Mechanics Research Communications 42 (2012) 32–39

Contents lists available at SciVerse ScienceDirect

Mechanics Research Communications

journal homepage: www.elsevier.com/locate/mechrescom

Pollen tube growth: Getting a grip on cell biology through modeling

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ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form
10 November 2011
Available online 22 November 2011

Keywords:
Feedback mechanism
Intracellular transport
Oscillation
Modeling
Pollen tube growth

ABSTRACT

Cellular growth in plant, fungal and bacterial cells is based on the mechanical deformation of the cellular envelope by the hydrostatic turgor pressure. Shape generation is therefore a mechanical problem whose biological control is poorly understood. The pollen tube is an attractive model system for the investigation of the growth process in walled cells. The geometry, mechanics and kinetics of the growth process represent intriguing features that are well investigated experimentally. In particular, the presence of regular pulsations in the growth rate, an indicator of non-linear feedback regulation, has attracted the attention of modelers from the engineering, mathematical and physical communities. Here, we summarize important hallmarks characterizing pollen tube growth, and we illustrate how modeling and mathematical analysis have become an integral part of the research programs targeting this cell type.

1. Introduction

The development of a multicellular organism from embryo to adult relies on three essential processes: cell division, cellular differentiation and cell growth. The latter is fundamentally a mechanical problem since cellular volume is increased and consequently the outer envelope of the cell needs to be pushed outwards both to increase its surface and/or to change its shape and geometry. In most mammalian cells the only resistance to this process is that of the plasma membrane surrounding the cell body, an extremely pliable material made of a phospholipid bilayer that is easily pushed and shaped into the desired form. In mammalian cells, the force necessary for deforming the surrounding membrane is exerted by actors such as polymerizing actin filaments, i.e. rod-shaped proteins forming a dense meshwork that expands and contracts. In cells surrounded by a polymeric wall such as those of plants, fungi and bacteria, on the other hand, the cell shaping process requires considerable internal forces to deform the wall with the purpose to increase its surface or change its shape. The nature of the wall material varies between kingdoms, but it generally consists of long chain polymers. The deforming forces are generated by the turgor, the internal hydrostatic pressure that is generated by a difference in osmotic water potential between the outside and inside of the cell. Given that pressure is a scalar, the eventual shape of the cell is regulated by the material properties of the wall and/or cytoskeletal elements (Geitmann and Ortega, 2009; Jiang et al., 2011; Money, 1997; Steinberg, 2007).

Similar to mammalian cells, the inner portion of plant and fungal cells, the protoplast, is lined by the plasma membrane that separates the polymer wall from the cytoplasm. The cytoplasm is an aqueous solution densely filled with proteins and bigger cellular components such as the nucleus (that contains the DNA), the vacuole (a large liquid-filled, membrane-bound compartment) and other organelles (smaller, membrane-bound compartments with different metabolic functions). Cellular growth requires the addition of new membrane and cell wall material to the cellular envelope. Both are produced in certain types of organelles and transported to the cell surface by transport vesicles, very small, membrane-bound spheres. The addition of new cell wall material can either soften or harden the existing wall, depending on its biochemical nature. The expansion of the cell wall is promoted by addition of soft material that allows the turgor pressure to stretch the wall and thus increase its surface. This expansion can occur over the entire cellular surface, but it can also be spatially confined by targeting the delivery of new cell wall to selected surface areas. Such a local softening causes the formation of a polar protuberance. Delivery of cell wall material thus enables the cell to modulate the mechanical properties and the geometry of the wall and hereby to control growth activities in time and space. Many of the biological agents that are involved in regulating the cellular growth process have been identified, but how they act on the cellular mechanics is largely unknown. Mechanical modeling has therefore proven very useful to direct the attempts of biologist to identify the mechanical principles governing cell morphogenesis in plants, fungi and bacteria,

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doi:10.1016/j.mechrescom.2011.11.005

and to understand the link between biochemistry and morphology.

Walled cells come in a wide variety of shapes ranging from simple spheres and rods to complex star-shaped structures. In the quest to advance the understanding of cellular growth in walled cells, numerous studies have focused on those cellular systems that present a simple geometry thus reducing the degree of complexity. Tip growing cells such as fungal hyphae, root hairs and pollen tubes grow essentially unidirectionally since they form finger-like protrusions that are generated by a deformative expansion of the cell wall that is spatially confined to the very end of the cell. In a stable environment, the shape of the advancing tip is typically self-similar in time (Goriely and Tabor, 2003b), but this pattern is easily disturbed either by changing environmental conditions or through the effect of an inhibitor or mutation-induced alteration in the cellular behavior (Geitmann et al., 1996; Klahre et al., 2006; Kost, 2008; Kost et al., 1999; Zerzour et al., 2009).

One of the fastest growing walled cells is the pollen tube, a widely used experimental model system. Fast growth combined with the relative ease with which pollen tubes can be cultured *in vitro* has drawn the attention of many experimental and theoretical biologists. The pollen tube is formed by the pollen grain, the male gametophyte generated in the anthers of flowering plants. The pollen grain has the purpose of transporting the male gametes from one flower to another. Each pollen grain carries two sperm cells, or their precursor, the generative cell. Upon contact with a receptive pistil, the vegetative cell forming the bulk of the pollen grain forms a tubular protuberance that elongates into the pistil towards the ovary to eventually reach an ovule. There it explodes to release the two sperm cells which then fertilize the egg and central cell, respectively, in a process termed double fertilization. Speed of protuberance elongation is a direct selection factor for the reproductive success of a pollen grain. This explains why the growth rates that pollen tubes have developed during evolution are among the fastest for any cell in the plant kingdom with up to 250 μm/min.

Similarly to the other tip growing cells, the pollen tube displays a remarkably clear and distinctive spatio-temporal growth pattern: it grows unidirectionally with a radially symmetric shape. Remarkably, its growth dynamics are typically characterized by an oscillatory change of the growth rate that was found to be accompanied by oscillations of numerous cellular features such as cytoskeletal dynamics and cell wall thickness (Chebli and Geitmann, 2007; McKenna et al., 2009). Particular emphasis has been placed on the transmembrane fluxes of ions which display both distinct spatial patterns and temporal variations (Feijó et al., 2001; Holdaway-Clarke and Hepler, 2003). Nearly four decades after the initial discovery of the oscillations in the ion fluxes entering pollen tubes (Weisenseel et al., 1975) and two decades after the first detailed description of the oscillatory growth behavior of the pollen tube (Pierson et al., 1995; Plyushch et al., 1995; Tang et al., 1992), it is appropriate to review the work devoted to the theoretical description of the spatio-temporal dynamics of pollen tube growth, and to assess how theoretical efforts have supported and inspired experiments. Although most of the work reviewed here pertains to pollen tubes, we will occasionally include work referring to root hairs and fungal hyphae since the general principles governing the growth process in these cells are similar.

2. The geometry of tube growth

The pollen tube has a cylindrical shape with a diameter that varies between species but is very constant within the pollen population of a given species. Typical dimensions are between 5 μm (e.g. *Arabidopsis*) and 20 μm (e.g. *Camellia*). The tube is capped by an approximately hemisphere shaped dome, the apex, to which all

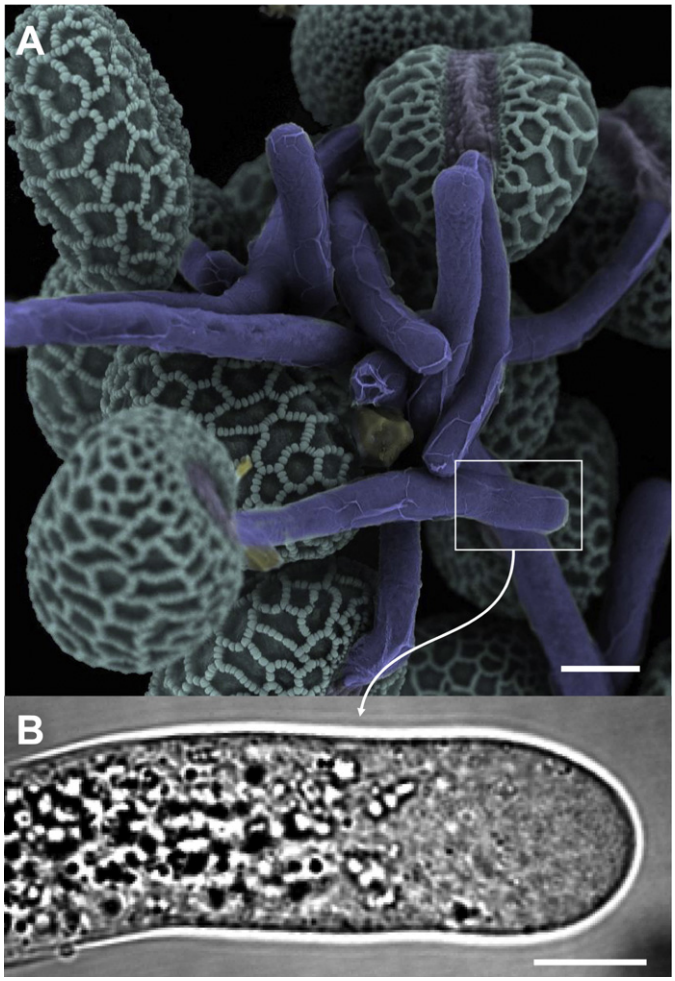

Fig. 1. Pollen tube morphology. (A) False colored scanning electron micrograph of lily pollen grains germinated *in vitro*. The spherical, ornamented objects are the pollen grains, the cylindrical protuberances are the pollen tubes, or cellular protuberances emerging from the grains. Bar = 30 μm. (B) Brightfield micrograph of the apical region of a lily pollen tube. Most of the tube is filled with bigger organelles, but the outermost end of the tube, the growing apex, is filled mostly with delivery vesicles, spherical bodies below the diffraction limit of the optical microscope. Bar = 10 μm.

growth activity is confined (Figs. 1 and 2). To sustain the growth process over the attainable lengths, up to 30 cm, a balance must be achieved between the mechanical deformation of the existing viscoplastic cell wall and the continuous addition of new cell wall material. The pollen tube cell wall consists primarily of pectin, a structural heteropolysaccharide, that is synthesized in the Golgi bodies, micron-sized organelles that move within the cytoplasm along the cytoskeletal network. The material synthesized in these organelles is then delivered to the apical region by transport vesicles with a diameter of 100–150 nm. These vesicles empty their contents towards the outside through exocytosis, a process during which the vesicle membrane fuses with the plasma membrane. The delivered polysaccharidic material is used to assemble the expanding cell wall. In the mature region of the tube, the wall is further reinforced by the deposition of cellulose microfibrils and callose, produced by membrane-associated enzymes (Geitmann and Steer, 2006).

The spatial confinement of the growth activity to the apex is reflected in a polar zonation of the cytoplasm. The apical or tip region is termed the “clear zone” since it is almost exclusively filled with vesicles. Since bigger organelles are excluded from this region and since the size of the vesicles is below the resolution capacity of

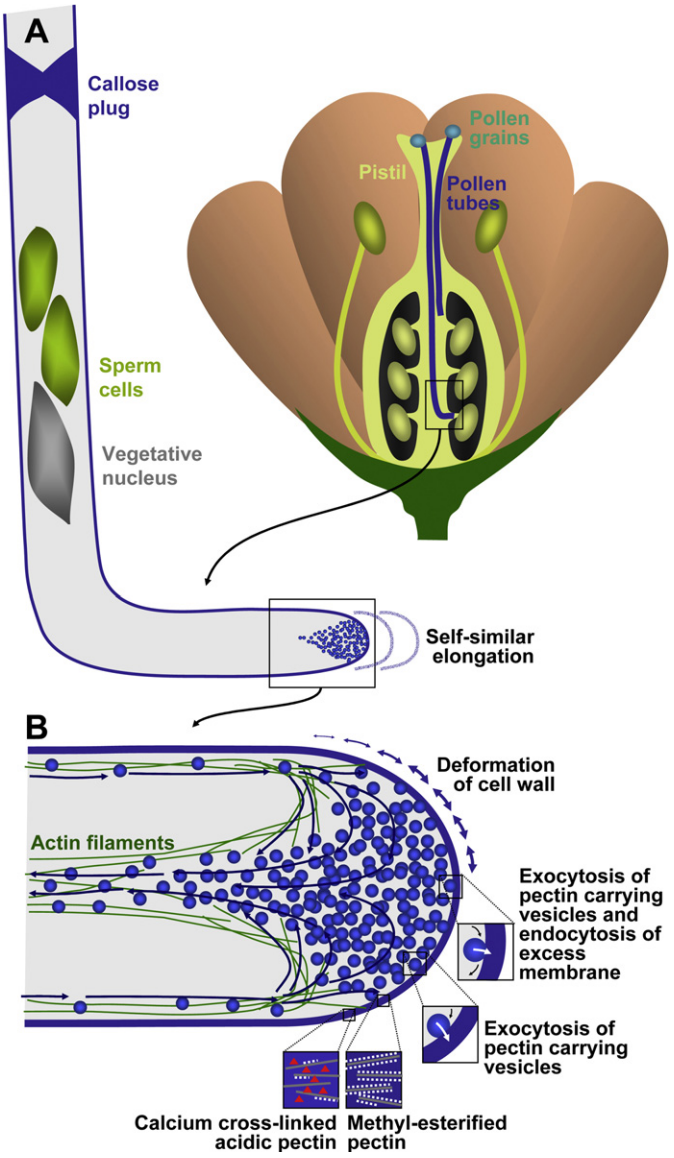

Fig. 2. Pollen tube cytoarchitecture. (A) The pollen tube is formed as a cellular protuberance from a pollen grain upon contact with the pistillar tissue of a receptive flower. The elongation of this tube is guided towards an ovule where it delivers the sperm cells through bursting. (B) Sketch of the apical region of a pollen tube. Cell wall expansion occurs only here (blue double arrows) and vesicles are targeted to this region to deliver cell wall material through fusion with the plasma membrane (dark-blue arrows). Vesicle transport is mediated by the actin cytoskeleton (green), rod shaped proteins that serve as transport rails. The actin cytoskeleton is responsible for actively moving vesicles in the tubular region of the cell and in the apical actin fringe, but in the apical cytoplasm vesicles move solely by diffusion and convection. Secretion events take place at the pole of the cell and in an annular region around it. Simultaneously, endocytotic events remove excess membrane material from the plasma membrane. Endocytosis is thought to occur at the pole and in the shank (not shown). The pectic wall material is delivered in methyl-esterified form and becomes demethyl-esterified upon maturation in the shoulder region of the apex. This biochemical change stiffens the wall and is hence responsible for the generation of a tubular shape. For clarity, organelles other than vesicles are not shown. Objects are not drawn to scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the optical microscope, this zone appears smooth under brightfield illumination (Fig. 1B). Labeling the vesicles with lipophilic styryl dyes has revealed that the space they occupy in the apical region has the shape of an inverted cone filling the extreme apex and pointing towards the rear of the cell (Bove et al., 2008; Malhó et al., 2005; Parton et al., 2001) (Fig. 2).

The cylindrical region adjacent to the clear zone and ending 3–5 tube radii behind is densely packed with various types of bigger organelles such as mitochondria, plastids, Golgi bodies, and endoplasmic reticulum. Further distal from this region the male germ unit comprising the vegetative nucleus and the two sperm cells occupies a large portion of the pollen tube interior over a length of tens of micrometers. Behind this zone vacuoles typically appear. The oldest region of the tube degenerates and loses turgor pressure after its separation from the advancing apical cytoplasm by thickenings of the wall, the callose plugs (Fig. 2A).

In the vital part of the tube, vesicles constantly move, mostly in paths that are parallel to the long axis of the cell. This motion is driven by an energy consuming transport mechanism that is based on the motor protein myosin pulling organelles along actin filaments. The actin arrays responsible for this are oriented parallel to the long axis with filaments in the periphery of the tube pointing towards the apex with their barbed ends and filaments in the center pointing backwards (Geitmann and Emons, 2000; Lenartowska and Michalska, 2008). The resulting organelle motion forms an inverse fountain pattern with forward movement occurring in the periphery of the tube and rearward flow in the center of the tube (de Win et al., 1999; Yokota and Shimmen, 1994) (Fig. 2B). Bigger organelles that can easily be observed in the optical microscope reverse their movement direction at a certain distance from the growing tip. Vesicles on the other hand enter the apical zone where many of them deliver their contents to the expanding wall, whereas others turn around to flow rearward (Bove et al., 2008; Geitmann and Dumais, 2009).

The cone shaped apical vesicle region, characteristic of angiosperm pollen tubes, is largely free of prominent filamentous actin cables (Lovy-Wheeler et al., 2005). The vesicles are transported to this apical cone by an actin array consisting of a fine mesh of actin filaments dominating the subapical cytoplasm, the actin fringe. This fringe is always in close proximity to the continuously advancing apex of the cell, but unless perturbed, the actin array does not invade the very tip of the tube. The high cytoplasmic calcium concentration found at the tube tip is likely to prevent filamentous actin (F-actin) polymerization in this region. Unlike the rapid organelle movement in the shank of the cell, vesicle motion in the apical cytoplasm is more erratic and random. Spatio-temporal image correlation spectroscopy revealed that in the apex the average velocities of the vesicles vary over small distances (Bove et al., 2008). Both the absence of prominent actin cables in the tip and the erratic character of vesicle dynamics suggest that vesicle movement in this region is not propelled or guided by an actin-myosin mechanism, but controlled by diffusion and possibly convection. Theoretical modeling has demonstrated that the observed motion patterns can be explained by the varying orientation in which vesicles are release into the clear zone by the actin microfilaments forming the fringe (Kroeger et al., 2009). This model assumes that the longitudinal actin filaments in the center and in the periphery of the tube have opposite polarity, whereas those filaments forming the fringe gradually change their orientation along the line perpendicular to the long axis (Fig. 2B). The spatial differences in actin filament orientation also explain the dynamics of the fringe itself which moves forward together with the expanding tip through continuous polymerization of the individual filaments. Since this polymerization activity is critical in determining the shape of the actin-free clear zone, this model offers an explanation for the observed differences between pollen tubes from species belonging to the angiosperms (flowering plants) and gymnosperms (conifers) regarding the shapes of their clear zones (Fig. 3). The model correctly predicts that in gymnosperm pollen tubes, which show a fountain-shaped rather than an inverse fountain-shaped vesicle movement, the vesicle accumulation zone is crescent shaped (Fig. 3B). The differences between angiosperm

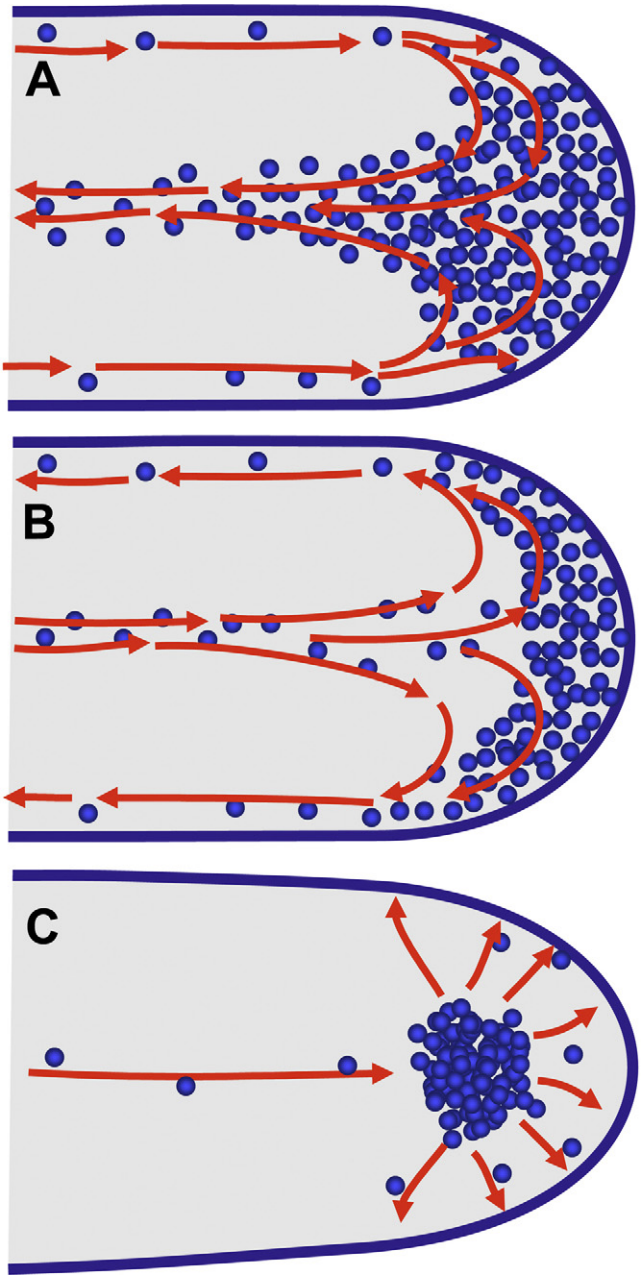

Fig. 3. Aggregation of vesicles in different types of tip growing cells. (A) Typical angiosperm pollen tube with inverse fountain-like streaming pattern and cone-shaped vesicle aggregation. (B) Typical gymnosperm (conifer) pollen tube with fountain-like streaming pattern and crescent-shaped vesicle aggregation. (C) Typical fungal hypha. Vesicles are organized in the Spitzenkörper, an apical structure composed of vesicles and cytoskeletal elements. From here vesicles are thought to be sent to the cellular surface. The shape of fungal hyphae is typically more tapered than that of pollen tubes. The vesicle motion patterns in (A) and (B) have been modeled by Kroeger et al. (2009). (C) has been modeled by Bartnicki-Garcia et al. (1989), Gierz and Bartnicki-Garcia (2001) and Tindemans et al. (2006).

and gymnosperm pollen tubes seem to suggest that the motion patterns of vesicles in tip-growing cells is not critical for shape generation of the expanding wall as long as the vesicles are delivered to the tip region. This is supported by a comparison between pollen tubes and fungal hyphae. Rather than being driven by a bi-directional flow, vesicles in fungal hyphae typically diffuse from a sphere-shaped complex termed Spitzenkörper that is located in the apical cytoplasm (Steinberg, 2007). Mathematical modeling of fungal tip-growth with vesicle diffusion occurring radially from a centrally located vesicle-supply center correctly reproduces the characteristic hyphoid fungal tip shape (Bartnicki-Garcia et al., 1989; Gierz and Bartnicki-Garcia, 2001; Tindemans et al., 2006).

Because of the variability in vesicle delivery patterns, the question whether or not the precise vesicle motion and site of exocytosis matter for morphogenesis is interesting. It is well understood that the delivery of vesicles to the apical region is indispensable for tube elongation. This has been demonstrated through the interference with cytoskeletal functioning. Drugs such as latrunculin B and cytochalasin D prevent actin filaments from polymerizing and result in an apical swelling of the tube likely caused by a lack of precise targeting of the vesicles (Camacho and Malhó, 2003; Zerzour et al., 2009). Consistent with this, a change of growth direction that occurs upon exposure to a directional trigger is preceded by a tilting of the vesicle cone and of the actin fringe, apparently resulting in a tilted or asymmetric delivery pattern of these vesicles (Bou Daher and Geitmann, 2011). These studies suggest that the precise location of vesicle delivery is critical for pollen tube growth and shape generation. Experiments based on fluorescence recovery after photobleaching indicate in fact that the main exocytosis activity may occur preferentially in an annulus with a width of a couple of micrometers that is located few micrometers from the pole of the tube (Bove et al., 2008; Geitmann and Dumais, 2009). For root hairs, this annulus coincides with the region of highest cell wall curvature and likely with the region under the highest mechanical stress (Dumais et al., 2004, 2006). However, for pollen tubes, a complete model describing the mechanics of material addition in combination with cell wall expansion has not been developed so far and is urgently needed to advance the understanding of pollen tube growth.

The membrane trafficking events in the pollen tube apex are complex. Although exocytosis is thought to be highest in an annulus around the pole, the pole itself is also likely to display a significant amount of vesicle secretion events. Simultaneously, the pole seems to be the site of endocytosis, the reuptake of membrane material into the cytoplasm by vesicle budding from the plasma membrane (Bove et al., 2008; Zonia, 2010). This removal of membrane material from the plasma membrane is necessary, because exocytosis of small vesicles delivers an excess of membrane as a consequence of the high surface (membrane) to volume (cell wall polymers) ratio of these organelles. While high exocytosis (and endocytosis) in a region of high surface expansion is obviously necessary for sustained growth, the biological mechanism controlling the precise location and kinetics of exocytosis remains obscure. A recent modeling study proposes the implication of two activation and de-activation agents, and replicates the observed pattern of exocytosis activity, thus providing a guide for future experimentation (Chavarría-Krauser and Yejie, 2011). The study presents a detailed description of the vesicle flux in the cytoplasm, membrane flow along the cell wall, and activation and de-activation agents for the vesicle cytosis. With these ingredients, the model reproduces the annular spatial distribution of the exo- and endocytosis activities in a growing pollen tube. While the biological nature of these putative activation and de-activation agents remains to be identified, the question of how vesicle secretion is confined to a specific target area appears critical to the understanding of pollen tube growth. A novel perspective on vesicle-trafficking, based on the mathematical analysis of different proteins involved in the vesicle trafficking machinery, predicts the growth rate of wild-type and knockout type *Arabidopsis* pollen tubes (Kato et al., 2010).

3. The mechanical constraints of pressure driven pollen tube growth

Whereas cells that are bound only by lipid membranes are reshaped by the action of polymerizing or contracting actin

filaments, the expansion of walled cells is driven by the hydrostatic turgor pressure. Using a pressure probe, the turgor was measured to be up to 1 MPa (Benkert et al., 1997) in pollen tubes and up to 1.2 MPa in fungal hyphae (Money, 1990). Turgor driven expansion of walled cells was first proposed in the context of fungal hyphae (Reinhardt, 1892), and early models of tip growth addressed primarily this cell type (Bartnicki-Garcia et al., 1989; da Riva Ricci and Kendrick, 1972; Denet, 1996). More recently, a detailed mathematical analysis coupling geometry, stress and elastic deformation was developed for the steady growth of fungal hyphae (Goriely and Tabor, 2003a,b). Another early model of pressure driven cell growth relates cell wall geometry (radius of curvature), cell wall thickness, pressure and elastic properties through scaling laws (Boudaoud, 2003). These scaling laws are similar to those obtained from thin shell theory and appear to fit experimental data on a diverse set of cells ranging from bacteria to giant algae, cochlear hair, plant root hair, fungi, and yeast cells.

The important contribution of a mechanical model lies in its capacity to identify biological features that are of relevance to the mechanical behavior of the cell. With this in mind, a model for pollen tube growth based on finite element analysis was established (Fayant et al., 2010). This modeling method has the advantage that it can easily be adapted for the simulation of more complex shapes. Several key parameters were tested to identify the spatial distribution of material properties in the cell wall that produce both temporal self-similar expansion and a strain pattern that corresponds to microscopic observations. Comparison with the spatial distribution of known cell wall components revealed that in pollen tubes de-esterification and subsequent gelation of pectin molecules are key processes that determine cellular diameter and shape. De-esterification is mediated by the endogenous enzyme pectin methyl esterase and occurs upon maturation of the cell wall material in the shoulder of the apical dome of the pollen tube (Fig. 2B) (Fayant et al., 2010; Röckel et al., 2008). The spatial distributions of the enzyme, its inhibitor, and of the two different configurations of the pectin polymers correspond very accurately to the predicted mechanical profile and thus suggest an important role for this process in pollen tube morphogenesis. Recently, the impact of the aging process of the cell wall material on steady-state growth has been investigated through modeling (Eggen et al., 2011). The cell wall aging process, whether biochemical or mechanical in nature, must be counterbalanced by addition of material through vesicle secretion. Modeling of the aging process allows, therefore, to narrow-down the requirements for the spatio-temporal control of the vesicle secretion such that it will counteract the aging process.

A stiff cell wall is necessary to prevent the tube from bursting, since the tensile stress exerted on the cell wall, related to the turgor pressure through the wall thickness and curvature, has been calculated to reach up to 45 MPa (Dumais et al., 2004, 2006). The first model for the mechanical deformation of the plant cell wall as a result of the turgor pressure was developed by Lockhart (1965). It was proposed that a viscous cell wall in a growing plant cell would deform plastically if the turgor is greater than a certain yield value. According to the Lockhart equations, the strain rate is proportional to the difference between the tensile stress applied on the cell wall, due to the turgor pressure, and the yield stress. In the absence of a yield value, the Lockhart equation is identical to the Stokes law of viscous flow. If the value of the tensile stress lies below the yield stress, the cell wall deforms elastically and returns to its initial state as soon as the turgor subsides. Although able to describe tip growth in certain conditions, the original formulation of the Lockhart equation did not consider the spatial variation in the cell wall's physical and rheological properties or their change over time as the cell wall stretches and thins. Detailed mathematical relations between the cell wall's hemispherical geometry, its varying rheological properties and its thickness were obtained from thin shell theory (Dumais et al., 2006). All models mentioned above are steady-state models and are uncoupled from growth markers whose dynamics change during growth. They can therefore not address phenomena such as an oscillatory growth rate or changes in growth direction that can be caused in a tip growing cell by exposing it to a directional trigger. This capacity to reorient, for example with the purpose to follow a chemical gradient, is also called tropic growth. Coupling the Lockhart equation to dynamical equations for the hydrostatic pressure (Ortega, 1990) or viscosity (Kroeger et al., 2011) allowed for the description of a range of new phenomena while coupling the Lockhart formalism to spatially varying vesicle secretion suggested that the latter is the main factor contributing to the difference in shape between tip-growing cells such as pollen tubes, root hairs, fungal hyphae and budding yeasts (Campas and Mahadevan, 2009). The Lockhart formalism was also used to describe the growth and buckling of fission yeast cells. It allowed for the extraction of the elastic surface modulus and the effective turgor pressure from experimental data, and was used to predict the onset of buckling of the tubular cell when elongation was blocked mechanically (Minc et al., 2009).

From the experimental perspective, the application of the Lockhart model to pollen tube growth is supported by the observations that decreasing the turgor below a critical level stops pollen tube growth (Benkert et al., 1997; Zerzour et al., 2009) and that modulating the osmolarity induces instantaneous and transient variations in the growth rate (Li et al., 1996). Turgor is therefore generally accepted as the mechanical force that leads to tube elongation, but the average pollen tube growth rate does not appear to be directly proportional to the global turgor pressure (Winship et al., 2010). This, however, is consistent with other plant cell systems (Passioura and Fry, 1992). Despite the observation that externally applied changes in turgor pressure affect the growth behavior, this does not mean that the cell regulates its growth through manipulation of this parameter. Although periodic increases in the turgor pressure have been postulated to precede phases of rapid growth rate (Zonia, 2010; Zonia et al., 2006), both pressure probe and micro-indentation measurements clearly demonstrate that oscillatory growth in pollen tubes is not accompanied by measurable variations in the turgor (Benkert et al., 1997; Zerzour et al., 2009). Because of a lack of correlation between average growth rate and magnitude of turgor, the work by Benkert et al. led to challenges of the Lockhart model. However, a recent theoretical study demonstrates that, due to inherent non-linearities and thresholds, the average growth rate is not expected to be directly proportional to the turgor pressure even if the pollen tube cell wall behaves according to the Lockhart equation (Kroeger et al., 2011). Lockhart's model implies that during oscillatory growth, either the turgor or the cell wall's rheological properties change in time. Indeed, a study using micro-indentation to probe the stiffness of the cell wall during growth suggests that the latter is the case (Zerzour et al., 2009). Of course, both could oscillate simultaneously, with the amplitude being below the detection levels of the pressure probe and micro-indenter, and the debate is far from settled (Kroeger and Geitmann, 2011; Winship et al., 2010, 2011; Zonia and Munnik, 2009, 2011).

4. The temporal aspect of the pollen tube growth cycle

In 1975, Weisenseel et al. observed distinct and regular pulses in the magnitude of the ionic fluxes crossing the plasma membrane of lily pollen tubes growing *in vitro* (Weisenseel et al., 1975). In the following decades, regular oscillations were observed in key features assessed at the tip of growing tubes: the growth rate, the pH, the cell wall thickness, the cell wall rheology, the concentration of cytosolic calcium (Bosch and Hepler, 2005; Chebli and Geitmann,

2007; Geitmann et al., 1996; Holdaway-Clarke and Hepler, 2003; McKenna et al., 2009; Messerli and Robinson, 2003; Pierson et al., 1994; Zerzour et al., 2009). All these features oscillate with the same periodicity, but with different phase delays, and their collaborative action was coined the "pollen tube growth cycle". One hypothesis for the origin of the growth rate oscillations is a disequilibrium in the force balance between cell wall strength and turgor, controlled through positive and negative feedback with chemical signaling cascades (Feijó, 1999). Feedback is believed to be involved in the regulation of pollen tube growth as it presents an attractive framework to explain the oscillations observed in many biological systems. Oscillatory systems based on feedback loops can display chaotic behavior upon slight modification of experimental parameters. Period doubling, for example, is a distinctive signature of underlying feedback, and was observed in tobacco pollen tubes (Zonia, 2010; Zonia et al., 2006). However, other changes in frequency resulting from manipulation of the pollen tube growth environment occur gradually (Feijó et al., 2001; Messerli et al., 1999, 2000; Messerli and Robinson, 1998, 2003).

While many of the variables governing pollen tube growth have been identified, their precise spatio-temporal interaction and the feedback loops that ensure the stability of this far-from-equilibrium and dynamical process remain elusive. A good starting point for the investigation of the causal relationships between the components of the feedback loops is their phase delay in the growth cycle (Chebli and Geitmann, 2007). The maximum in the cytosolic calcium concentration, for example, is phase delayed by 10° to 40° with respect to the maximum growth rate. The cell wall thickness, which is a good indicator of the level of vesicle exocytosis activity, is phase delayed by −124° in tobacco pollen tube and by −98° in lily pollen tubes (McKenna et al., 2009). Maximum proton (H^+) and potassium influx are phase delayed by 90–100° with respect to the maximum growth rate while the maximum chloride (Cl^-) efflux is in phase with the growth rate (Holdaway-Clarke and Hepler, 2003). A meaningful modeling approach should be able to reproduce these phase delays at least qualitatively.

A first theoretical model of the pollen tube growth rate oscillations was built on the assumptions that the turgor pressure is constant and that variations in the elasticity of cell wall create the oscillations in the growth (Kroeger et al., 2008). In this model, a high growth rate induces the opening of stretch-activated calcium channels located in the tube apex, leading to massive secretion of vesicles and assembly of new cell wall. While the model explains important aspects of the calcium dynamics, the predicted direct correlation between calcium concentration and vesicle exocytosis does not agree with experimental data. More recently, two other mathematical models have discussed the interaction between pollen tube growth rate and calcium concentration (Liu et al., 2010; Yan et al., 2009). Yan et al. focus on the interactions between ROP1 GTPases, F-actin polymerization and calcium, and explain how drugs interfering with the functioning of the actin cytoskeleton, latrunculin B and jasplakinolide, disrupt the calcium oscillations. Liu et al. model the interactions between different ionic currents, their voltage dependent gating variables and the trans-membrane potential. They show that these currents can sustain oscillations even in the absence of tube growth, a phenomenon that has been observed experimentally (Parton et al., 2003). Kroeger et al. (2008) couple the growth rate to the cell wall thickness and the cytosolic calcium concentration. Specifically, they assume that stretch-activated calcium channels open when the growth rate rises, and thus the stress on the apical membrane increases beyond a certain threshold value. The model explains why the maximum in cytosolic calcium concentration occurs after the peak in growth rate, a phenomenon that had puzzled biologists hitherto. The main difference between the three models is the mechanism that is assumed to be responsible for calcium entry at the tube apex, i.e. through voltage-gated channels (Liu et al., 2010), stress-activated channels (Kroeger et al., 2008) or the action of the scaffolding proteins (Yan et al., 2009). These mechanisms are not mutually exclusive and it is possible that all of them contribute to calcium entry and thus the oscillation in cytosolic calcium concentration. These mechanisms might even operate in concert to render pollen tube growth robust against external perturbations. However, more experimental work is clearly necessary to establish whether, or when, one of these mechanisms dominates calcium dynamics in the growing pollen tube. The importance of calcium for tube growth is undeniable, nevertheless, and while changing extra-cellular calcium concentration does affect the periodicity of the oscillations (Geitmann and Cresti, 1998; Messerli and Robinson, 2003), the important phase delay between the calcium and exocytosis maxima suggests that vesicle secretion is not primarily dependent on calcium concentration. A recent model of the growth rate oscillations proposes that the vesicle secretion rate is negatively correlated with the pollen tube growth rate (Rojas et al., 2011). The phenomenological description in this model is based on the experimentally determined temporal relation between exocytosis and tube growth rate (McKenna et al., 2009). The model yields the correct phase difference between exocytosis, cell wall thickness and tube growth rate. The explanation for the phenomenological equation is that a high growth rate allows massive calcium influx, as proposed by Kroeger et al. (2008, 2011), but that rather than triggering exocytosis directly, calcium prompts the depolymerization of F-actin into G-actin (Yan et al., 2009) and prevents the advancement of the actin fringe. Consistent with this, slight interference with actin polymerization without inhibiting growth prevents the tube from oscillating (Geitmann et al., 1996). The final assumption, that ties together the tube growth rate and the vesicle secretion, is that when the actin fringe is far from the tip, vesicle secretion is low while an actin fringe in close proximity to the tip leads to increased secretion activity. The idea that calcium influx prevents cytoskeleton advancement, and thus decreases exocytosis, is supported by observations that calcium entry reduces the growth rate (Malhó et al., 1994; Malhó and Trewavas, 1996; Messerli and Robinson, 2003) and lower cytoplasmic calcium concentration leads to shorter growth rate oscillations (Messerli and Robinson, 2003), as predicted by a mathematical model coupling dynamical calcium influx and tube growth rate (Kroeger et al., 2011). This assumption is also supported by experiments analyzing turning events during pollen tube elongation (Bou Daher and Geitmann, 2011), but the signaling pathway leading to this differential cytoskeletal activity remains poorly understood.

Clues from the different experimental and theoretical studies suggest the following picture for vesicle exocytosis: exocytosis is favored on a target region forming an annulus on the tip of growing tubes, possibly because the plasma membrane in this region contains the active machinery necessary for vesicle docking, priming and fusion, whereas the machinery is absent or inactive elsewhere. This is based on experimental observation (Bove et al., 2008; Zonia and Munnik, 2008) and has been addressed in a theoretical model (Chavarría-Krauser and Yejie, 2011). Furthermore, exocytosis is more probable if the actin fringe is in close proximity to this target zone and less probable if the actin fringe is further from this region. A close proximity between actin fringe and exocytosis target zone would lead to a high concentration of vesicles, and therefore a high probability of exocytosis, whereas a larger distance between fringe and target zone would lead to low vesicle concentrations and exocytosis probability. While the temporal correlation between exocytosis rate and vesicle concentration was observed experimentally (McKenna et al., 2009), the correlation between exocytosis rate and fringe position was suggested in a theoretical model (Rojas et al., 2011) and by experimental data (Bou Daher and Geitmann, 2011). If such a picture was further supported experimentally, it

suggests a purpose for the growth rate oscillations. Being able to control growth at low and high growth rates, as well as low and high exocytosis rates, might allow the pollen tube to turn at sharp angles while maintaining high average growth rates. In order to turn, a pollen tube must reorient the region of major cellular expansion to produce an off axis outgrowth. This picture could also reconcile the observations that calcium is a chemo-attractant for pollen tubes (Iwano et al., 2004; Mascarenhas and Machlis, 1962) while excessive calcium concentrations in the growth medium lead to a decreased growth rate (Bou Daher and Geitmann, 2011). In summary, the position of the actin fringe with respect to the annular growth region presents itself as the ideal candidate for the regulation of growth rate and potentially tropic growth. The oscillation mechanism, a tight feedback regulation loop involving non-linear mechanical and chemical interactions, allows to shift rapidly from low to high growth rates without bursting or growth arrest, and therefore turn the growth direction without losing control. In effect, instabilities in the growth regulation might be responsible for high maneuverability, a situation akin to supermaneuverability due to aerodynamic instabilities in advanced fighter airplanes.

5. Conclusions

Modeling cellular behavior is by definition a reductionist approach, since considering the entirety of all cellular molecules and reactions is not only impossible but also needlessly complex. It is therefore important to focus on those processes that seem to be the key agents and essential steps in the reactions leading to cellular morphogenesis. The fact that different sets of assumptions, translated into mathematical models, allow the simulation of a phenomenon such as oscillatory growth, indicates that either the process is regulated by redundant mechanisms or that at least some of the models do not necessarily focus on key features regulating the mechanism. Whether any of the models are even remotely close to reality requires validation by experimental testing. The most important function of these models is thus the guidance and direction of future experimentation towards the identification of a minimal set of processes leading to oscillations, and their causal relation. Furthermore, it is critical for the convergence of modeling ideas that future models produce "signature" predictions, i.e. predicted phenomena particular to the new interaction or mechanism that they propose. Most current modeling approaches lack such predictions or do not state them clearly.

Astonishingly, while numerous theoretical models of pollen tube growth have succeeded in simulating the oscillatory behavior, none provides an unambiguous explanation for the biological function of this phenomenon. Various suggestions have been brought forward for explaining this behavior. On one side the oscillations could be a simple artifact of the *in vitro* growth conditions that are suboptimal as indicated by the relatively low growth rate compared to the *in vivo* situation in the pistil of the flower. These suboptimal conditions could push this non-linear dynamical system into a limit-cycle regime. In such a case, the oscillations could be a stabilization effort in response to changes in the environmental conditions that prevents complete failure, i.e. growth arrest or precocious bursting. In a similar vein, if it was advantageous for the processes involved in growth to proceed in a certain order, the oscillations could regulate the sequence of events. On the other hand, the oscillations may actually have a purpose and the rapid growth phases may aid the cell in invading the pistillar tissues that represent a mechanical obstacle to the elongating tube (Chebli and Geitmann, 2007; Geitmann, 1999). This hypothesis is supported by the finding that period thickenings in the pollen tube cell wall, likely caused by the oscillations, also occur in *in vivo* growing tubes (Li et al., 1995). Moreover, there seems to be a correlation between the presence of a solid style filled with transmitting tissue (as opposed to an open canal) that presents a mechanical obstacle to pollen tube growth, and the display of pollen tube growth oscillations. In plant species with hollow styles, this behavior is less frequently observed.

One way to gain a better understanding of the oscillatory behavior may be to establish a set of mathematical equations that correctly describe the phase difference between the different actors of the pollen tube growth cycle, and, importantly, that provide falsifiable predictions for the response of growing pollen tubes to transient or prolonged perturbation of the growth environment. While the geometric and mechanical aspects of pollen tube growth have enjoyed much attention and are well described by extensions of the Lockhart model, the insertion of new cell wall material through vesicle secretion, a complex, dynamical phenomenon closely intertwined with the dynamics of the actin cytoskeleton, might provide the key to the understanding of pollen tube growth in particular and tip-growth in general. Very understandably, modelers have started by first "picking the low-hanging fruit". While initial models addressed steady state growth and more recent ones focused on growth varying in time, future models will likely explore growth that varies in time and space, or that deviates from the straight growth pattern, thereby hopefully shedding new light on long-standing questions.

Acknowledgements

Research in the Geitmann lab is supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

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