

# Tip Growth

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## Advanced article

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**Pollen tubes and root hairs grow by a highly focused deposition of new wall and membrane materials at their growing apex. Comparison of the machinery that localises such growth between these cell types has revealed common components, providing important insight into how plant cells control cell expansion. Such elements include the small GTPases (e.g. ROPs and RABs), gradients and intricate spatial patterning in the fluxes of ions (e.g.  $\text{Ca}^{2+}$  and  $\text{H}^+$ ) and partitioning of membrane lipids (such as the phosphoinositides). These regulators are coupled to focused action of the secretory machinery (e.g. the exocyst) and cytoskeletal dynamics, with integral roles emerging for actin, tubulin and their associated motor proteins. These components form an integrated regulatory network that imposes robust spatial localisation of the growth machinery and so supports the production of an elongating tube-like growth form where cell expansion is limited to the very apex, that is, tip growth.**

## Introduction

Plant cell growth occurs when the bonds between the cell wall polymers that normally resist the force of the cell's own turgor pressure are weakened, allowing hydrostatic pressure to cause the cell to expand. Uncontrolled expansion leads to eventual rupture of the cell and so this process of weakening of the wall is highly regulated, for example, by tightly restricted reduction in wall pH that activates growth permissive proteins such as the expansins. See also: [Plant Cell Growth and Elongation](#). The wall is then stretched under the driving force of internal pressure, enabling the cell volume to irreversibly increase. However, to this general mechanism for controlling growth, we must also add

regulatory processes that allow plant cells to generate the intricate shapes that they eventually adopt. For example, pavement cells of the leaf epidermis often show a lobed growth pattern that requires localised control of expansion at different points along the cell surface. Control systems relating to auxin, actin, small monomeric G-proteins (*Rho of plants*, Rops) and membrane trafficking have all been implicated in the machinery that allows one part of the epidermal cell to expand while an adjacent part of the wall is restricted in growth (reviewed in Chen *et al.*, 2015).

Although we are still far from a complete understanding of how these kinds of complex patterns of cellular growth are imposed at the molecular level, one specialised form of local growth control, tip growth, has yielded many clues as to the underlying regulatory network. As with pavement cell morphogenesis, tip growth involves regulation by a host of factors such as the dynamics of membrane trafficking, G-proteins and the cytoskeleton. In this article, we present an overview of some of these molecular components and cellular processes that are thought to drive such highly spatially controlled growth. We concentrate on common themes of regulation emerging from two of the most heavily studied tip growing systems: pollen tubes and root hairs. Although the biological functions of these two cell types are very different and elements of cell structure, such as their cell wall composition, diverge from one another, these tip growing cells still show remarkable parallels in the machinery that drives their growth. A prime example of such conservation is the ability of researchers to use comparative transcriptional profiling between root hairs and pollen tubes to help define a shared apical growth transcriptional signature (Hafidh *et al.*, 2012; Becker *et al.*, 2014). These analyses highlight elements also seen in pavement cell regulatory networks such as G-protein-based signalling and a major role for cell wall function, reinforcing the likely key role for such components in a core localised growth machinery, as outlined in the following sections.

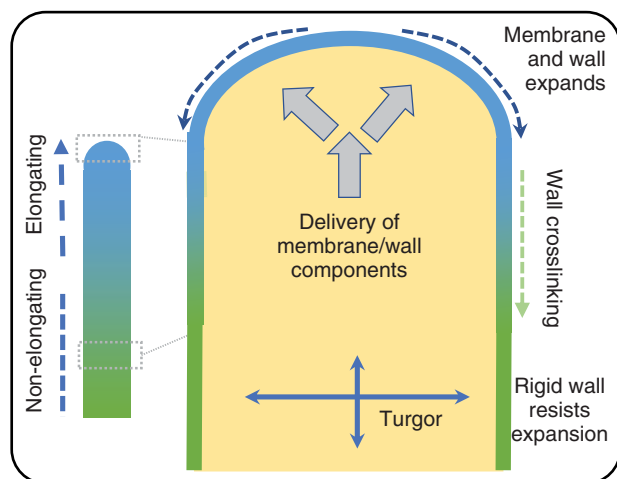
## Tip Growing Cells: Pollen Tubes versus Root Hairs

Tip growth represents the highly localised deposition of new wall and membrane that leads to an expanding apical dome. A nonexpanding, cylindrical cell body is 'left behind' as the tip extends as outlined in **Figure 1**. This growth form can be extremely dynamic, with elongation rates in root hairs readily

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**Figure 1 General features of tip growth.** In tip growth, highly localised delivery of new membrane and wall materials to the tip support localised, turgor-driven expansion. As the tip grows on, the wall that is left behind becomes progressively more cross-linked and able to withstand the forces of turgor in both the longitudinal and radial directions, restricting cell expansion. This pattern of growth control results in a rigidified tube behind a constantly expanding tip. The tube therefore gets longer by constant addition of new tube material at the elongating apex.

maintaining  $1 \mu\text{m min}^{-1}$  and pollen tubes reaching in excess of 10 times that speed. For pollen, tip growth generates a tube able to grow down through the stylar tissues of the flower to deliver sperm to the ovary as part of the fertilisation process (**Figure 2a**). The distance between the site where the pollen lands on the flower (stigmatic surface) and the egg cells to which sperm must be delivered can be in excess of 30 cm in corn, and so the rapidly elongating tip growth of the pollen tube fits well with its functional role in reproduction. Similarly, in root hairs, growth is highly localised to the growing apical dome, leading to an elongated hair-like projection. In this case, the tip growth is initiated in the epidermal layer of the root, producing outgrowths that greatly increase the root's surface area. The tip growth habit is again well suited to the role of this cell type in increasing the root's capacity for nutrient and water uptake as well as improving anchorage of the root to the soil (**Figure 2b**).

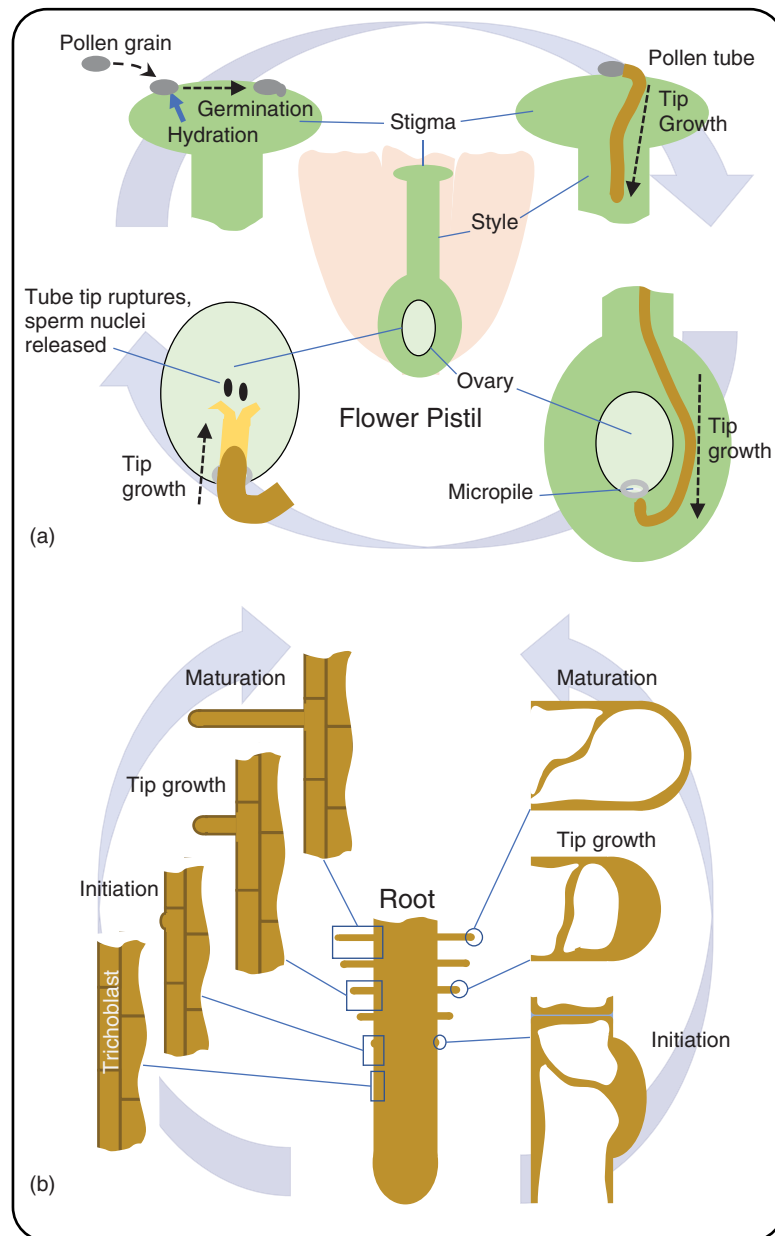
In root hairs, the cellulose fibres of the shank are aligned transversely to the axis of expansion, whereas those at the tip are shorter and more randomly arranged (reviewed in Gu and Nielsen, 2013). This wall structure can help explain why expansion can only occur at the tip, with the fibres of the shank resisting the turgor forces that drive cell enlargement, but how are properties promoting expansion maintained at the apex? How are they restricted from encroaching on the shank regions? In addition, as the cell elongates, regions of the wall that were formerly at the growing tip must transition to become rigidified in the shank (**Figure 1**). This precise regulation of cell expansion-related properties emerges from the interplay between the network of cellular components that control positioning and activity of the growth machinery (**Figure 3**), a theme we explore in the following sections.

## The Tip Growth Regulatory Network

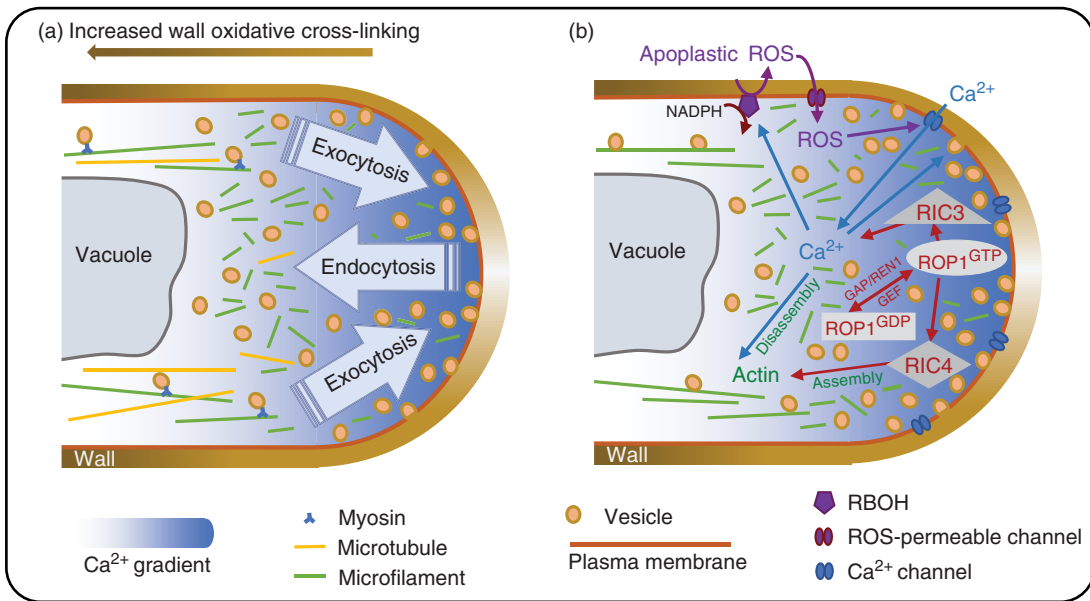
### Ionic gradients

Calcium ions are ubiquitous regulators of cellular function. **See also: Calcium Signalling in Plants.** Basal  $\text{Ca}^{2+}$  levels in the cytoplasm are maintained at around 100 nM, but the opening of channels in the plasma and organelle membranes can rapidly cause this level to increase to the  $\mu\text{M}$  range as  $\text{Ca}^{2+}$  floods into the cytosol. Plant cells possess an array of  $\text{Ca}^{2+}$ -responsive proteins capable of triggering regulatory cascades in response to this rise in ion level. However, a sustained, elevated cytosolic  $\text{Ca}^{2+}$  level is cytotoxic, and so a series of buffering and sequestration systems rapidly bring these higher  $\text{Ca}^{2+}$  levels back to basal values. In both pollen tubes and root hairs, a tip-focused gradient of elevated  $\text{Ca}^{2+}$  levels has been observed with highest  $\text{Ca}^{2+}$  at the very apex (reviewed in Michard *et al.*, 2009). This gradient is dynamic and can oscillate as growth occurs. Elevated  $\text{Ca}^{2+}$  towards the growing tip should lead to a gradient in the activity of  $\text{Ca}^{2+}$ -responsive elements such as the protein kinases and calmodulin and calmodulin-like proteins that have been shown to be key components of maintaining tip growth (Myers *et al.*, 2009; Gutermuth *et al.*, 2013; Yang *et al.*, 2014; Zhou *et al.*, 2014; Steinhorst *et al.*, 2015; Wang *et al.*, 2015). This gradient of activated signalling proteins provides one mechanism to promote spatially localised interactions that may help restrict growth. Elevated  $\text{Ca}^{2+}$  levels also facilitate membrane fusion and regulate cytoskeletal dynamics (see the following discussion), providing a further set of components of the tip growth machinery that will exhibit localisation in activity due to the  $\text{Ca}^{2+}$  gradient.

What are the  $\text{Ca}^{2+}$  influx channels driving this tip-focused gradient? In pollen tubes, the calmodulin and cyclic nucleotide gated channels (CNGCs, such as CNGCs 7, 8 and 18; Chang *et al.*, 2007; Frietsch *et al.*, 2007; Tunc-Ozdemir *et al.*, 2013; Gao *et al.*, 2016) and glutamate receptor-like channels (GLRs, such as GLRs1.2 and 3.7; Michard *et al.*, 2011) have been shown to be important for initiating and maintaining tip growth. calcium-dependent protein kinase (CPK) 32 has been shown to activate CNGC18 (Zhou *et al.*, 2014), providing a potential feed-forward amplification loop where the kinase activates the  $\text{Ca}^{2+}$  channel leading to more  $\text{Ca}^{2+}$  influx and so more kinase activity. This kind of amplification loop would provide one element of a self-reinforcing system to localise the growth machinery activity in space, provided there was some limitation on channel or CPK distribution or of the spread of  $\text{Ca}^{2+}$  within the cell to limit runaway propagation of the  $\text{Ca}^{2+}$  signal outside of the growing tip. Localisation of channels may be one element restricting the gradient. For example, in root hairs there are voltage/reactive oxygen species (ROS) regulated  $\text{Ca}^{2+}$  channel activities that are likely key elements of the  $\text{Ca}^{2+}$ /tip growth system, especially considering the likely role for the interaction between ROS and  $\text{Ca}^{2+}$  in tip growth discussed below. These channels are restricted to the growing tip (Laohavisit *et al.*, 2012, and references therein), providing a spatial component to limiting the extent of  $\text{Ca}^{2+}$  entry. Similarly, elements of  $\text{Ca}^{2+}$  sequestration systems such as the  $\text{Ca}^{2+}$ /ATPases (adenosine triphosphatase)



**Figure 2 Tip growth in pollen tubes and root hairs.** (a) Pollen tube tip growth fits well with the requirements of plant fertilisation. A pollen grain lands on a receptive stigmatic surface, takes up water and germinates, producing a pollen tube that emerges from the operculum, a preformed pore in the pollen wall. The tube then enters tip growth and using chemical cues both within the style and from the ovary is guided to the micropile, a pore allowing entry to the ovary. The fine balance between simultaneously allowing growth but also resisting the expansive forces of turgor within the growing tube tip now shifts towards expansion and the tip of the tube bursts, releasing the sperm inside the pollen tube to effect fertilisation. (b) Tip growth in root hairs starts with the process of initiation, where a bulge forms in a precisely controlled point in the lateral wall of a trichoblast, an epidermal cell genetically preprogrammed to be able to produce root hairs. Cytoplasm accumulates in the bulge which then transitions to tip growth, a genetically distinct process from initiation. Apically growing root hairs have a cytoplasm-rich tip where the machinery of tip growth is localised. Growth continues until the hair reaches a mature length at which point the tip growth machinery dissipates, the vacuole protrudes to the cell apex and the apical wall becomes strengthened and no longer able to support cell expansion.



**Figure 3 Regulatory networks at the growing apex.** (a) Exocytosis of new wall polymers and insertion of new plasma membrane material supports expansion focused to the apex of tip growing cells. Simultaneously, endocytosis recycles excess membrane delivered to the tip. The actin cytoskeleton plays a key role in mediating this membrane trafficking system. (b) Examples of regulatory loops controlling tip growth activities in pollen tubes and root hairs.  $\text{Ca}^{2+}$  influx has been shown to lead to a tip-focused  $\text{Ca}^{2+}$  gradient promoting multiple processes at the growing apex including exocytosis, regulation of actin dynamics and activation of the ROS producing NADPH oxidases of the RBOH family. RBOH produces ROS to the apoplast promoting wall rigidification. ROS also enter the cytosol via channels in the plasma membrane (possibly aquaporins) where they regulate  $\text{Ca}^{2+}$  channel activity. These activities are modulated by parallel, overlapping regulatory cassettes. For example, in pollen tubes a tip-focused accumulation of the active (GTP-bound) form of the ROP1 GTPase acts (in part) through RIC3 and RIC4 to modulate both  $\text{Ca}^{2+}$  and actin dynamics (RIC3 promoting disassembly and RIC4 assembly). ROP activity itself is modulated by regulators such as Guanine nucleotide Exchange Factors (GEF) that determine whether ROP1 is bound to GTP (active:  $\text{ROP}^{\text{GTP}}$ ) or GDP (inactive:  $\text{ROP}^{\text{GDP}}$ ). REN1: ROP1 ENHANCER, a Rop GTPase Activating Protein (GAP). REN1 contains a pleckstrin homology (PH) domain implying it can interact with the phosphoinositide regulatory system.

that pump  $\text{Ca}^{2+}$  from the cytosol, and so limit the extent of  $\text{Ca}^{2+}$  increases, appear integral to sustaining tip growth. Thus, the plasma membrane autoinhibited  $\text{Ca}^{2+}$ -ATPase ACA9 is critical for pollen tube function (Schiott *et al.*, 2004) and the  $\text{Ca}^{2+}$  gradient at the tip seems intimately linked to organelle  $\text{Ca}^{2+}$  sequestration systems, such as to the endoplasmic reticulum (Iwano *et al.*, 2009).

Other ions including  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{H}^+$  also show complex patterns of movements into and out of tip growing cells (reviewed in Michard *et al.*, 2009). For example, pH has been shown to change dynamically during tip growth in both pollen tubes and root hairs (Feijo *et al.*, 1999; Monshausen *et al.*, 2007; Certal *et al.*, 2008), likely regulated by  $\text{H}^+$ -ATPase activity and distribution (Certal *et al.*, 2008). Potential targets for these ions is harder to define than for  $\text{Ca}^{2+}$  as, for example, pH changes are likely to simultaneously affect a wide array of cellular activities through a plethora of molecular interactions.

The  $\text{H}^+$  pumps on the plasma and internal membranes likely show regulation through  $\text{Ca}^{2+}$  (Haruta *et al.*, 2015) with, for example, the  $\text{Ca}^{2+}$ -dependent regulators CALCINEURIN B-LIKE (CBL) 2 and CBL3 that modulate the activity of the vacuolar  $\text{H}^+$  pump proving to be integral to normal pollen tube growth (Steinhorst *et al.*, 2015). Similarly, a tip low anion gradient seen in pollen tubes is dependent on regulation of the anion channel SLAH3 by CPK2 and CPK20 (Gutermuth *et al.*,

2013), integrating the dynamics of these other ions with the  $\text{Ca}^{2+}$  gradient. Further, the mechanosensitive anion channel MSL8 appears integral to pollen function (Hamilton *et al.*, 2015), suggesting a further potential regulatory link, in this case between anion fluxes and the local mechanical forces inherent in tip-focused, turgor-driven growth.

## The cytoskeleton

Both the microfilament and microtubule cytoskeletons appear to play roles in tip growth with their spatial patterning supporting focus of the growth machinery to the tip. Thus, in root hairs and pollen tubes, actin forms diffuse arrays towards the growing apex, with thicker bundles running the length of the shank (reviewed in Ketelaar, 2013; Fu, 2015). This patterning of actin cables and bundles is thought to provide a mechanism to transport membrane and cell wall materials to the tip (Figure 3a). Disrupting actin dynamics disrupts apical growth, typically causing arrest or delocalisation of expansion. Similarly, mutants in actin-dependent motor proteins, such as those of the myosin XI family exhibit root hair growth arrest (Park and Nebenfuhr, 2013 and references therein). These proteins are thought to support the vesicle transport function that underlies the actin-based delivery system of new wall and membrane components required to sustain tip expansion (Figure 3a). An array of actin-binding proteins related to tip growth, such as the formins and actin depolymerisation

factor govern actin nucleation and dynamics (Ketelaar, 2013; Fu, 2015) and are themselves sensitive to factors such as  $\text{Ca}^{2+}$  and small GTPases (guanosine triphosphatase) (**Figure 3b** and see below), integrating actin dynamics into the tip-focused regulatory network.

The precise role of microtubules in tip growth is less clear. Cortical and endoplasmic microtubules run the length of the shank but do not protrude to the very apex of the tip growing cells (Fu, 2015). Disrupting microtubule function through either pharmacology (Bibikova *et al.*, 1999; Ketelaar *et al.*, 2002) or by mutants in microtubule associated proteins, such as the plus-end microtubule-associated motor ARMADILLO REPEAT KINESIN-1 (ARK1; e.g. Yoo and Blancaflor, 2013; Eng and Wasteneys, 2014 and references therein), does not simply arrest growth as expected for an integral element of the growth machinery. These treatments and mutants do however cause the directional component of tip growth to be lost, revealing a waving phenotype or even branching. Thus, the microtubule cytoskeleton may be playing a role in stabilising the direction and positioning of the growth machinery. **See also: Plant Microtubules: Their Role in Growth and Development**

## Small G-proteins

Small G-proteins of the ROP family also appear to be key regulators of localised growth in plants. These proteins are GTPases that cycle between a GTP-bound (generally the active) and GDP-bound (generally the inactive) form. Arabidopsis has 11 ROPs with, for example, ROPs 1, 3 and 5 acting in supporting pollen tube growth, and other family members such as Rops 2, 4 and 11 affecting root hair expansion. For example, expressing a constitutively active form of AtRop11 delocalises root hair growth (Bloch *et al.*, 2011). In pollen tubes, members of the Rop Interacting Crib-domain containing (RIC) family of proteins act along with ROP1 to modulate actin filament dynamics. RIC1 and RIC3 interact with the tip-focused  $\text{Ca}^{2+}$  gradient to promote actin disassembly, an activity counteracted by RIC4 (Gu *et al.*, 2005; Zhou *et al.*, 2015). The RICs may help to stabilise an apical fringe of short actin filaments that accompanies tip growth and that may play a role in focusing secretion to the very apex of the expanding tip. **See also: Rop GTPases: Polarity and Cell Shape in Plants**

A host of regulators affect ROP activity, with GTPase activating proteins (GAPs) facilitating the intrinsic GTPase action that converts the GTP bound to the ROPs to GDP; GTP exchange factors (GEFs) promoting the replacement of the bound GDP with GTP, and guanine nucleotide dissociation inhibitors (GDIs) preventing GDP dissociation. These regulators act to control Rop activity and localisation that in turn affects other elements of the tip growth regulatory machinery (**Figure 3b**). **See also: Rop GTPases: Polarity and Cell Shape in Plants**

In addition to the Rops, members of the RAB family of monomeric small GTPases (plant homologues of the RAS in the Brain GTPase superfamily) are important regulators of membrane trafficking and have proven to be key to maintaining tip growth. For example, RAB-B family members are highly expressed in pollen where they likely help mediate endomembrane trafficking to sustain tube growth (Cheung *et al.*, 2002). Similarly, RAB-A4 GTPases are localised to the apex of pollen

tubes and root hairs where they too appear to be critical players in maintaining membrane dynamics and the tip growth process (reviewed in Gu and Nielsen, 2013). In root hairs, RAB-A4B interacts with the lipid kinase PI-4K $\beta$ 1 that along with the PI-4P phosphatase RHD4 coordinately regulate the levels of the membrane lipid phosphatidylinositol-4 phosphate that also appears critical for supporting membrane function related to tip growth. Indeed, phosphatidylinositol-phosphate kinases, phospholipases and phospholipid transfer proteins all appear important to sustaining tip growth in both root hairs and pollen tubes (reviewed in Heilmann and Ischebeck, 2016). Phosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PIP2) show highly polarised accumulations at the tip of both pollen tubes and root hairs, where they may act to generate specialised microdomains within the apical membrane that target and anchor key elements of the apical growth machinery to this region.

Again, these lipid-related elements likely form part of an integrated network of regulation with other key elements of the tip growth machinery. For example, ADP ribosylation factor GTPase-activating protein 1 has been shown to be critical for normal root hair tip growth and cytoskeletal dynamics (Yoo and Blancaflor, 2013). This protein contains a phosphoinositide-binding domain and interacts with the phosphoinositide regulatory system. This GTPase regulator is also required for the correct targeting of ROP2 and RABA4b and acts alongside the microtubule-related tip growth system through the ARK1-dependent pathway outlined above, providing a point of crosstalk between several elements of the tip growth regulatory network (Yoo and Blancaflor, 2013 and references therein). Similarly, the exocyst is a vesicle tethering complex that appears intimately involved in directing secretory vesicles to the growing tip of both pollen tubes and root hairs (Zhang *et al.*, 2016 and references therein). The exocyst is also likely using the phosphoinositide system to provide some spatial control. For example, the localisation of the sec3a exocyst subunit appears to be mediated in part by interactions with PIP2 (Bloch *et al.*, 2016). The exocyst also interacts with small GTPases, continuing with the theme that each element of the regulatory network interacts with multiple others to coordinate spatial patterning of the growth machinery's activities.

## The cell wall

The cell walls of pollen tubes and root hairs are structurally different, but the interactions of the tip growth machinery and the wall are fundamental to polarised growth control in both. Cellulose synthases of the CesaA-like (CSL) superfamily support tip growth with, for example, CSLD2 and CSLD3 being localised to the root hair's apex (reviewed in Gu and Nielsen, 2013). Although cellulose deposition is also important in pollen tubes, the secretory vesicles supporting tip expansion in these cells are also rich in pectin and this wall polymer is important for elongation of both pollen tubes and root hairs. In pollen tubes, pectin methyl esterase (PME) activity accumulates along the shank, with the pectin methyl esterase inhibitor protein localising to the tip (reviewed in Gu and Nielsen, 2013). Pectin methyl esterases remove methyl esters from the pectin polymer, revealing carboxylic acids that can then bind  $\text{Ca}^{2+}$ , rigidifying the wall. Thus, high PME levels



in the shank and low activity at the tip should promote localised expansion to the tube apex. It is important to note here that the cell wall is a complex carbohydrate- and protein-rich environment with many other structural and regulatory elements that in addition to cellulose and pectin are known to be integral elements of the tip growth process. **See also: Plant Cell Walls; Cellulose: Structure and Distribution.** To maintain tip growth control therefore requires feedback between these wall elements and the regulatory networks that sustain tip growth. We are far from understanding how the status of the cell wall is relayed to the growth regulatory machinery. However, one hint as to how such feedback may occur comes from studies of the interactions between receptor-like kinases (RLKs) and ROS production to the cell wall, a system outlined in the following section.

## ROS and the cell wall

ROS are now known to play important roles in pollen tube and root hair growth and a key insight into some of the core cellular machinery underlying such ROS action came from the cloning of the gene for the Arabidopsis *root hair defective 2* mutant (Foreman *et al.*, 2003). This mutant failed to sustain root hair tip growth and is caused by a lesion in the gene for the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase RESPIRATORY BURST OXIDASE HOMOLOGUE C (RBOHC). RBOHC is a plasma membrane enzyme that produces ROS to the cell wall. This family of NADPH oxidases is under a complex set of posttranscriptional regulatory influences but notably for its role in tip growth, RBOHC is activated by its endogenous  $\text{Ca}^{2+}$  binding EF-hand domains (Takeda *et al.*, 2008). Tip growth is associated with oscillations in ROS (Monshausen *et al.*, 2007) which, although slightly delayed relative to the  $\text{Ca}^{2+}$  oscillations, do mimic the frequency of the  $\text{Ca}^{2+}$  peaks, suggesting a model where  $\text{Ca}^{2+}$  gradient activity may help pattern RBOHC-dependent ROS production to the wall. Further, RBOHC is localised to the growing tip of root hairs in a microfilament-dependent manner (Takeda *et al.*, 2008), again allowing integration of RBOH-dependent events with the other controlling elements of the tip growth regulatory network.

It is important to note that ROS likely have complex roles not only in modulating wall architecture (serving to both cross-link and sever bonds within the wall) but also acting as cytosolic signalling molecules and as stress response compounds. Therefore, tight regulation of their production in time and space is to be expected. **See also: Oxidative Stress and Redox Signalling in Plants.** Treatments that alter ROS levels can arrest both root hair and pollen tube growth (Monshausen *et al.*, 2007; Potocky *et al.*, 2012). Taken together with the finding that the root hairs of *RbohC* mutants burst (Monshausen *et al.*, 2007; Macpherson *et al.*, 2008), these observations suggest apoplastic ROS may play a role in strengthening of the cell wall to avoid runaway expansion at the growing tip. Interestingly, the cellulose synthesis *cls2* mutant seems to show repeated rounds of bursting and restarting tip growth (Bernal *et al.*, 2008), reinforcing the idea of a subtle, cyclical balance within the wall between facilitating expansion and resisting catastrophic, turgor-driven failure. An intriguing observation is that *ANNEXIN 1* mutants have impaired root hair growth and lack a ROS-activated  $\text{Ca}^{2+}$  conductance normally

seen at the tip of root hairs (Laohavisit *et al.*, 2012), suggesting one further candidate for a pathway integrating cytosolic  $\text{Ca}^{2+}$ - and cell wall ROS-related events. In addition to  $\text{Ca}^{2+}$ , ROS production in pollen tubes is known to be modulated by phospholipids and Rac/Rop GTPases (Potocky *et al.*, 2012), again stressing the network-like nature of the regulators controlling such localised growth. Also, alterations in extracellular pH can rescue the *rh2/rbohC* phenotype (Monshausen *et al.*, 2007), highlighting a further element of integration between these regulators in the likely interplay between the wall, ROS and pH dynamics at the growing root hair tip.

A role for ROS-wall interactions has also emerged in the regulation of the progression of tip growth by RLKs such as FERONIA (FER; Cheung and Wu, 2011). FER likely plays a role in relaying information about cell wall integrity and/or wall mechanical properties to the growth machinery (Shih *et al.*, 2014). Interestingly, the ligand for FER is the small peptide RAPID ALKALINISATION FACTOR (RALF, Haruta *et al.*, 2014). RALF was originally identified as a novel wall peptide that inhibits root growth and alters extracellular pH, potentially providing a mechanistic link between wall integrity signals, wall pH and the growth regulatory machinery. RALF also modulates cytosolic  $\text{Ca}^{2+}$  levels (Haruta *et al.*, 2008) providing a further point of crosstalk in the elements linked to the tip growth regulatory network. ANXUR1 and 2 are kinases related to FER that also play roles in modulating tip growth and ANXUR-related modulation of cell wall integrity at the pollen tube tip has been shown to operate via the RBOHH and RBOHJ NADPH oxidases (Boisson-Dernier *et al.*, 2013; Lassig *et al.*, 2014). These RBOHs are likely acting to modulate the ROS production that fine-tunes  $\text{Ca}^{2+}$  dynamics at the growing tip to restrict growth. These ideas fit well with the observation that ROS also appear crucial for the pollen tube to rupture at the point sperm must be released during fertilisation (**Figure 2a**). For the rupture process, FER is thought to operate by altering ROS production that in turn triggers  $\text{Ca}^{2+}$  channel activation. This elevated  $\text{Ca}^{2+}$  leads to unrestricted tip expansion, that is, bursting (Duan *et al.*, 2014). FER is also thought to modulate root hair growth via its effects on ROS production and in this case the regulatory network involves its action on the Rop2 regulatory cassette (Duan *et al.*, 2010).

## Conclusion

The regulatory network that sustains the tip growth process appears to form a complex set of feedback and -forward interactions that generate a tight web of interacting controls. Such integration can be seen playing out not only in the posttranslational regulatory loops described above but also, for example, at the transcriptional level. Thus, ROOT HAIR DEFECTIVE SIX-LIKE4 is a transcriptional regulator that is required for root hair growth (Vijayakumar *et al.*, 2016). This protein coordinately controls expression of elements drawn from many of the regulatory categories described above: the cytoskeleton (*SUPPRESSOR OF ACTIN 1*), the exocyst (*EXOCYST SUBUNIT 70A1*), wall ROS modulation (*PEROXIDASE7*) and  $\text{Ca}^{2+}$ -dependent regulation (*CPK11*). Coordinate modulation of these genes likely facilitates balancing the level of each regulatory cassette, promoting

the generation of the tightly coordinated, dynamic network that appears to be the hallmark of tip growth regulation. Indeed, the dynamic, multicomponent nature of this control system presents a major challenge to designing experiments to dissect how the network imposes its regulatory influence. Monitoring the activity or dynamics of a single component may not reveal how the network as a whole is operating. Combining new techniques such as Green Fluorescent Protein-based biosensors for visualising cellular activities with simultaneous imaging of the cytoskeleton and membrane flow should allow multiparameter dynamics to be collected, providing one way to approach this problem. Integrating such analyses with quantitative modelling of cellular regulatory networks and the biophysics of the tip growing system offers great potential to describe both regulatory network architecture and dynamics. Many of the processes emerging as important regulators of localised cell expansion from work on root hairs and pollen tubes are conserved throughout land plants, for example, the cytoskeleton, secretory apparatus and the Rops are all key components of apical growth phenomena in mosses (Vidali and Bezanilla, 2012). Thus, these studies on tip growth hold the promise of revealing some potentially generally applicable principles about the molecular networks used by plants to impose spatial and temporal controls on growth.

## Acknowledgements

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