Chapter 7
Pollen Tip Growth: Control of Cellular Morphogenesis Through Intracellular Trafficking

Hana Rakusová and Anja Geitmann

Abstract The control of cellular growth in pollen tubes occurs through the fine-tuning of intracellular transport and secretion processes. This does not only apply to the basic genesis of the cylindrical cell through polar expansion but also to the pollen tube's specialized skills including its capacity to respond to directional guidance cues and its ability to perform invasive growth. The control of these specialized activities by intracellular trafficking occurs through the strategic deposition of cell wall material and cell wall modifying agents that soften or stiffen the wall with the aim to regulate the cell wall's mechanical properties both in time and space. Directional and invasive growth of the pollen tube is crucial for successful sperm delivery and fertilization. The mechanisms underlying the regulation and logistics of the endomembrane trafficking in the pollen tube therefore have a direct impact on pollen tube elongation and male fertility. Here, we relate pollen tube morphogenesis and its biological functionality to the intracellular processes that control cellular growth behavior and allow the cell to respond to environmental cues.

Keywords Pollen tube • Tip growth • Cell wall mechanics • Vesicle trafficking • Exocytosis

Abbreviations

CalSs	Callose synthases
CESAs	Cellulose synthases A
FRAP	Fluorescence recovery after photobleaching
HG	Homogalacturonan
PGs	Polygalacturonases
PME	Pectin methyl esterase
PMEIs	Pectin methyl esterase inhibitors

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G. Obermeyer, J. Feijó (eds.), *Pollen Tip Growth*,
DOI 10.1007/978-3-319-56645-0_7

RIPs	ROP-interacting partners
ROPs	Rho family of GTPases
SNAREs	Soluble NSF attachment protein receptors
SYP	Syntaxin of plants
TGN	Trans-Golgi network

7.1 Introduction

The fertilization success of the male gametophyte in plants relies on the speed with which it outcompetes its competitors on the way to the finite number of receptive ovules, its efficiency to find these ovules and deliver the sperm cells, and its capacity to drill its way through or around any mechanical obstacles along its path. Pollen tube elongation occurs in highly polar manner and requires the delivery of cell wall and membrane material as well as enzymes and other agents to the growing tip. This applies to the basic growth process that produces the characteristic cylindrical morphology of the cell and becomes even more critically important when the pollen tube has to accomplish specific tasks such as responding to a guidance cue or navigating a mechanical obstacle. These specific tasks require a tight regulation of the morphogenetic process, which in turn is controlled by the cellular machinery delivering and assembling the cellular envelope (Kroeger et al. 2009; Bou Daher and Geitmann 2011; Qin and Yang 2011). The present chapter explains how the pollen tube forms a tube, how it accomplishes specific tasks, and how the intracellular transport machinery regulates all of these processes.

7.2 Mechanics of Tubular Cellular Expansion

Pollen tubes have a cylindrical shape with a diameter of 5–20 μm, depending on the plant species. Their length is indeterminate and can reach several centimeters. In some species, the pollen tube can grow tens of centimeters long, although the living portion of the cytoplasm is confined to the tip region of the cell. The rapid polar cell growth requires structural and functional organelle compartmentalization and high rates of cell wall assembly (Cheung and Wu 2007). An exquisitely orchestrated intracellular transport mechanism ensures the delivery of the material necessary for the construction of the expanding cellular envelope—cell wall polymers and phospholipids. These materials are delivered to the growing tip in small vesicles (Fig. 7.1). Larger organelles such as mitochondria and Golgi bodies are predominantly segregated to the cytoplasm distal to the subapical region. The apex of the rapidly elongating cell is roughly hemisphere shaped, and all growth activity is confined to this region implying that the bulk of new soft cell wall material is added here. The result of this polar mode of growth is a cell wall, the thickness of which does not vary much around the cell (100–200 nm) but which shows a high

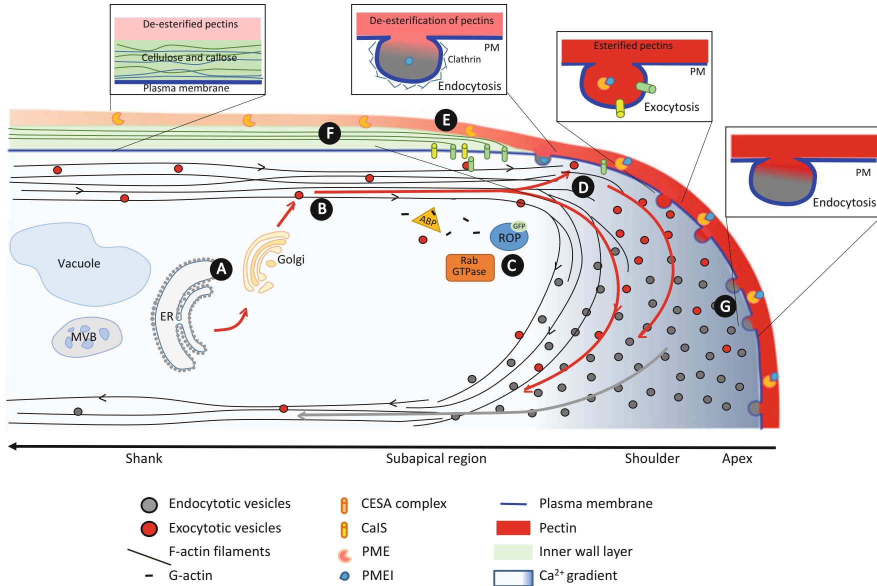

Fig. 7.1 Spatial distribution of pollen tube cell wall components is regulated by vesicular trafficking. Components of pollen tube envelope (plasma membrane and cell wall) are synthetized in the ER and Golgi (**A**) and transported to the cell surface via vesicular trafficking. The vesicles are transported preferentially along the actin filaments (**B**). Exocytosis is regulated by GTPases such as Rab or ROP (**C**) and in some species takes place preferentially in the shoulder region of the apex (**D**). CESA and CalS enzymes are incorporated in the plasma membrane and start to synthetize cellulose and callose. At the same time, endocytosis of PMEI (PME inhibitor) (**E**) allows activation of PME leading to pectin de-esterification and a stiffened cell wall (**F**). The endocytosis process helps to regulate the proportion between the plasma membrane and cell wall component secretion at the pollen tube apex (**G**)

degree of nonuniformity along the longitudinal axis concerning the distribution of its components (Chebli et al. 2012). Mechanical modeling has predicted (Fayant et al. 2010) and micro-indentation has confirmed that the mechanical properties of the cell wall change along the length of the tube, although absolute values for cell wall stiffness are elusive for the tip region (Bolduc et al. 2006; Zerzour et al. 2009; Chebli et al. 2012; Vogler et al. 2013). The cell wall of the shank has been measured to possess a Young's modulus of 350 MPa, a value that resembles that of Teflon (Sanati Nezhad et al. 2013b), and scanning electron microscopy has shown that microfibrils are oriented at a helical shallow angle in this portion of the cell (Aouar et al. 2010). The latter has been speculated to protect the tube from kinking when it is bent, thus ensuring that sperm cell transport is not hampered.

The nonuniform distribution of cell wall components and the resulting polarity of wall mechanical properties are a prerequisite for the successful formation of a cylindrical tube (Fayant et al. 2010). Just as other plant cells, pollen tubes grow by controlled yielding of the wall to the internal turgor pressure (Geitmann and Ortega

2009; Guerriero et al. 2014). The spatial gradient in mechanical properties ensures that the yielding occurs in one direction only. Since turgor is a scalar and pressure is exerted equally in all directions, turgor-driven cell wall expansion at the very apex of the pollen tube must exhibit spatial regulation in biomechanical properties of the cell wall between the apex and the shank of the cell (Geitmann and Steer 2006). Typical pressures in plant cells range between 0.1 and 1 MPa, which corresponds to the pressure in a car or bicycle tire. The only absolute value for turgor in pollen tubes was measured in lily (Benkert et al. 1997). Turgor is a hydraulic pressure maintained by osmosis—the movement of water across a semipermeable membrane along a concentration gradient (Hill et al. 2012). In addition to driving growth, turgor plays a role as a hydroskeleton that maintains plant cell shape against external forces. In the pollen tube, turgor is therefore not only necessary to drive growth; it is the pressure that prevents the cell from collapsing under the lateral compression force of the pistillar tissues (Sanati Nezhad et al. 2013a, Fig. 7.2). Finally, the hydrostatic pressure is also thought to provide the force necessary for the invasive penetration of the pistillar tissues (Sanati Nezhad and Geitmann 2013). The importance of turgor as a driving force for pollen tube elongation is easily illustrated by exposure of these cells to altered osmotic conditions (Liu and Hussey 2014), but this does not mean that the growth rate is actually regulated by the turgor. Rather, it is thought

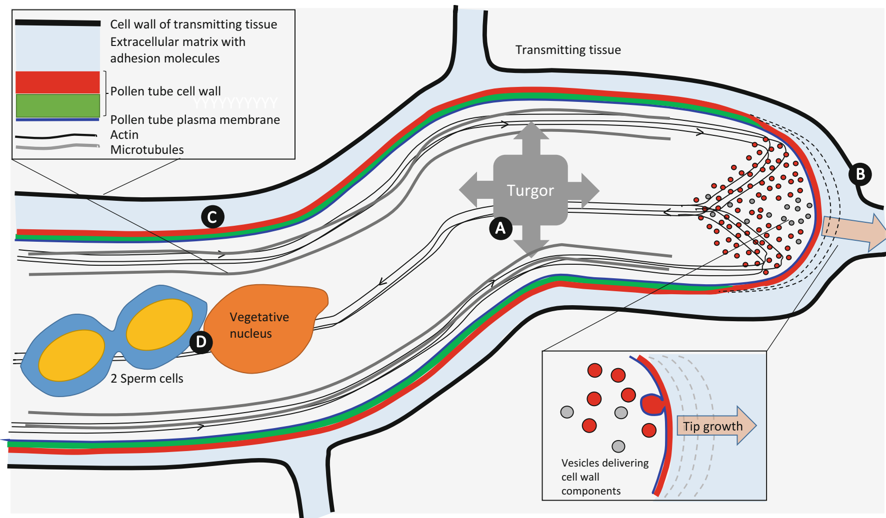

Fig. 7.2 Pollen tube invasive growth within the flower tissue. The pollen tube cytoplasm is compartmentalized—at the very tip, the softer cell wall yields to the turgor pressure (**A**), and simultaneously, cell wall assembly occurs (**B**); in the shank of the pollen tube, the cell envelope is stiffened in order to resist against compressive stress provided by the turgor (**C**). The two sperm cells and the vegetative nucleus are transported along the cytoskeleton toward the tip region. Their transport can occur even in bending tubes since the helical winding of the cellulose microfibrils prevents kinking (**D**)

that the mechanical properties of the cell wall and its assembly rates control the velocity with which pollen tube growth occurs (Winship et al. 2011). Consistent with this notion, it was shown experimentally (Benkert et al. 1997) and explained by modeling (Kroeger et al. 2011) that the average growth rate is uncorrelated from the turgor pressure.

The nonuniform distribution of mechanical properties required for polar growth is generated by a precisely controlled distribution of the individual polymers composing the pollen tube wall (Dardelle et al. 2010; Chebli et al. 2012). It has been shown in lily (Roy et al. 1997), tobacco (Li et al. 1995; Ferguson et al. 1998), and *Arabidopsis thaliana* (Lennon and Lord 2000; Dardelle et al. 2010; Chebli et al. 2012) that the pollen tube cell wall in the shank of the cell is composed of two layers: a fibrillar outer layer and a weakly electron-dense inner wall. The former is rich in pectins and can contain cellulose. Cellulose microfibrils can also be found in the inner layer in certain species, but the main component is callose (Taylor and Hepler 1997). At the pollen tube tip, this inner layer is generally lacking (Roy et al. 1997; Lennon and Lord 2000). The relative abundance of certain cell wall components in the different regions of the cell is therefore one of the features that underlies the spatial gradient in mechanical properties.

The different components of the pollen tube cell wall are synthesized and assembled in different ways. Pectin and hemicellulose are synthesized at the Golgi apparatus and secreted in their final forms. Cellulose microfibrils and callose on the other hand are synthesized directly at the plasma membrane by synthase complexes (Driouich et al. 2012). The deposition and enzymatic modification of all components occurs at precisely defined locations relative to the pollen tube tip. The latter is particularly important for pectins which are complex polymers including homogalacturonan (HG). In most angiosperms, HG is present in highly methyl-esterified form at the tip, whereas the shank of the cell displays acidic or weakly methyl-esterified HG epitopes (Dardelle et al. 2010; Chebli et al. 2012). Given its lower ability to cross-link, the methyl-esterified pectin in the apical cell region is believed to provide sufficient plasticity to provide a strong but malleable surface that permits rapid cell expansion (Cheung and Wu 2008) consistent with predictions made through mechanical modeling (Fayant et al. 2010). Such spatial change in the chemical configuration of a single type of cell wall polymer is therefore the second important feature that contributes to the gradient in mechanical properties.

HG synthesis is carried out in the Golgi apparatus, and the polymers are secreted via Golgi-derived vesicles (Driouich et al. 2012). Their deposition at the apical cell wall occurs in a highly methyl-esterified form explaining the high concentration at this location. As the apical wall matures and becomes part of the shank, the HG is de-esterified, and because the molecules are now negatively charged, they attract Ca^{2+} ions that cross-link the polymers in a process called gelation (Micheli 2001; Geitmann and Steer 2006). The de-esterification is accomplished by the enzyme pectin methyl esterase (PME), which is co-secreted with the esterified pectins in the same secretory vesicles (Bosch and Hepler 2005; Kim and Brandizzi 2014). PME activity has to be tightly regulated in space and time to allow pollen tube growth to proceed. Exogenous application of the enzyme or overexpression inhibits pollen

tube growth, likely by causing the soft apical wall to stiffen prematurely (Bosch et al. 2005).

To prevent PME from acting prematurely (in the secretory vesicles or at the apical wall), its activity is regulated by inhibitors (pectin methyl esterase inhibitors, PMEIs) which are co-secreted (Röckel et al. 2008). The inhibitor is present in the apical wall only, leading to the speculation that it is internalized through endocytosis in the shoulder of the tip coinciding with the location of beginning pectin maturation and PME activity. Treatment of pollen tubes with exogenous PMEI results in an abnormal germination and bursting of pollen tubes in *A. thaliana* confirming the role of HG in the mechanical properties of the cell wall (Lehner et al. 2010; Mollet et al. 2013; Leroux et al. 2015). PMEIs and their endocytosis are therefore thought to play a crucial role enabling polar growth in pollen tubes through confining the "soft spot" in the cell wall to the pollen tube apex (Zhang et al. 2010b; Palin and Geitmann 2012).

The pollen tube also produces other pectin-modifying enzymes such as polygalacturonases (PGs) and pectate lyases. They cleave the HG backbone thus affecting the rigidity of the cell wall (Micheli 2001). However, their main target may not be the tube itself but the substrate through which it has to grow. PGs are detected in the tip region of pollen tubes during papillar cell penetration (Dearnaley and Daggard 2001) suggesting that PGs are probably involved in the loosening of the stigma and transmitting tract cell walls to facilitate the penetration of the pollen tube during pollination.

The stiffening of the cylindrical portion of the pollen tube cell wall is not only accomplished by pectin cross-linking but also by the addition of polymers such as cellulose and callose. Callose is a β-glucan with β-1,3-linkages and is produced, for example, to temporarily block the flow in the phloem during dormancy (Sager and Lee 2014) or as a defense mechanism against invading pathogens (Lee and Lu 2011; Voigt 2014). It is abundant in the pollen tube cell wall but absent from the growing tip (Parre and Geitmann 2005; Derksen et al. 2011; Chebli et al. 2012). Callose clearly has a stabilizing function since treatments with lyticase, a callose-digesting enzyme, cause an increase in the cellular diameter, a reduction in cellular stiffness, and an increase in the cellular viscoelasticity (Parre and Geitmann 2005; Aouar et al. 2010). Callose is also employed to plug the active part of the pollen tube cytoplasm from the distal region, thus enabling the cell to maintain a relative constant cytoplasmic volume despite its continuous expansion (Geitmann and Steer 2006; Abercrombie et al. 2011).

Callose is synthesized at the plasma membrane by callose synthases (CalSs) (Ferguson et al. 1998) and activated in vitro by proteolysis and detergents (Brownfield et al. 2007). Immunogold labeling indicated that CalSs localize in the ER, Golgi bodies, and vesicles located adjacent to the plasma membrane in the distal pollen tube region. CalSs are incorporated into the plasma membrane in the shank and presumably sites of future callose plugs (Ferguson et al. 1998; Brownfield et al.

2008). CalS in *Nicotiana alata* pollen tubes consists of a 220 kDa polypeptide, which is likely to be delivered to the plasma membrane by exocytosis of Golgi membranes (Brownfield et al. 2008). The delivery and accumulation of CalS in the distal region depends on actin, while microtubules seem to be involved in the distribution and maintenance of distal CalS (Cai et al. 2011). Thus, CalSs are involved in cell wall modification in the pollen tube tip and formation of callose plugs in the very distal part of the pollen tube (Cai et al. 2011).

Cellulose, a β-glucan with β-1,4-linkages, is a major component in most plant cell walls. It is present in pollen tubes but in relatively low amounts (Schlüpmann et al. 1994; Cai et al. 2011). Despite its low abundance, cellulose plays an important role in stabilizing the pollen tube tip wall especially in the transition zone between the tip and the shank (Geitmann 2010; Derksen et al. 2011) where it contributes to the maturation of the wall and to its ability to resist the tensile stress generated in the cell wall by the internal turgor pressure (Aouar et al. 2010). This is evident from the fact that cellulase treatments as well as pharmacological or mutational inhibition of cellulose crystal formation result in larger pollen tube diameters, tip swelling, and eventually bursting (Anderson et al. 2002; Lazzaro et al. 2003; Aouar et al. 2010; Derksen et al. 2011). Similar to other plant cells, pollen tubes are able to compensate to a certain degree for a failure in the production of one cell wall component by overproducing another. Partial cellulose digestion results in pollen tubes with increased amounts of pectins, illustrating a certain flexibility in the cellular construction process (Aouar et al. 2010). Most pollen tubes do not possess much cellulose in the apical cell wall, but some do (Chebli et al. 2012; Hao et al. 2013). Whether or not there is a causal relationship between the relatively slow growth rate in these species and the presence of cellulose in the apex has not been investigated (Lazzaro et al. 2003).

Cellulose microfibrils are produced by transmembrane cellulose synthases A (CESAs) deposited into the plasma membrane in a form of rosette terminal complex (Doblin et al. 2002). Different CESA families are implicated in cellulose biosynthesis in plants, depending on tissue and cell type (Doblin et al. 2002; Persson et al. 2007; Chebli et al. 2012). In the pollen tube, cellulose synthases are inserted into the plasma membrane in the apical flanks, and deposition of cellulose begins immediately, if not already prior to insertion. In *A. thaliana* pollen tubes, crystalline cellulose was found in the trans-Golgi network and in cytoplasmic vesicles suggesting that the synthesis of cellulose microfibrils could be initiated before the incorporation to the plasma membrane. This could potentially enable the pollen tubes to optimize cell wall assembly to promote rapid elongation (Chebli et al. 2012). Alternatively, the presence of crystalline cellulose in cytoplasmic vesicles could be indicative of a progressive removal of this polymer from the shank of the cell by endocytosis and a relocalization to the apex (Chebli et al. 2012). Both explanations remain speculative.

7.3 Generation of a Biochemical Gradient in the Pollen Tube Cell Wall by Targeted Intracellular Transport

From the description of the growth mechanism, it becomes obvious that the genesis of a perfectly cylindrical cell with a uniform diameter requires the insertion and removal of polysaccharide building blocks, synthesizing and modifying enzymes, as well as their inhibitors at precisely defined locations at the plasma membrane. This is illustrated even better with a pollen tube that changes its growth direction in order to steer toward or away from an attractant or repulsive agent, respectively. Such a reorientation requires the spatial redefinition of the subcellular region that yields to the turgor pressure and grows. By consequence, the delivery of new cell wall building material and regulatory agents has to be targeted toward newly defined surface areas (Bou Daher and Geitmann 2011). Other substances that need to be synthesized and delivered to the apex include digestive enzymes and signaling molecules that enable the pollen tube to dissolve the apoplast of the pistillar tissue and to communicate with the female partner. All of these delivery processes occur through intracellular trafficking. Exocytosis and endocytosis of cell wall components and their regulated enzymes both contribute to maintaining membrane and cell wall domains over time in the elongating pollen tubes (Onelli and Moscatelli 2013). When this traffic is altered experimentally, the spatial distribution in the mechanical properties of the cell is affected, and as a consequence, cell shape changes from the cylindrical default, either by swelling or tapering of the tube or even by rupture of the wall. Such interference can, for example, result from manipulation of the transporting machinery, the cytoskeleton (Ischebeck et al. 2011; Abenza et al. 2015). Despite the need for a precisely tuned mechanical gradient (Fayant et al. 2010), there is some plasticity built into the system, as is evident when moderately interfering with cell wall assembly (Chebli et al. 2013a, b). Understanding how material is transported and used for morphogenesis is therefore an important endeavor to understand how plant cells are regulated in general.

Depending on the species, endocytosis occurs either in a single apical domain (Guan et al. 2013) or two membrane domains—the extreme apex and the shank region adjacent to the apex (Bove et al. 2008; Zonia and Munnik 2008; Moscatelli and Idilli 2009; Zonia 2010; Onelli and Moscatelli 2013). These two domains seem to be separated by a ring-shaped domain in the shoulder of the apex in which exocytosis dominates (Geitmann and Dumais 2009). It is thought that vesicles endocytosed at the tip region mix with vesicles that are delivered to the apex but fail to exocytose in the inverted cone in which vesicle motion appears to be governed by diffusion (Kroeger et al. 2009) and possibly convection. Secretory vesicles and all other organelles in the pollen tube are moved actively by motor proteins in the shank of the cell. The resulting drag forces move the surrounding cytosol and cause it to move resulting in a fluid flow that transports dissolved substances over significant distances in the “go with the flow” manner (Lord and Russell 2002). The overall cytoplasmic streaming, or movement of cytosol and organelles, shows different streaming patterns in angiosperm and gymnosperm pollen tubes. In angiosperms,

organelles move forward on cortical arrays and backward on a centrally located array resulting in a reverse fountain-like streaming pattern in the tip region (Bove et al. 2008; Geitmann and Nebenführ 2015). In gymnosperm pollen tubes, the direction of movement is reverse, where a fountain-like streaming pattern is governed by forward movement along the central axis and backward movement in the periphery of the cell (Lazzaro 1996). The opposite streaming pattern in angiosperm and gymnosperm pollen tubes was suggested to be generated by an opposite polarity of the actin filaments (Lenartowska and Michalska 2008; Kroeger et al. 2009). Albeit in differently shaped aggregates, both directions of fountain streaming are associated with an accumulation of vesicles in the apical region (Parton et al. 2001; Bove et al. 2008). The bigger organelles such as mitochondria and Golgi compartments generally do not reach the apical cytoplasm but turn around in the subapical region of the cell.

Whether they are delivered through the periphery or the central region of the cytoplasm, the Golgi-derived secretory vesicles containing cell wall components (Toyooka et al. 2009; Kang et al. 2011) have to reach the apical plasma membrane and deliver their cargo (Young et al. 2008; Chebli et al. 2013b; Gendre et al. 2013). It is interesting that the highest rate of cell wall assembly and expansion does not necessarily occur at the extreme apex of the tip but in some species at least at an annular region surrounding the apex (Geitmann and Dumais 2009; Zonia and Munnik 2009). This mechanism could serve to provide a greater resilience against factors that might disturb the steering mechanism. These exocytosis patterns point at the necessity to understand how exactly vesicles are delivered to their target region. Monitoring individual secretory vesicles in living cells is challenging since their size is typically between 60 and 150 nm—below the diffraction limit of the optical microscope. Direct observation and quantitative tracking are difficult, even by TIRF microscopy, since in the pollen tube these vesicles move rapidly and are very densely packed, and even if the pollen tube lies directly on a coverslip, the key processes are located several micrometers away from the glass surface (due to the curvature of the pollen tube apex). To circumvent these challenges, vesicle dynamics in growing pollen tubes have been analyzed quantitatively using fluorescence recovery after photobleaching (FRAP) and spatiotemporal image correlation spectroscopy (STICS) (Bove et al. 2008; Zonia and Munnik 2008). These data have informed theoretical modeling approaches that have been employed to make predictions on the functioning of the transport mechanism (Kroeger et al. 2009). The modeling has been particularly useful to understand how vesicle motion might be driven in cellular regions devoid of cytoskeletal arrays. The simulations have shown that the motor-driven movement of vesicles in the shank of the tube and the flanks of the apex is sufficient to explain the fountain and inverse fountain-shaped motion patterns through the apex even if only diffusion is assumed in apical cytoskeleton-free regions (Kroeger et al. 2009). Another conclusion from the simulations is that the vesicle motion patterns are very stable even when the growth rate changes. This is consistent with microscopic observations made in various species.

7.4 Cytoskeletal Control of Intracellular Trafficking

Motor-driven transport of organelles and vesicles is mediated by a cytoskeletal array that consists of microtubules and actin filaments oriented predominantly parallel to the long axis of the cylindrical cell (Geitmann and Emons 2000; Idilli et al. 2013). Generally, as is typical for plant cells, microtubules play a less prominent role in organelle transport processes (Gossot and Geitmann 2007; Cheung et al. 2008). They are involved in the movement of the male germ unit (Miyake et al. 1995), but drugs such as oryzalin and colchicine do not interfere with cytoplasmic streaming. However, recently nocodazole, another inhibitor of tubulin polymerization, has been demonstrated to impede plasma membrane internalization of the vesicles in the apex of tobacco pollen tubes (Idilli et al. 2013), but more detailed analyses are wanted. No doubt exists that actin plays a crucial role for transport processes in pollen tubes, including the long-distance transport of secretory vesicles (Zhang et al. 2010a, Moscatelli et al. 2012). Actin polymerization and depolymerization therefore need to be tightly regulated to ensure accurate delivery of vesicles to the tip for exocytosis (Cheung et al. 2008; Kroeger et al. 2009). This regulation is performed by numerous actin-binding proteins covered in excellent reviews (Ren and Xiang 2007; Chen et al. 2009; Staiger et al. 2010).

Vesicle trafficking does not only consist of long-distance transport but also comprises processes such as budding, sorting, navigating, and fusing. These processes involve the molecular machineries necessary for specific interactions such as contact of a vesicle with the plasma membrane. The effect of failure or deficiencies in any of these processes are illustrated when analyzing pollen tubes of mutants affected at the various steps in the vesicular trafficking pathway including cytoskeletal functioning (Zhu et al. 2013), endocytosis (Kitakura et al. 2011), or secretion (Cole et al. 2005; Silva et al. 2010; Richter et al. 2012; Doyle et al. 2015). In most of the cases, this affects the assembly of the pollen tube cell wall and as a consequence results in altered morphogenesis of the cell.

For both polar and diffuse plant cell growth, vesicle trafficking involves protein complexes that catalyze vesicle formation (clathrin), transport (RABs/cytoskeleton), tethering (exocysts), and docking and fusion (RABs/SNARE) (Sanderfoot and Raikhel 1999). Vesicle trafficking and fusion in the pollen tube are known to be regulated by small Arf or Rab GTPases (Saito and Ueda 2009; Szumlanski and Nielsen 2009; Kato et al. 2010). RabA4d localizes in the tip-localized membrane compartments in growing pollen tubes, and its function is important for polarized membrane trafficking during tip growth. Mutations *raba4d* and *rab11b* inhibit targeting of exocytotic and recycled vesicles to the pollen tube inverted cone region and compromise the delivery of secretory vesicles; this results in defects in the cell wall composition as well as pollen tube guidance, suggesting that Rab functions in selective delivery of cargo to the pollen tube tip (de Graaf et al. 2005; Szumlanski and Nielsen 2009).

The plant-specific Rho family of GTPases (ROPs) strictly localizes at the tip of the pollen tube thus providing a spatial signal function at the intersection of polarity

and vesicle trafficking (Lee and Yang 2008). ROP GTPases control polar growth by regulating the actin cytoskeleton and Ca^{2+} signaling in pollen tubes and root hairs which in turn regulate vesicle transport and fusion (Molendijk et al. 2001; Chen et al. 2003; Lee and Yang 2008; Jamin and Yang 2011). ROP1 also regulates the temporal control of exocytosis as its concentration varies during oscillatory growth (Hwang et al. 2005; Lee et al. 2008; Lee and Yang 2008). Brefeldin A, a drug inhibiting the secretory pathway, reduces pollen germination and eliminates growth and polar plasma membrane localization of ROPs and ROP-interacting partners (RIPs) (Molendijk et al. 2001) by disrupting the secretory vesicles at the tip. All this leads to significantly decreased content of pectin in the apical region (Wang et al. 2005; Richter et al. 2012). Other polarity markers are RIC3 and RIC4. The spatially regulated presence and activity of signaling agents such as ROP1, RIC3, and RIC4 are believed to be involved in such spatial regulation of the vesicular secretion in the pollen tube through regulation of actin dynamics (Gu et al. 2005; Lee et al. 2008). An increase in Ca^{2+} concentration suppresses ROP1 function and thus balances the actin-RIC4 ROP1 apical activation (Yan et al. 2009), but the ROP1-Ca^{2+}-RIC3 interaction is not clearly characterized.

Tethering and fusion of the vesicles is mediated by the exocyst complex (Hala et al. 2008) and soluble NSF attachment protein receptors (SNAREs) (Sanderfoot and Raikhel 1999). When the vesicle reaches its destination, vesicle (v)-SNAREs interact with target (t)-SNAREs to mediate vesicle fusion (Saito and Ueda 2009). Chemical inhibition of V-ATPase activity at the trans-Golgi network (TGN) by concanamycin A leads to secretory defects and related growth phenotypes (Dettmer et al. 2005, 2006; Brux et al. 2008; Viotti et al. 2010; Guo and McCubbin 2012) suggesting that the vesicular trafficking from TGN plays a central role in polysaccharide secretion to the tip. Also *Arabidopsis* SYP124 and SYP125, pollen-specific SNARE subfamilies of syntaxin-like proteins required for docking and fusion of secretory vesicles, participate in vesicle trafficking between the vacuole and the TGN, mediating exocytotic membrane fusion at the pollen tube apex (Enami et al. 2009; Kato et al. 2010; Silva et al. 2010). Secretory vesicles are exocytosed involving the exocyst complex (a multiprotein tethering factor) important for secreting vesicles to get delivered to specific exocytosis sites (Cole et al. 2005) suggesting that the exocyst is one of the major determinants of pollen tube growth and, hence, of cell morphology (Cole et al. 2005; Hala et al. 2008).

The second messenger Ca^{2+} is believed to be one of the central intracellular modulators of vesicular trafficking and subsequently regulating tip growth in pollen tubes (Qin and Yang 2011; Steinhorst and Kudla 2013). Both the cytoplasmic Ca^{2+} concentration and also the pH change significantly over short distances within the apical and subapical region of the pollen tube cytoplasm (Chebli and Geitmann 2007; Qin and Yang 2011). The concentration of cytosolic Ca^{2+} is high at the growing pollen tube tip and decreases rapidly in the subapical region. The magnitude of this gradient oscillates with the same periodicity as pollen tube growth (Pierson et al. 1996; Holdaway-Clarke et al. 1997; Messerli et al. 2000). Previous observations suggest that the Ca^{2+} gradient is essential for polarity of ROP1 and that it regulates its tip growth polarity (Hwang et al. 2005, 2008). Tip-localized

ROP1 GTPase effectors RIC3 and RIC4 are proposed to promote Ca^{2+} influx through the plasma membrane and thus to establish the Ca^{2+} gradient (Gu et al. 2005) and regulate the actin dynamics at the tip by way of actin-binding protein activity, which in turn might promote exocytosis (Gu et al. 2005; Lee et al. 2008; Bou Daher and Geitmann 2011). Most importantly, when intracellular trafficking is modulated by experimentally blocking the release of intracellular Ca^{2+}, cell wall structure is altered significantly as callose accumulates at the tip and the tip-localized methyl-esterified HG is de-esterified (Chen et al. 2008). The roles of Ca^{2+} in the regulation of pollen tube growth are therefore multiple—through its regulation on actin dynamics and hence secretion and through its action on the mechanical properties of pectin.

Pollen tube assembly requires not only exocytotic but also endocytotic activities. The latter are necessary for the controlled removal of agents (e.g., PMEI) but also to recover excess membrane material. Because of their different geometry, the cellular envelope—cell wall and plasma membrane—and the delivery vesicles have a different ratio of the two materials (polysaccharides/phospholipids). As a consequence, the relative amount of membrane material delivered to the outside is too high and has to be endocytosed (Bove et al. 2008). Therefore, a tight correlation between endocytosis and exocytosis during pollen tube growth is necessary, both in time and in space (Moscatelli and Idilli 2009). The immediate reinternalization of membrane material could potentially also be accomplished by “kiss-and-run” endocytosis (Bove et al. 2008) similar to the endocytosis described in synapses as a clathrin-independent internalization process (Alabi and Tsien 2013), but evidence for this process in plant cells is still lacking.

7.5 Invasive Growth

The regulation of pollen tube growth dynamics through intracellular trafficking becomes a particularly intriguing question if one considers that pollen tubes do not usually grow in the uniform and artificial growth medium provided in a Petri dish but within the complex, maze-like structure existing in the pistil (Lennon et al. 1998; Lord and Russell 2002; Chapman and Goring 2010). This complex environment requires the tube to have the abilities (1) to follow guidance cues by reorienting its growth direction, (2) to navigate or penetrate mechanical obstacles, and, in some species, (3) to adhere to the pistillar tissue. All three mechanisms can be linked to intracellular trafficking. The adhesion mechanism is thought to enable the pollen tubes to attach to the transmitting tissue lining the hollow style in certain species (Zinkl et al. 1999; Park et al. 2000). Arabinogalactan proteins (Cheung and Wu 1999; Nguema-Ona et al. 2012), pectic polysaccharides, and stigma/stylar cysteine-rich adhesin (Lord 2000) have been identified in this context, and all clearly require a secretion mechanism to reach the cellular surface.

Pollen tube reorientation and the calibration of the invasive force exerted by the pollen tube are linked to intracellular trafficking since cell wall mechanics is regulated through controlled secretion either in space (reorientation) or time (invasion). To invade the pistillar tissue in solid styles, an advancing pollen tube has to exert an invasive force that is higher than the resistance posed by the apoplast of the invaded tissue. The invasive behavior of the pollen tube has been studied using the TipChip, a microfluidic platform (Agudelo et al. 2013). This lab-on-a-chip device allows the researcher to expose individual pollen tubes to precisely calibrated mechanical obstacles with simultaneous high-resolution and fluorescence imaging. When pollen tubes were presented with slit-shaped openings, they had to exert a force that could be measured quantitatively (Sanati Nezhad et al. 2013a). Intriguingly, pollen tubes maintained a constant growth speed despite increasing mechanical resistance. The growth speed is likely modulated by all acting forces, the internal turgor pressure and in the opposite direction, the cell wall and the external mechanical obstacle. The stiffer the cell wall, the smaller the available force acting against the obstacle. The fact that growth speed remains constant despite increasing external resistance of the obstacle suggests, therefore, that, when required, the growing tip can soften its wall so that the pressure acts directly against the obstacle instead of being dissipated in the wall (Sanati Nezhad et al. 2013a).

The softening of the apical cell wall can also occur in repeated manner resulting in an oscillatory growth pattern that has been hypothesized to be employed as a sledge hammer (Geitmann 1999). Oscillatory pollen tube growth is generated by periodic changes in the extensibility of the apical cell wall (McKenna et al. 2009; Zerzour et al. 2009; Winship et al. 2010; Kroeger and Geitmann 2013). This in turn is regulated by periodically changing rates of exocytosis which are subject to control by a feedback mechanism that is hypothesized to involve mechanosensors located in the apical plasma membrane (Kroeger et al. 2008).

A change in growth orientation required to either follow a chemical guidance cue (Sprunck et al. 2012) or circumvent an obstacle (Gossot and Geitmann 2007) has been shown to be preceded by a spatial reorientation of the secretory machinery in the pollen tube (Bou Daher and Geitmann 2011). Both the fluorescent signals for the apical vesicle cone and the dynamics of the actin cytoskeleton show asymmetric distribution prior to a visible change in pollen tube shape symmetry hence suggesting a causal relationship between these parameters. This behavior is consistent with that of other walled cell types such as fission yeast (Abenza et al. 2015). The turning response can also be triggered by directly modulating the cell wall mechanical properties. Local application of PME asymmetrically stiffens the apical cell wall and thus causes the tube to turn away from the agent, to the side where the wall remains soft (Sanati Nezhad et al. 2014).

7.6 Conclusion and Perspective

Understanding the intracellular underpinnings of the invasive and directional growth of pollen tubes requires analyzing how the massive amount of cell wall deposition and assembly processes are coordinated to promote fast cell elongation. Tight control of cell wall remodeling during the morphogenetic process ensures that the mechanical properties of the cell wall are regulated in space and time. Cell wall assembly and maturation are directly influenced by spatial control of vesicular delivery of cell wall components and related enzymes. The functioning of the pollen tube including its growth, its targeting, and its force generation is therefore all connected through these intracellular membrane transport processes.

The research on pollen tubes is highly interdisciplinary because it combines cell biology with quantitative and mechanistic modeling and draws upon novel technological developments such as microfluidics and high-resolution live-cell imaging to address questions of global cell biological relevance. The challenge is now to integrate the multitude of different types of molecular, biological, and mechanical data to conceive a meaningful picture of pollen tube biological functioning.

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