

Pollen cytoskeleton during germination and tube growth

Giampiero Cai*, Cecilia Del Casino, Silvia Romagnoli and Mauro Cresti

Dipartimento Scienze Ambientali, Università degli Studi di Siena, via Mattioli 4, 53100 Siena, Italy

Sexual reproduction in higher plants requires the development of a special cell protrusion, the so-called pollen tube, which is generated by the male gametophyte. Like other plant cells, the pollen tube contains a conspicuous cytoskeletal apparatus that regulates and promotes most of its biological functions, the most important of which is the transport of sperm cells. The study of the pollen tube cytoskeleton has been intensified during the last few years on account of the critical importance of the pollen tube as a cell model. This review will focus on several aspects of the cytoskeleton in the pollen tube, from the way it assembles and organizes to discussing its role in organelle motility and tip growth.

Keywords: Cell polarity, cytoskeleton, pollen tube, reproduction, tip growth.

THE pollen grain is the male gametophyte of seed plants (gymnosperms and angiosperms) and represents a crucial evolutionary step that allowed plants to leave the aqueous environment during sexual reproduction. The consequence is that seed plants have progressively populated the dry land, thus becoming the most abundant plant species on earth. The pollen tube is not a real cell but is a cylindrical protuberance that the pollen grain produces upon landing on the stigma of a receptive flower (in angiosperms) or on macrosporangium (in gymnosperms). However, the role of the pollen tube is critical as it transports the male gametes (sperm cells) towards the egg cell. Because of this importance and because of the ease with which it can be obtained and handled, the pollen tube has progressively become a model system for the study of different physiological features of plant cells, such as cell polarity, apical growth, movement of organelles, cell-cell recognition and signal transduction¹. Like every other plant cell, the pollen tube contains a conspicuous network of filamentous proteins, termed the cytoskeleton, which is the structural basis of its internal organization. The cytoskeleton controls how the pollen tube grows and how the cytoplasm dynamically reorganizes itself during tube elongation. In addition, the cytoskeleton is also responsible for the transport of male gametes – the most important function of the pollen tube.

The cytoskeleton of the pollen tube is constituted by actin filaments and microtubules and most of its charac-

teristics are the same as those of a typical interphase cell. However, since the pollen tube is a tip-growing cell, it has some peculiarities that differentiate it from other plant cells. The most obvious and unique feature is that the pollen tube contains additional cells, the sperm cells, and that these cells move inside the pollen tube. The cytoskeletal apparatus of the pollen tube is consequently adapted to support such movement. The second important feature is polarization: actin filaments and microtubules are arranged along the elongation axis of the pollen tube and their orientation determines the cytoplasmic polarization of the tube. In addition, actin filaments and microtubules show a gradient of organization that matches the gradient of ions and molecules found in the pollen tube. A third characteristic of the pollen tube cytoskeleton is the molecular composition: some cytoskeletal proteins (for example, a number of tubulin isotypes) are expressed only in the male gametophyte, which therefore represents an exclusive example of proteome. This review will essentially focus on the structure and organization of the angiosperm pollen and pollen tube, since they have been studied in greater detail than those of gymnosperms.

The nature of cytoskeleton in ungerminated pollen and its reorganization during germination

The study of the cytoskeleton in the pollen grain is not simple, mainly because of the technical complexity in labelling actin filaments and microtubules inside the grain. Consequently, only a few reports are available on this specific subject^{2,3}. It appears that both actin filaments and microtubules are organized as short fibres inside the pollen grain. Such strands may represent precursors of both the cytoskeletal systems, either as reservoirs of protein subunits or as units for the assembly of longer filaments. Moreover, the pollen grain contains most of the protein subunits used subsequently during tube elongation. Although the synthesis of new actin and tubulin may take place during tube growth^{4,5}, the level of actin and tubulin during tube elongation is steady⁴. This evidence suggests that the turnover of both proteins is precisely regulated; nevertheless, the assembly of novel actin filaments and microtubules during tube elongation should also require the disassembly of older cytoskeletal fibres.

The polarization of both actin filaments and microtubules initiates with the emergence of the pollen tube.

*For correspondence. (e-mail: cai@unisi.it)

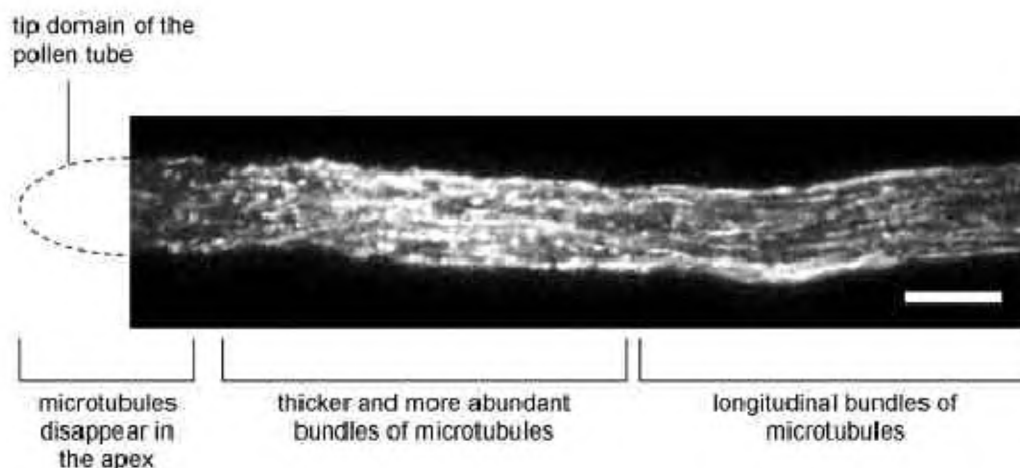


Figure 1. Immunocytochemistry of microtubules in the pollen tube of tobacco. The image was recorded close to the tube apex (which is only partially shown on the left and indicated by the dotted line). The different density of microtubules alongside the pollen tube is clearly visible. Bar: 15 μm .

Both cytoskeletal systems organize as longer bundles and enter the emerging tube^{2,3}. This process recalls the question of the origin of actin filaments and microtubules. No data exist on the localization of microtubule-organizing sites or on actin filament-assembly sites in the pollen grain. Analogous to other plant cells and to the pollen tube^{6,7}, both cytoskeletal systems may initiate from distinct origin sites scattered all along the plasma membrane of the pollen grain.

The organization of microtubules and actin filaments during tube growth

The organization of actin filaments and microtubules within the pollen tube has been the subject of several studies. With the introduction of immunocytochemistry, it was possible to analyse how actin and tubulin organize in the pollen tube and to correlate their distribution to their function. Both systems have a comparable organization, as they are mainly structured in bundles that approximately have the same direction of the tube axis⁸. The density of bundles is not constant along the pollen tube. For example, microtubules are more abundant in the terminal part of the pollen tube close to the growth region and progressively disappear in the older parts of the tube upon sealing by the callose plugs^{9,10} (Figure 1). The concentration of microtubule bundles also changes during the passage of the generative cell¹⁰. Microtubules can be depolymerized by treatment with several drugs or cold, although they are inclined to resist in the cortical domain, where microtubules are likely to be associated with the plasma membrane through membrane-microtubule interacting proteins¹¹. The presence of microtubules in the growth region (the apical domain) is still debated. Although standard immunocytochemistry after chemical fixation has shown the occurrence of short and twisted microtubules¹⁰, electron microscopy after

freeze-fixation has not confirmed such finding¹². One speculative hypothesis suggests that the apical domain may be the site of origin of pollen tube microtubules¹³, but the subapical domain¹⁴ and the plasma membrane in general⁷ may play the same role as well.

The organization and regulation of actin filaments in the pollen tube are known in greater detail. The use of a specific dye, such as rhodamine-phalloidin, has allowed researchers to distinguish helically organized bundles that permeate the cytoplasm of pollen tubes¹⁵. It is likely that actin bundles are generated by the activity of villin-like proteins¹⁶ that assemble actin filaments into bundles with identical polarity¹⁷. As Ca^{2+} -calmodulin inhibits the activity of villin¹⁸, actin bundles are not consequently found in the tube apex (a region with higher Ca^{2+} concentration). This finding correlates well with the evidence that the apical domain of pollen tubes does not contain actin bundles but has a mesh of short actin filaments¹⁹. Following the advent of molecular biology techniques, the presence of such actin mesh in the apical domain of pollen tubes has been confirmed²⁰. The balance between G-actin, short actin filaments in the apex and actin bundles in the older region is maintained through the activity of several factors^{21–26}, as also discussed elsewhere in this article.

The specific role of microtubules and actin filaments during pollen tube growth

The role of both actin filaments and microtubules during pollen tube growth may be schematically divided into different categories according to the specific cell regions considered or according to the specific organelles transported. Schematically, we can consider the involvement of the cytoskeleton in (i) the process of apical secretion (and thus tube elongation), (ii) cytoplasmic streaming (that is organelle transport), and (iii) transport of the male

gametes along the pollen tube. We shall discuss all the three processes separately, but in the last part we will attempt to incorporate the separate results into a single model.

Apical secretion and growth

Secretion is defined as the discharge of new material outside the cell through the activity of specific membrane-bound vesicles, which are usually generated by the Golgi bodies. During secretion, the membrane of secretory vesicles fuse with the plasma membrane and the content of vesicles is released out of the cell. In the pollen tube, secretory vesicles mainly contain precursors of the cell wall, such as pectins, other glycoproteins and more specific proteins such as phosphatases²⁷. The process of secretion can be divided into two distinct phases: the production of vesicles (which is independent of the cytoskeleton) and the delivery of vesicles to the final destination (which conversely needs the presence of a well-structured cytoskeleton). The two phases can be distinguished and studied separately using different chemical substances that interfere distinctly with the two processes²⁸. The cytoskeleton is mainly responsible for delivering the newly-formed secretory vesicles to the apical plasma membrane; however, the activity achieved by the cytoskeleton cannot be merely restricted to the delivery activity. The secretion of new material is a precise balance between accumulation of new vesicles and the release of old ones from the exocytosis site²⁹; in addition, membrane recycling is known to occur in the pollen tube³⁰. Consequently, the activity of Golgi bodies is not regulated according to the number of vesicles required for tube growth. The actin cytoskeleton is apparently essential for the proper growth of pollen tubes³¹ and such requirement may be necessary at two distinct levels. First, actin and myosin may function cooperatively to drive vesicles to the secretion site; second, an intact actin cytoskeleton may be necessary to facilitate and maintain the accumulation of secretory vesicles into the apical domain. The second function is supported by evidences that tube elongation and tube germination can be uncoupled using distinct dosages of actin antagonists³², which suggests that germination is less sensitive to actin antagonists than extension and that tube elongation requires a more precise balance between monomeric and filamentous actin. To infer the role of actin filaments in the secretion process, it was originally proposed that actin filaments physically separate the growth region from the base region through the formation of an actin ring³³. This structure should act like a filter to physically divide the small-sized vesicles from the population of larger organelles. This preliminary hypothesis has been recently reviewed using techniques of molecular biology, which have led to the identification of a dynamic actin collar²¹. The function and existence of the collar is not definitely determined but it should pre-

sumably work to separate the growth domain from the domain of organelle movement. The transition from the domain of growth to the base domain is presumably controlled by several proteins, which collectively contribute to regulate the polymerization state of actin. The growth domain likely contains short and highly dynamic actin filaments, which are converted into rigid bundles in the base domain. The regulation of actin dynamics is critical for the proper growth of pollen tubes³¹ and is maintained by the concerted action of a number of factors, such as actin depolymerising factors (ADF)²², phosphatidylinositol-4,5-bisphosphate (PIP2)³⁴, Rho and Rac proteins^{35,36,23,24,26}, together with a number of accessory proteins like profilin³⁷, villin¹⁶ and actin filament-severing protein²⁵. The concerted activity of all these proteins ensures that the polymerization state of actin is accurately maintained to promote the elongation of pollen tubes. Ca^{2+} , which is essential for the proper growth of pollen tubes³⁸, also controls the dynamics of actin filaments but its concentration in the apical domain is also regulated by the polymerization state of actin³⁹, suggesting that the two systems reciprocally regulate each other for a finer control of tube growth.

The role of microtubules during tube growth and specifically in the process of secretion is likely to be less important. Classical experiments in which pollen tubes were treated with microtubule-depolymerizing drugs have shown that the elongation rate of pollen tubes is not affected⁴⁰. However, some exceptions exist and they must be mentioned. The anti-microtubule drug carbetamide can stop the elongation of pollen tubes in the style (thus *in vivo* conditions)⁴¹; benomyl, another anti-microtubule drug, also generates drastic effects on both the morphology and the elongation rate of pollen tubes⁴²; oryzalin, a different microtubule drug, has been shown to alter the pulsatory growth of pollen tubes but it does not affect the elongation rate⁴³. These results collectively suggest that microtubules may contribute to some phases of pollen tube growth, although it is not exactly clear what phase is affected. Furthermore, the effects of the inhibitory treatment do not clarify if the role of microtubules in the elongation activity is direct or indirect, such as in the organization of the pollen tube cytoplasm. To sum up, most data indicate that actin filaments have a clear and definite role in the process of elongation, whereas the role of microtubules is less important or still to be defined.

Cytoplasmic streaming

One of the most evident features of pollen tube is the prominent movement of organelles from the grain towards the apex and vice versa⁴⁴ (Figure 2). This flow of organelles, which recalls the cytoplasmic streaming of plant cells, is fundamental for the growth activity of pollen tubes. The involvement of the cytoskeleton in the movement of organelles and vesicles during tube growth is es-

established by several studies. Some of these findings are derived from investigations on other plant cells, whereas some are pertinent to the pollen tube system. The motility of pollen tube organelles along actin cables was clearly demonstrated using the alga, *Chara*⁴⁵. In addition, the analysis of organelle movement along actin cables revealed that such a movement was dependent on the concentration of Ca^{2+} and that myosin was the most plausible candidate to drive such movement⁴⁶. The role of actin filaments in the cytoplasmic streaming of pollen tubes

was further suggested by several pharmacological evidences⁴⁷. No doubts remain that actin filaments and myosin are the protein systems that promote the movement of organelles and vesicles. Treatment of pollen tubes with cytochalasins induced several alterations in the tube structure and in the distribution of organelles⁴⁸ and the organization of actin filaments was well correlated with the pattern of organelle movement in different tube regions⁴⁹. Pollen tube growth is also strongly affected by different actin antagonists, such as latrunculin B³², and profilin/DNase I³¹. In the last case, tip growth was shown to be more sensitive than streaming in response to treatment with inhibitors suggesting that tube growth requires that tip actin is assembled in a process independent of cytoplasmic streaming. The requirement of actin filaments and the accurate equilibrium between the G- and F-forms of actin has also been shown recently, suggesting that actin polymerization promotes the reversal of streaming in the apex of the pollen tube⁵⁰. In other cases, curved bundles of actin filaments have been shown in the subapical region of pollen tubes, which should represent a prerequisite for the reversal of organelle streaming⁵¹.

Myosins have also been identified in pollen tubes of different species. Unfortunately, proteins related to myosin have been mainly characterized using heterologous antibodies, leading to the composition of a myosin puzzle, in which the molecular mass and the distribution of myosins were essentially dependent on the type of antibody used. Consequently, myosins of 185 kDa were identified in the pollen tube tip and in association with the generative cell⁵²; conversely, myosins of 175 kDa were detected in association with vesicles and organelles (such as mitochondria)⁵³. Different classes of myosins were also discovered using antibodies to specific myosin classes (I, II and V)⁵⁴. The latter finding suggested that the movement of individual membrane-bounded objects (vesicles, organelles, generative cell) was dependent of specific myosins. However, this finding was not confirmed by molecular analysis, which showed that fewer and different myosin classes are present in the *Arabidopsis* genome⁵⁵. Consequently, some of the results previously published should be reviewed and integrated with the molecular data. The only pollen tube myosin identified so far is a 170 kDa polypeptide isolated from lily pollen⁵⁶. The protein was purified by a standard biochemical approach based on selective binding to actin filaments and was shown to have the typical properties of myosins, such as the actin-dependent ATPase activity and the ability to glide actin filaments. After immunolocalization in the pollen tube of lily, the protein was found in specific tube districts, suggesting that it may not be associated with all organelle classes or that its localization depends on the specific physiological state of the pollen tube examined⁵⁷. However, these findings represent the more powerful evidence of myosins in the angiosperm pollen tubes, although they do not prove definitely that the 170-kDa polypeptide is responsible for the cyto-

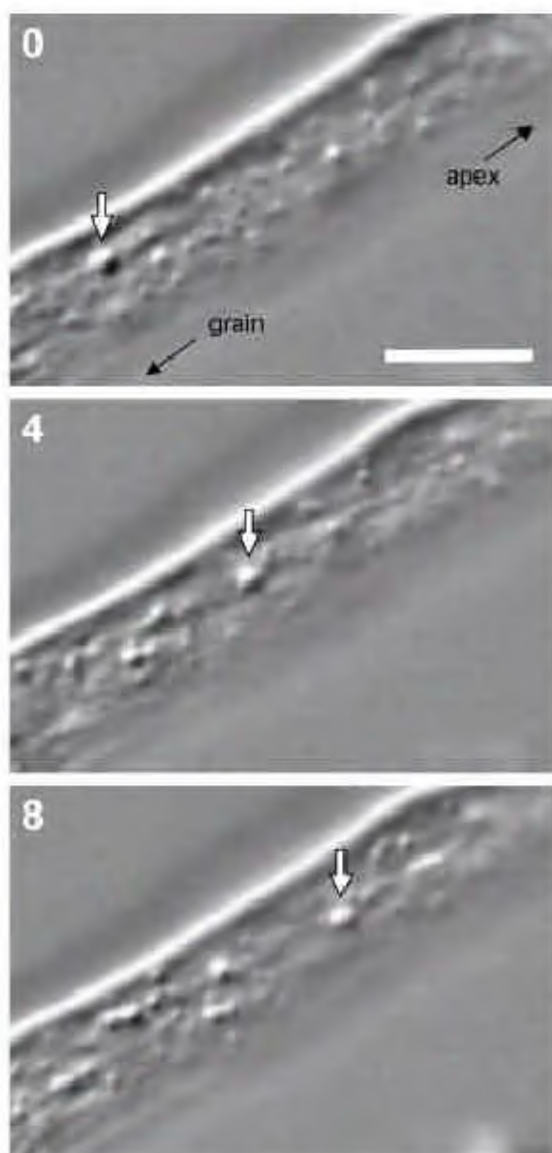


Figure 2. Video microscopy analysis of a living pollen tube of tobacco. Images correspond to video frames captured every 4 sec and show the considerable flow of organelles observed in the pollen tube. The white arrow indicates specifically one larger organelle that moves directionally towards the tube apex with a mean velocity of 2 $\mu\text{m}/\text{sec}$. Such movement is expected to occur along actin cables. Only the movement towards the apex is observed because the focus of each image corresponds to the cortical region of the pollen tube. Bar: 10 μm .

plasmic streaming of pollen tubes. The evidence that the myosin activity is dependent on Ca^{2+} concentration and that myosin binds to calmodulin in a Ca^{2+} -dependent way suggested a hypothetical role for the 170-kDa myosin in the pollen tube⁵⁸. As the concentration of Ca^{2+} is lower in the base region of pollen tubes, the 170-kDa myosin may be active in that region and contribute to the movement of pollen tube organelles. The activity of the 170-kDa myosin may be strongly reduced and inhibited in the subapical and apical domains of pollen tubes through the Ca^{2+} -dependent removal of calmodulin. This mechanism could be the basis for the regulation of organelle movement in the pollen tube.

The involvement of microtubules in organelle transport is not exactly defined but microtubules may support a different type of transport compared with actin filaments. Microtubule-dependent motors have been identified and partially characterized in the pollen tube^{59,60,61}. In addition, pollen tube organelles have been shown to move along microtubules under *in vitro* motility assays⁶². These findings cooperatively suggest that the pollen tube has the proper protein machinery to move organelles along microtubules. However, the precise physiological consequence of the microtubule-based organelle movement in the pollen tube is not clear. The speed of organelles along microtubules is much lower compared with the velocity of organelles along actin filaments, which suggests that microtubules are not important in the cytoplasmic streaming. Consequently, the role of microtubules in organelle transport should be searched in the regulation of organelle positioning or in the precise delivery of organelles to their final site.

Transport of the generative cell and vegetative nucleus

The fundamental function of the pollen tube is the transport of the sperm cells from the pollen grain to the female gametophyte. Such activity is strictly coordinated with the elongation rate of the pollen tube. The transport of the sperm cells depends on a number of molecular mechanisms only partially characterized. No doubts exist that the sperm cells are not able to move by themselves. The internal cytoskeleton, mainly constituted by microtubules⁶³, seems to be responsible for maintenance of the ellipsoidal shape of the sperm cells, which in turn facilitates their migration through the pollen tube. Consequently, the motor capacity of the sperm cells should be searched in the protein machinery outside the sperm cells (specifically, on the outer surface or in the vegetative cytoplasm). A first clue on the presence of molecular motors was the identification of myosin-related polypeptides associated with the sperm cells⁶⁴. Such findings have been subsequently confirmed by additional studies⁶⁵, suggesting that the sperm cells could move along actin cables similar to cytoplasmic or-

ganelles. Nevertheless, other models have been proposed to account for the movement of sperm cells in case of insufficient presence of myosins on the outer surface⁶⁶. Consequently, it is still difficult to assume that sperm cells move because of interactions between actin filaments and myosins. Furthermore, this simple model does not integrate the evidence that microtubules also have a critical role in the transport of the sperm cells. Treatment with microtubule antagonists resulted in a significant decrease in the translocation rate of sperm cells⁶⁷, indicating that the cytoplasmic microtubules participate in such an activity. We do not know how microtubules promote the movement of sperm cells, through the activity of dependent motor proteins or by arranging the pollen tube cytoplasm to facilitate cell movement.

An integrated model of organelle movement and tube growth

The pollen tube is a cell that grows at a specific site (the tip) through the continuous delivery of new material carried by secretory vesicles; simultaneously, organelles move forward and backward to distribute evenly along its axis. The two processes are likely based on distinct molecular mechanisms that interact with each other and that are collectively regulated by a central controller. The controller, which ensures the correct growth of pollen tubes, is not likely to be represented by a single protein or a single factor, but rather by a network of proteins and factors. We will focus on those elements that regulate the organization of the cytoskeleton in connection with both the growth process and the flow of organelles. The central point of the regulation process is represented by the transition from G-actin (or short actin mesh) in the tube apex to the actin bundles in the base region. This differentiation also separates the growth region from the rest of the pollen tube. Ca^{2+} is a central factor in this transition because it controls many distinct activities. Apart from promoting the fusion of secretory vesicles in the tip, Ca^{2+} also controls the polymerization state of actin in cooperation with other protein factors. Specifically, the Rho/Rac complex in the plasma membrane, which also controls the activity of actin-depolymerizing factors, likely through regulating the concentration of Ca^{2+} . Ca^{2+} directly participates in the regulation of actin dynamics by activating actin filament-severing proteins. The assembly of actin filaments into actin bundles is also controlled by Ca^{2+} , since the Ca^{2+} -calmodulin complex removes villin from actin bundles and thus converts actin bundles into single actin filaments, which are likely to be more susceptible to depolymerization. In the base region, actin cables, stabilized by villin, are the main tracks for the myosin-dependent movement of organelles and vesicles (and of sperm cells). Another regulatory activity of Ca^{2+} is the inhibition of myosin activity. The higher concentration of

Ca^{2+} in the apical domain is supposed to inhibit the myosin-mediated movement of organelles along actin cables, while such movement is facilitated in the base region by the lower Ca^{2+} concentration. All together, these data suggest that Ca^{2+} has a critical role in controlling the polymerization and organization of actin filaments and in regulating the activity of actin-dependent motor proteins.

The simple model described above does not involve the microtubule cytoskeleton. This is due to insufficient information on the role of microtubules in the control of the growth process. Apart from mediating the movement of organelles, it is still hard to state what exactly microtubules do during pollen tube growth. The regulation of

their activity as well as the factors that regulate their organization are completely unknown. Therefore, the proposed model (schematically sketched in Figure 3) is still partial, as it does not take into account the second major cytoskeletal system.

Microtubules and the control of cell wall deposition

A traditional function of plant cell microtubules is their involvement in the deposition of the cell wall. The classical model proposes that microtubules restrict the diffusion of the cellulose-synthesizing complex in the plasma membrane, thus indicating how the new cellulose fibrils should be deposited out of the cell⁶⁸. This model, which is far from being finished and accepted in interphase cells, is even more difficult to adapt to the pollen tube system. Unlike somatic cells, the orientation of microtubules and of cellulose fibrils corresponds to the growth direction of pollen tubes. In somatic cells, the orientation of microtubules determined the direction of cell expansion by affecting the local stiffness of the cell wall. Conversely, in the pollen tube the growth direction is mainly determined by the local accumulation of secretory vesicles in the tube tip. Nevertheless, the cylindrical shape of the pollen tube should be constantly maintained during growth and this process is likely to depend on the correct deposition of the cell wall components, such as pectins, cellulose and callose. How the cytoskeleton controls the deposition of these building blocks is unidentified but it is expected that microtubules and actin filaments may contribute in some measure to such activity.

Prospects

The pollen tube plays a key role in sexual reproduction. It is a fascinating cell model through which researchers can understand most of the cellular activities of plant cells. We expect that the research on the cytoskeleton of pollen tubes will move progressively deeper in the coming years until we understand how this protein network allows the pollen tube to grow, to reach the final destination and to deliver its precious content. Our current state of knowledge emanates from scattered bits of information on different aspects and functions of the cytoskeleton (regulation, organelle movement, growth, and so forth). The integrated use of mutants, cell biology, DNA engineering, and proteomic techniques is expected to provide us with a clear picture of the relationships between the cytoskeletal genes and proteins that regulate and operate in the pollen tube. One of the most important expectations is the discovery of the interactions between different cytoskeletal components. For example, how do actin filaments and microtubules interact with each other and how do the extracellular signals precisely regulate the structure and activity of the cytoskeleton, and what is the identity of the proteins that syn-

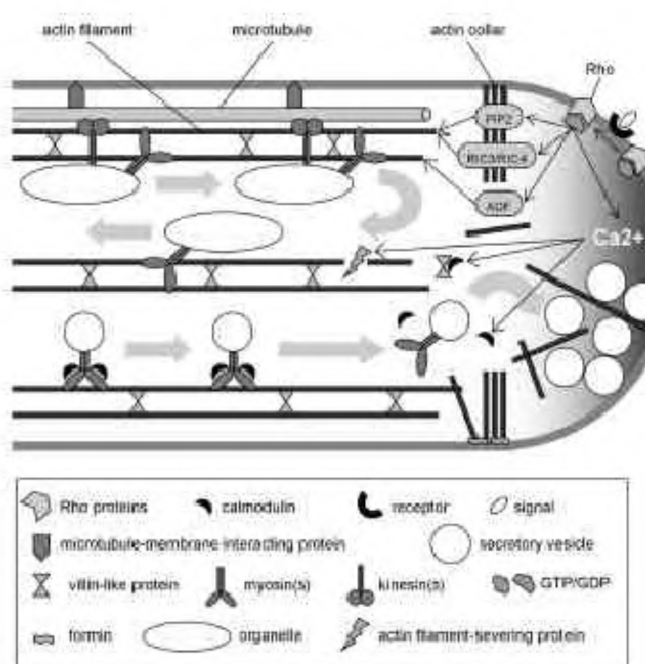


Figure 3. Schematic drawing of the putative interactions between the Ca^{2+} gradient, regulatory proteins and the cytoskeleton in the pollen tube. The illustration attempts to collect information from different papers (and from different pollen species) into a single model. Objects in the picture are not drawn to scale. The starting point is the generation of the Ca^{2+} gradient, which is under the control of several factors, such as Rho proteins. These small GTP-binding proteins are likely to interact with (putative) receptor proteins in the apical plasma membrane and thus transduce extracellular signals into variations of the Ca^{2+} concentration. The activity of Rho proteins is also achieved at the level of actin filaments, whose polymerization/depolymerization is controlled by a cascade of proteins including ADF, RIC3/RIC4. Ca^{2+} also participates in the control of actin assembly by regulating accessory proteins, such as the actin filament-severing proteins. The role of Ca^{2+} is also achieved in the organization of actin filaments; the association with Ca^{2+} -calmodulin complex negatively regulates villin (an actin bundling protein). Ca^{2+} also regulates the activity of myosin through binding and removing calmodulin from the myosin motor. Consequently, the vesicle-associated myosin is not further able to move along actin filaments and thus vesicles accumulate in the tip domain. Other structural factors (such as the actin collar) should contribute to the process of vesicle accumulation. These structural restrictions do not apply to larger organelles, which are consequently free to flow back in the reverse direction. Microtubules and their dependent motors are likely to participate in regulating the positioning of organelles in the pollen tube.

chronize the growth of the pollen tube with the movement of organelles and the transport of sperm cells?

1. Mascarenhas, J. P., The male gametophyte of flowering plants. *Plant Cell*, 1989, **1**, 657–664.
2. Tiwari, S. C. and Polito, V. S., The initiation and organization of microtubules in germinating pear (*Pyrus communis* L.) pollen. *Eur. J. Cell Biol.*, 1990, **53**, 384–389.
3. Tiwari, S. C. and Polito, V. S., An analysis of the role of actin during pollen activation leading to germination in pear (*Pyrus communis* L.): treatment with cytochalasin D. *Sex. Plant Reprod.*, 1990, **3**, 121–129.
4. Sorri, O., Åström, H. and Raudaskoski, M., Actin and tubulin expression and isotype pattern during tobacco pollen tube growth. *Sex. Plant Reprod.*, 1996, **9**, 255–263.
5. Mascarenhas, J. P., Gene activity during pollen development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1990, **41**, 317–338.
6. Cheung, A. Y. and Wu, H. m., Overexpression of an *Arabidopsis* formin stimulates supernumerary actin cable formation from pollen tube cell membrane. *Plant Cell*, 2004, **16**, 257–269.
7. Palevitz, B. A., Liu, B. and Joshi, C., γ -tubulin in tobacco pollen tubes: association with generative cell and vegetative microtubules. *Sex. Plant Reprod.*, 1994, **7**, 209–214.
8. Pierson, E. S., Derksen, J. and Traas, J. A., Organization of microfilaments and microtubules in pollen tubes grown *in vitro* or *in vivo* in various angiosperms. *Eur. J. Cell Biol.*, 1986, **41**, 14–18.
9. Raudaskoski, M., Åström, H., Perttinen, K., Virtanen, I. and Louhevaara, J., Role of microtubule cytoskeleton in pollen tubes: an immunocytochemical and ultrastructural approach. *Biol. Cell*, 1987, **61**, 177–188.
10. Del Casino, C., Li, Y., Moscatelli, A., Scali, M., Tiezzi, A. and Cresti, M., Distribution of microtubules during the growth of tobacco pollen tubes. *Biol. Cell*, 1993, **79**, 125–132.
11. Cai, G., Ovidi, E., Romagnoli, S., Vantard, M., Cresti, M. and Tiezzi, A., Identification and characterization of plasma membrane proteins that bind to microtubules in pollen tubes and generative cells of tobacco. *Plant Cell Physiol.*, 2005, **46**, 563–578.
12. Lancelle, S. A., Cresti, M. and Hepler, P. K., Ultrastructure of cytoskeleton in freeze-substituted pollen tubes of *Nicotiana tabacum*. *Protoplasma*, 1987, **140**, 141–150.
13. Cai, G., Moscatelli, A., Del Casino, C., Chevrier, V., Mazzi, M., Tiezzi, A. and Cresti, M., The anti-centrosome mAb 6C6 reacts with a plasma membrane-associated polypeptide of 77-kDa from the *Nicotiana tabacum* pollen tubes. *Protoplasma*, 1996, **190**, 68–78.
14. Heslop-Harrison, J. and Heslop-Harrison, Y., Sites of origin of the peripheral microtubule system of the vegetative cell of the Angiosperm pollen tube. *Ann. Bot.*, 1988, **62**, 455–461.
15. Tang, X. J., Lancelle, S. A. and Hepler, P. K., Fluorescence microscopic localization of actin in pollen tubes: comparison of actin antibody and phalloidin staining. *Cell Motil. Cytoskeleton*, 1989, **12**, 216–224.
16. Vidali, L., Yokota, E., Cheung, A. Y., Shimmen, T. and Hepler, P. K., The 135 kDa actin-binding protein from *Lilium longiflorum* pollen is the plant homologue of villin. *Protoplasma*, 1999, **209**, 283–291.
17. Yokota, E. and Shimmen, T., The 135-kDa actin-binding protein from lily pollen tubes arranges F-actin into bundles with uniform polarity. *Planta*, 1999, **209**, 264–266.
18. Yokota, E., Muto, S. and Shimmen, T., Calcium-calmodulin suppresses the filamentous actin-binding activity of a 135-kilodalton actin-binding protein isolated from lily pollen tubes. *Plant Physiol.*, 2000, **123**, 645–654.
19. Miller, D. D., Lancelle, S. A. and Hepler, P. K., Actin filaments do not form a dense meshwork in *Lilium longiflorum* pollen tube tips. *Protoplasma*, 1996, **195**, 123–132.
20. Kost, B., Spielhofer, P. and Chua, N.-H., A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualize the actin cytoskeleton in growing pollen tubes. *Plant J.*, 1998, **16**, 393–401.
21. Fu, Y., Wu, G. and Yang, Z., Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. *J. Cell Biol.*, 2001, **152**, 1019–1032.
22. Chen, C. Y., Wong, E. I., Vidali, L., Estavillo, A., Hepler, P. K., Wu, H. m. and Cheung, A. Y., The regulation of actin organization by actin-depolymerizing factor in elongating pollen tubes. *Plant Cell*, 2002, **14**, 2175–2190.
23. Chen, C. Y., Cheung, A. Y. and Wu, H. m., Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth. *Plant Cell*, 2003, **15**, 237–249.
24. Gu, Y., Vernoud, V., Fu, Y. and Yang, Z., ROP GTPase regulation of pollen tube growth through the dynamics of tip-localized F-actin. *J. Exp. Bot.*, 2003, **54**, 93–101.
25. Fan, X., Hou, J., Chen, X., Chaudhry, F., Staiger, C. J. and Ren, H., Identification and characterization of a Ca^{2+} -dependent actin filament-severing protein from lily pollen. *Plant Physiol.*, 2004, **136**, 3979–3989.
26. Gu, Y., Fu, Y., Dowd, P., Li, S., Vernoud, V., Gilroy, S. and Yang, Z., A Rho family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes. *J. Cell Biol.*, 2005, **169**, 127–138.
27. Ibrahim, h., Pertl, H., Pittersschatscher, K., Fadl-Allah, E., El Shahed, A., Bentrup, F. W. and Obermeyer, G., Release of an acid phosphatase activity during lily pollen tube growth involves components of the secretory pathway. *Protoplasma*, 2002, **219**, 176–183.
28. Ruten, T. L. and Knuiman, B., Brefeldin A effects on tobacco pollen tubes. *Eur. J. Cell Biol.*, 1993, **61**, 247–255.
29. Parton, R. M., Fischer-Parton, S., Watahiki, M. K. and Trewavas, A. J., Dynamics of the apical vesicle accumulation and the rate of growth are related in individual pollen tubes. *J. Cell Sci.*, 2001, **114**, 2685–2695.
30. Pictou, J. M. and Steer, M. W., Membrane recycling and the control of secretory activity in pollen tubes. *J. Cell Sci.*, 1983, **63**, 303–310.
31. Vidali, L., McKenna, S. T. and Hepler, P. K., Actin polymerization is essential for pollen tube growth. *Mol. Biol. Cell*, 2001, **12**, 2534–2545.
32. Gibbon, B. C., Kovar, D. R. and Staiger, C. J., Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell*, 1999, **11**, 2349–2364.
33. Heslop-Harrison, J. and Heslop-Harrison, Y., Actomyosin and movement in the angiosperm pollen tube: an interpretation of some recent results. *Sex. Plant Reprod.*, 1989, **2**, 199–207.
34. Monteiro, D., Liu, Q., Lisboa, S., Scherer, G. E. F., Quader, H. and Malho, R., Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[\text{Ca}^{2+}]_c$ and membrane secretion. *J. Exp. Bot.*, 2005, **56**, 1665–1674.
35. Kost, B., Lemichez, E., Spielhofer, P., Hong, Y., Tolias, K., Carpenter, C. and Chua, N. H., Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J. Cell Biol.*, 1999, **145**, 317–330.
36. Li, H., Lin, Y., Heath, R. M., Zhu, M. X. and Yang, Z., Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell*, 1999, **11**, 1731–1742.
37. Vidali, L. and Hepler, P. K., Characterization and localization of profilin in pollen grains and tubes of *Lilium longiflorum*. *Cell Motil. Cytoskeleton*, 1997, **36**, 323–338.
38. Rathore, K. S., Cork, R. J. and Robinson, K. R., A cytoplasmic gradient of Ca^{2+} is correlated with the growth of lily pollen tubes. *Dev. Biol.*, 1991, **148**, 612–619.
39. Wang, Y. F., Fan, L. M., Zhang, W. Z., Zhang, W. and Wu, W. H., Ca^{2+} -permeable channels in the plasma membrane of *Arabidopsis*

- pollen are regulated by actin microfilaments. *Plant Physiol.*, 2004, **136**, 3892–3904.
40. Heslop-Harrison, J., Heslop-Harrison, Y., Cresti, M., Tiezzi, A. and Moscatelli, A., Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. *J. Cell Sci.*, 1988, **91**, 49–60.
 41. Joos, U., van Aken, J. and Kristen, U. The anti-microtubule drug carbetamide stops *Nicotiana sylvestris* pollen tube growth in the style. *Protoplasma*, 1995, **187**, 182–191.
 42. He, Y., Palevitz, B. A. and Wetzstein, H. Y., Pollen germination, tube growth and morphology, and microtubule organization after exposure to benomyl. *Physiol. Plant.*, 1996, **96**, 152–157.
 43. Geitmann, A., Li, Y.-Q. and Cresti, M., The role of cytoskeleton and dictyosome activity in the pulsatory growth of *Nicotiana tabacum* and *Petunia hybrida* pollen tubes. *Bot. Acta*, 1995, **109**, 102–109.
 44. Heslop-Harrison, J. and Heslop-Harrison, Y., Organelle movement and fibrillar elements of the cytoskeleton in the angiosperm pollen tube. *Sex. Plant Reprod.*, 1988, **1**, 16–24.
 45. Kohno, T. and Shimmen, T., Accelerated sliding of pollen tube organelles along *Characeae* actin bundles regulated by Ca^{2+} . *J. Cell Biol.*, 1988, **106**, 1539–1543.
 46. Kohno, T., Chaen, S. and Shimmen, T., Characterization of the translocator associated with pollen tube organelles. *Protoplasma*, 1990, **154**, 179–183.
 47. Mascarenhas, J. P. and Lafountain, J., Protoplasmic streaming, cytochalasin B, and growth of the pollen tube. *Tissue Cell*, 1972, **4**, 11–14.
 48. Lancelle, S. A. and Hepler, P. K., Cytochalasin-induced ultrastructural alterations in *Nicotiana* pollen tubes. *Protoplasma*, 1988, **2**, 65–75.
 49. de Win, A. H., Pierson, E. S. and Derksen, J., Rational analyses of organelle trajectories in tobacco pollen tubes reveal characteristics of the actomyosin cytoskeleton. *Biophys. J.*, 1999, **76**, 1648–1658.
 50. Cardenas, L., Lovy-Wheeler, A., Wilsen, K. L. and Hepler, P. K., Actin polymerization promotes the reversal of streaming in the apex of pollen tubes. *Cell Motil. Cytoskeleton*, 2005, **61**, 112–127.
 51. Li, Y., Zee, S. Y., Liu, Y.-M., Huang, B. Q. and Yen, L.-F., Circular F-actin bundles and a G-actin gradient in pollen and pollen tubes of *Lilium davidii*. *Planta*, 2001, **213**, 722–730.
 52. Tang, X., Hepler, P. K. and Scordilis, S. P., Immunochemical and immunocytochemical identification of a myosin heavy chain polypeptide in *Nicotiana tabacum* pollen tube. *J. Cell Sci.*, 1989, **92**, 569–574.
 53. Tirlapur, U. *et al.*, Confocal imaging and immunogold electron microscopy of changes in distribution of myosin during pollen hydration, germination and pollen tube growth in *Nicotiana tabacum* L. *Eur. J. Cell Biol.*, 1995, **67**, 209–217.
 54. Miller, D. D., Scordilis, S. P. and Hepler, P. K., Identification and localization of three classes of myosins in pollen tubes of *Lilium longiflorum* and *Nicotiana glauca*. *J. Cell Sci.*, 1995, **108**, 2549–2563.
 55. Lee, Y. R. J. and Liu, B., Cytoskeletal motors in *Arabidopsis*. sixty-one kinesins and seventeen myosins. *Plant Physiol.*, 2004, **136**, 3877–3883.
 56. Yokota, E. and Shimmen, T., Isolation and characterization of plant myosin from pollen tubes of lily. *Protoplasma*, 1994, **177**, 153–162.
 57. Yokota, E., McDonald, A. R., Liu, B., Shimmen, T. and Palevitz, B. A., Localization of a 170 kDa myosin heavy chain in plant cells. *Protoplasma*, 1995, **185**, 178–187.
 58. Yokota, E., Muto, S. and Shimmen, T., Inhibitory regulation of higher-plant myosin by Ca^{2+} ions. *Plant Physiol.*, 1999, **119**, 231–240.
 59. Tiezzi, A., Moscatelli, A., Cai, G., Bartalesi, A. and Cresti, M., An immunoreactive homolog of mammalian kinesin in *Nicotiana tabacum* pollen tubes. *Cell Motil. Cytoskeleton*, 1992, **21**, 132–137.
 60. Cai, G., Bartalesi, A., Del Casino, C., Moscatelli, A., Tiezzi, A. and Cresti, M., The kinetin-immunoreactive homologue from *Nicotiana tabacum* pollen tube: biochemical properties and subcellular localization. *Planta*, 1993, **191**, 496–506.
 61. Cai, G., Romagnoli, S., Moscatelli, A., Ovidi, E., Gambellini, G., Tiezzi, A. and Cresti, M., Identification and characterization of a novel microtubule-based motor associated with membranous organelles in tobacco pollen tubes. *Plant Cell*, 2000, **12**, 1719–1736.
 62. Romagnoli, S., Cai, G. and Cresti, M., *In vitro* assays demonstrate that pollen tube organelles use kinesin-related motor proteins to move along microtubules. *Plant Cell*, 2003, **15**, 251–269.
 63. Palevitz, B. A. and Tiezzi, A., Organization, composition and function of the generative cell and sperm cytoskeleton. *Int. Rev. Cytol.*, 1992, **140**, 149–185.
 64. Heslop-Harrison, J. and Heslop-Harrison, Y., Myosin associated with the surface of organelles, vegetative nuclei and generative cells in angiosperm pollen grains and tubes. *J. Cell Sci.*, 1989, **94**, 319–325.
 65. Zhang, Z., Tian, H. Q. and Russell, S. D., Immunogold scanning electron microscopic localization of myosin on isolated sperm cells of tobacco (*Nicotiana tabacum* L.). *Protoplasma*, 1999, **208**, 123–128.
 66. Zhang, Z. and Russell, S. D., Sperm cell surface characteristics of *Plumbago zeylanica* L. in relation to transport in the embryo sac. *Planta*, 1999, **208**, 539–544.
 67. Astrom, H., Sorri, O. and Raudaskoski, M., Role of microtubules in the movement of the vegetative nucleus and generative cell in tobacco pollen tubes. *Sex. Plant Reprod.*, 1995, **8**, 61–69.
 68. Wasteneys, G. O., Progress in understanding the role of microtubules in plant cells. *Curr. Opin. Plant Biol.*, 2004, **7**, 651–660.