2003) and related to the state of nutrition (Shin et al., 2005). Once the polarity is established, however, a proton gradient along the growing hair was no longer observed (Herrmann and Felle, 1995; Bibikova et al., 1997; Parton et al., 1997).

What difficulties lie behind measurements of H+? Because H+ is highly mobile by comparison with other ions in solution, local proton gradients are easily perturbed during measurement and lost. Other problems relate to the buffering capacity of pH indicators. Thus, an excess in the concentration of pH indicator dyes used during the experiments can have a disproportionate effect in shuttling protons, buffering and dissipation of local gradients. In short, questions of pH gradients in root hairs and their physiological correlates still need addressing. In fact, strong currents of protons have been measured in the extracellular vicinity of root hairs, with an influx towards the apex and a marked efflux toward the base of the hair (Gilroy and Jones, 2000), suggesting the presence of an internal proton gradient. It is worth noting, too, that an alkalinization of the cytosol was observed in conjunction with changes in growth direction of root hairs in response to Nod factors (Cárdenas et al., 2000).

Potassium

If proton gradients have a minor role in driving tip growth in root hairs compared to pollen tubes, potassium ions are
of fundamental importance to root hair tip growth as the predominant inorganic solute (Hepler, 2001). Recent studies of mutants with aberrant root hair phenotypes have served to underline this point, as well as raising many more questions, especially in relation to the potassium transporter TRH1. This transporter belongs to the AtKt/AtKUP/HAK gene family and was isolated from a mutant screen for altered root hair morphology and elongation. The Arabidopsis trh1 (tiny root hair 1) mutant plants failed to establish the cytoplasmic zonation characteristic of tip growth in root hairs (Rigas et al., 2001; Desbrosses et al., 2003). A link between TRH1 and auxin transport is also a feature of these mutants: subsequent studies of Vicente-Aguillo et al. (2004) indicated that trh1 plants show ectopic expression of an auxin-induced reporter gene in the root stele as a result of an impaired transport of auxin (Vicente-Aguillo et al., 2004). The effect of trh1 loss-of-function on root hair elongation, then, might be explained as a secondary effect due to suboptimal concentrations of auxin in the root epidermis. In short, it remains to be established whether the primary action of the TRH1 gene product is in auxin rather than in potassium transport.

The major channels responsible for K+ transport across the plasma membrane of root hairs themselves are the outward-rectifier K+ channel GORK and the inward-rectifier K+ channels AKT1 and AKT2/AKT3 as well as the ‘silent’ K+ channel AtKC1, identified from expression profile and electrical property studies (Iwashikina et al., 2001; Reintanz et al., 2002; Pilot et al., 2003). Among the inward-rectifier K+ channels, AKT1 underlies the predominant inward K+ current in root hairs and is probably modulated by AtKC1. Significantly, disrupting the AKT1 gene resulted in complete loss of the inward K+ current in these cells.

In root hairs AKT1 also seems to have a role in polar tip growth. Desbrosses et al. (2003) found that Arabidopsis plants homozygous for the akt1 loss-of-function mutation displayed altered root hair tip growth. Curiously, mutant root hairs were longer than in the wild type when the plants were grown in the absence of potassium, but shorter if grown in potassium concentrations higher than 10 mM. Thus, at low external concentrations of potassium AKT1 seemed negatively to regulate hair tip growth, exerting a function opposite to that of TRH1. Moreover, the root hair phenotype of the double akt1-trh1 mutant did not exhibit a clear epistasis, reinforcing the idea that the two channels affect root hair growth via very different mechanisms (Desbrosses et al., 2003).

If much is already known about potassium transport in root hairs, little is known about it in pollen tubes. Nevertheless, potassium ions also seem to play a role in tip growth in pollen tubes. Mutations in the pollen-specific Arabidopsis inward-rectifying K+ channel SPIK, also belonging to the Kv channel family, impaired the growth of the mutant pollen tubes with a consequent decrease in the competitive ability for fertilization (Mouline et al., 2002).

Chloride

Chloride ions have also been associated with tip growth. Intriguingly and in contrast to potassium, chloride is lost from the apex and it is recovered by an influx behind the tip (Zonia et al., 2001). Inhibition of the chloride flux via treatments with inhibitors of Cl− channel activity led to a complete block of tip growth and to an increase in pollen tube volume (Zonia et al., 2002). These observations are consistent with a role for chloride ions in supporting growth and the associated increase in cell volume of pollen tubes. Interestingly, the Cl− flux appeared to be regulated by inositol 3,4,5,6-tetrakisphosphate (Zonia et al., 2002). Phosphoinositides and phospholipids are also known to contribute to the regulation of vesicle trafficking and polar tip growth (Malhó, 1998; Zonia and Munnik, 2004; Monteiro et al., 2005; Vincent et al., 2005; for review see Martin, 1998).

Coupling tip growth with ion gradients and membrane trafficking

Understanding the temporal relationship of membrane traffic in relation to tip growth presents a number of challenges. Of these, one of the most intriguing puzzles relates to oscillations in Ca2+, associated ion flux and their coupling to growth and, presumably, to secretions that are characteristic of these cells, especially pollen (Pierson et al., 1996; Wymer et al., 1997). Much attention has focused on an examination of phase relationships associated with these oscillations on the assumptions that the calcium gradient at the apex of tip-growing cells participates in secretory processes (Battey et al., 1999) and that it should be possible to extract hierarchical data giving information regarding the identity of the primary regulator of tip growth (Feijó et al., 2001). Indeed, the Ca2+ gradient oscillates in magnitude in close relation to oscillations in growth rate (Holdaway-Clarke et al., 1997; Messerli and Robinson, 1997; Feijó et al., 2001) and styryl dye internalization (Parton et al., 2001). Nevertheless a direct correspondence between Ca2+ oscillations, vesicle delivery and turnover, and growth in pollen has proven more difficult to assess, since the peak in calcium oscillations lags the peak of growth rate by 4 s (Messerli et al., 2000). Still more confusing, the influx of calcium from extracellular medium also oscillates, but with a lag of 11–15 s on growth rate, and with a significant delay in relation to the fluctuations in cytoplasmic-free Ca2+ concentration (Holdaway-Clarke et al., 1997; Messerli et al., 1999). Proton flux, pH gradient, and chloride flux also oscillate in relation to growth, but none perfectly in phase with the growth rate. H+ influx shows a lag relative to growth rate of about 8 s (Feijó et al., 1999); chloride oscillations exhibit a lag of 3 s (Zonia et al., 2001). The counter-intuitive nature of these delays led Messerli et al. (2000) to argue that Ca2+ gradient oscillations and secretion are in fact uncoupled.
The problem is all the more complex in fact, since intracellular calcium stores and calcium chelation by cell wall proteins introduce noise in experimental measurements and thereby confound further analysis. Moreover, exocytosis of new membrane material at the tip and its retrieval by endocytosis can be expected to affect channel and other transporter populations and their patterns of localization. In short, analysing the cause/effect relationships among ion flux, ion gradients, and vesicle transport at this point will benefit from direct experimental monitoring and manipulation of membrane traffic and secretion. For example, studies of pollen tubes treated with Yariv reagent, which precipitates arabinogalactans proteins and interferes with the normal deposition of the cell wall, showed that pollen tube extension, at least, may be uncoupled from secretion in some circumstances (Roy et al., 1999). Intriguingly, these experiments also showed that the cells retained tip-focused calcium influx, locally high cytosolic calcium concentrations, and vesicle transport, despite the inhibition of growth.

It is worth considering two prevalent models for the integration of traffic with ion transport that centre (i) on the turnover of transporters in the membrane and (ii) on direct protein–protein interactions for the control of transport, although these alternatives are not mutually exclusive per se. In many animal cells, exo- and endocytosis of membrane transporters and receptors are regulated in response to external stimuli and lead to longer-term changes in their activities. Traffic of the EGF (Epidermal Growth Factor) receptor and the glucose transporter GLUT4 are two of the best characterized examples. The EGF receptor, a protein kinase, is negatively regulated by EGF in animal epithelia by evoked endocytosis of the EGF-receptor complex which is delivered to the lysosomes for degradation (for review see Katzmann et al., 2002). Traffic of the GLUT4 glucose transporter is tightly regulated in adipose and muscle cells in response to insulin (Zeigerer et al., 2002). In this case, the hormone triggers the exocytosis of GLUT4, and can generate a 4–5-fold rise in its population at the plasma membrane of cells in these tissues over a period of 10–20 min. As blood glucose is stabilized, GLUT4 recovers from the membrane dynamically by endocytosis and is recycled to endosomal compartments (Zeigerer et al., 2002; Karylowski et al., 2004). Thus, membrane traffic in these instances is strongly biased to transport control through what might be considered a ‘transport-passive’ mechanism.

By contrast, the second model is characterized by interactions between vesicle trafficking proteins and ion channels, and thus might be considered to be ‘transport-active’ in regulating ion flux. Such direct control is an important part of the mechanisms regulating neurotransmitter release. Notably, Ca\(^{2+}\) channel interactions with Q-SNAREs, including mammalian syntaxin 1A, affect the gating, and hence efficacy of Ca\(^{2+}\) channels that facilitate Ca\(^{2+}\) influx for neurotransmitter release as well as channel interactions with other second messengers (Stanley and Mirotkin, 1997; Bezprozvanny et al., 2000; Jarvis et al., 2002; Arien et al., 2003; Swayne et al., 2005; Yokoyama et al., 2005). Direct interactions of SNAREs with neuromuscular and epithelial K\(^{+}\) and Cl\(^{-}\) channels also impact on the activity of these channels (Cormet-Boyaka et al., 2002; Tsku et al., 2005).

At present there is little concrete evidence for either mechanism in plants, but there is no doubt that ion transport and membrane transport are closely linked. Transport-passive mechanisms of regulation have been proposed to account for the basipetal distribution of the PIN family of auxin transporters (Geldner et al., 2001, 2003; Friml et al., 2002; Blakeslee et al., 2004), for auxin-evoked changes in the activity of the H\(^{+}\)-ATPase (Hager et al., 1991), and for osmotically-driven changes in KAT1 K\(^{+}\) channel activity in guard cells (Hurst et al., 2004; Meckel et al., 2004). To date, none of these studies has offered substantial data that could demonstrate the molecular mechanics of the trafficking events, but they do set precedents for traffic-related changes in the population and distribution of transporters at the plasma membrane.

Support for transport-active mechanisms of regulation remains thinner still, but there is at least one hint of such control of ion channels mediated by a SNARE. The syntaxin NtSyp121 (=NtSyr1) was isolated by screening a tobacco cDNA library for sensitivity to abscisic acid (ABA) using *Xenopus* oocytes (Leyman et al., 1999). The basis of the screen in oocytes was an evoked, endogenous Cl\(^{-}\) conductance that is normally sensitive to Ca\(^{2+}\)-induced activation; however, a key line of evidence for function in the plant was that adding a truncated peptide, corresponding to the cytosolic domain of NtSyp121, suppressed ABA-mediated channel activation; however, a key line of evidence for function in the plant was that adding a truncated peptide, corresponding to the cytosolic domain of NtSyp121, suppressed ABA-dependent regulation of the K\(^{+}\) and Cl\(^{-}\) channel currents in *Nicotiana* guard cells. The simplest interpretation of these results was that the truncated peptide competed with the native, full-length protein for interacting partners, thereby suppressing ABA action on the ion channels. Subsequent studies (Geelen et al., 2002) indicated that the same peptide also suppressed vesicle traffic to the plasma membrane.

It might be argued in this case that the influence of the truncated peptide in suppressing ABA-mediated channel control reflected its action on ion channel populations, a transport-passive mechanism, rather than a more direct mechanism. The difficulty with this argument is that ion channel response to ABA is normally extremely rapid, typically complete within some tens of seconds for two of the three dominant channel currents, and its block was similarly rapid upon direct injection of the truncated peptide (Leyman et al., 1999). These effects are more than an order of magnitude faster even than insulin-evoked exocytosis of GLUT4 (above) and, hence, are difficult to explain in the context of vesicle traffic alone. In fact, recent work from this laboratory (Sutter et al., 2006b) has...
demonstrated that KAT1, the *Arabidopsis* homologue to one of these ion channels, is anchored within discrete microdomains in the plasma membrane when expressed in tobacco leaves. Intriguingly, this microdomain architecture, and channel anchoring, is severely disturbed when the KAT1 protein is co-expressed with the same truncated fragment of the SNARE that affects ABA control of the channels. These results indicate a role for the SNARE in stabilizing and localizing the channel at the plasma membrane and they raise some interesting questions about roles for the vesicle trafficking protein in regulation of the channel through its situation in the membrane. Indeed, it is very likely that spatial position and associations with other proteins are essential for correct transmission of evoked signals. It will be important now to identify the nature of the proteins that associate locally with these ion channels and the SNAREs.

A complementary model could be proposed for instance to explain the pattern of H⁺ flux around the tip of the growing pollen tube. According to this model, the polar distribution of the H⁺ flux maps to the distribution of the H⁺-ATPase that is thought to be intercalated within the plasma membrane by exocytosis at sites distal from the tip and then to migrate within the plasma membrane and to be actively retrieved outside the alkaline zone. Alternatives could entail targeted exocytosis of the H⁺-ATPase and/or localized endocytosis and depletion of H⁺-ATPase without significant lateral movements. These same ideas extend to the ion channels and other transporters that contribute to ion flux and gradients during tip growth, as shown in Fig. 3. For example, anchoring mechanisms involving cytoskeleton, binding proteins, and phospholipids could be important for local ‘trapping’ of selected, laterally-mobile transporters either at the apex or over more distal regions of the plasma membrane. Alternatively, these proteins could be spatially targeted with exo- and/or endocytosis ensuring their exclusion from the adjacent regions (Fig. 3B). Some hint for targeted exocytosis and endocytotic depletion may be found in recent studies of trafficking in root hairs and pollen using FM dyes (Ovečka *et al.*, 2005; Wang *et al.*, 2005). It remains to be seen whether these observations extend to the pattern of distribution of H⁺-ATPase and ion channels.

**Conclusions**

Although evidence is mounting for a close interplay between membrane vesicle traffic and the regulation of ion transport in plants, there are still large gaps in our current knowledge. Key elements of these processes relate to targeting and localized activities in many cell types, but none more so than in cells that exhibit tip growth. Detailed information on the characteristics of ion transport and intracellular ion gradients has been drawn from electrophysiological and fluorescence imaging studies of root hairs and pollen tubes over the past two decades. By contrast, our understanding of membrane traffic in these cells and the molecular players involved remains sparse. It is expected that recent advances in molecular tagging strategies and approaches to single-cell measurements, as well as mutant analyses, should aid significantly in resolving the links between ion gradients, ion flux, and membrane trafficking in polar growth.

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