Protoplasma (2006) 228: 21–30
DOI 10.1007/s00709-006-0162-6

PROTOPLASMA
Printed in Austria

Signalling pathways in pollen germination and tube growth

R. Malhó[1,*], **Q. Liu**[1], **D. Monteiro**[1], **C. Rato**[1], **L. Camacho**[1,2], and **A. Dinis**[3]

[1] Departamento de Biologia Vegetal, Instituto de Ciência Aplicada e Tecnologia, Faculdade de Ciências, Universidade de Lisboa, Lisbon
[2] School of Biological and Biomedical Sciences, University of Durham, Durham
[3] Departamento de Botânica, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra

Received March 22, 2005; accepted June 8, 2005; published online August 31, 2006
© Springer-Verlag 2006

Summary. Signalling is an integral component in the establishment and maintenance of cellular identity. In plants, tip-growing cells represent an ideal system to investigate signal transduction mechanisms, and among these, pollen tubes (PTs) are one of the favourite models. Many signalling pathways have been identified during germination and tip growth, namely, Ca^{2+}, calmodulin, phosphoinositides, protein kinases, cyclic AMP, and GTPases. These constitute a large and complex web of signalling networks that intersect at various levels such as the control of vesicle targeting and fusion and the physical state of the actin cytoskeleton. Here we discuss some of the most recent advances made in PT signal transduction cascades and their implications for our future research. For reasons of space, emphasis was given to signalling mechanisms that control PT reorientation, so naturally many other relevant works have not been cited.

Keywords: Calcium ion; Calmodulin; D-*myo*-Inositol-1,4,5-trisphosphate; Phosphatidylinositol-4,5-bisphosphate; Phosphatidic acid; Secretion.

Abbreviations: ABP actin-binding protein; AC adenylyl cyclase; $[Ca^{2+}]_c$ cytosolic free calcium; CaM calmodulin; CDPK Ca^{2+}-dependent protein kinase; IP3 D-*myo*-inositol-1,4,5-trisphosphate; MF microfilament; MT microtubule; PA phosphatidic acid; PIP2 phosphatidylinositol-4,5-bisphosphate; PLC phospholipase C; PLD phospholipase D; PKH pollen kinesin homologue; PT pollen tube.

Ca^{2+}, central signalling molecule

Cytosolic free calcium ($[Ca^{2+}]_c$) is a key element in the regulation of pollen tube (PT) elongation and guidance. A tip-focused $[Ca^{2+}]_c$ gradient has been imaged with a high 1–3 μM Ca^{2+} concentration in the tip region and a low 0.2–0.3 μM Ca^{2+} concentration in the subapical and basal part of the tube (Malhó et al. 1995, Pierson et al. 1996, Messerli and Robinson 1997). This gradient is absent in nongrowing PTs and its disruption leads to the inhibition of tube growth. Both the intracellular $[Ca^{2+}]_c$ gradient and extracellular Ca^{2+} influx suffer sinusoidal oscillations which are accompanied by oscillations in growth rate (Holdaway-Clarke and Hepler 2003). The phase relationships differ, with the gradient being delayed from growth by about 4 s, while the influx is delayed by about 11–13 s.

A substantial body of evidence reveals that Ca^{2+} controls cytoplasmic streaming. At basal levels (100–200 nM), streaming occurs normally; when the concentration is elevated to 1 μM or higher, streaming is rapidly but reversibly inhibited (Taylor and Hepler 1997). This applies also to PTs, which show a marked inhibition of streaming when the intracellular $[Ca^{2+}]_c$ is elevated and a recovery when the concentration returns to basal levels (Yokota et al. 1999). High Ca^{2+} is known to fragment filamentous actin (F-actin) (Yokota et al. 1998), which explains why thick microfilaments (MFs) are not visible in the tube apex. These inhibitory and fragmentary activities are certainly brought about by specific actin-binding proteins, namely, villin (Vidali et al. 2001). $[Ca^{2+}]_c$ at the tube apex is also sufficiently high to inhibit myosin motor activity and thus to inhibit streaming. Therefore, apical $[Ca^{2+}]_c$ may have an important role in slowing or stopping motion and, as a consequence, in the local regulation of vesicle docking and fusion with the plasma membrane. These events are intimately correlated with PT reorientation. As has been shown (Malhó and Trewavas 1996), modification of $[Ca^{2+}]_c$ at the tube apex induces bending of the growth axis toward the zone of higher $[Ca^{2+}]_c$.

PT growth depends on polarised exocytosis at the growing tip and Ca^{2+} was suggested to be involved in this

* Correspondence and reprints: Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.
E-mail: r.malho@fc.ul.pt

process (Malhó et al. 2000). Measurements of endo- and exocytosis in growing PTs with the fluorescent probe FM 1-43 showed a fluorescence hotspot at the apex (Camacho and Malhó 2003), and when the tube reorients, the side of the dome to which the cell bent became more fluorescent. This suggests vesicle relocation and an asymmetric distribution of the fusion events. The cargo which is released upon vesicle fusion provides the material for the construction of the new cell wall needed for growth (not simply elongation of existing wall). These results strongly resemble those obtained when $[Ca^{2+}]_c$ was mapped during this process (Malhó and Trewavas 1996). This suggests that $[Ca^{2+}]_c$ is a regulator of the coupling between growth and endo- and exocytosis. The results of Camacho and Malhó (2003) also suggest that cell growth is not strictly dependent on a Ca^{2+}-mediated stimulation of exocytosis. This is in agreement with the results of Roy et al. (1999) using the Yariv reagent thus suggesting that a Ca^{2+}-dependent exocytosis serves mainly to secrete cell wall components. This explains why rises in apical Ca^{2+} alone led to augmented secretion and reorientation of the growth axis but not to an increase in growth rates.

The mechanisms which regulate $[Ca^{2+}]_c$ at the PT apex are still controversial. In a study combining measurements of intracellular free Ca^{2+} and Ca^{2+} fluxes, Holdaway-Clarke et al. (1997) found an apparent discrepancy between internal Ca^{2+} measurements and external Ca^{2+} fluxes, with the fluxes an order of magnitude greater than the amount needed to support the observed intracellular apical Ca^{2+} gradient. On the basis of these findings, it was suggested that the cell wall could act as a buffer for Ca^{2+}. Pectin-methyl-esterases responsible for cross-linking unesterified pectins were proposed to control the availability of cell wall binding sites for Ca^{2+}. However, aequorin measurements of apical $[Ca^{2+}]_c$ (Messerli et al. 2000) suggest that peak values for $[Ca^{2+}]_c$ in the apical region are about one order of magnitude higher than previous estimates using Ca^{2+}-sensitive dyes thus challenging this hypothesis. An alternative explanation is that $[Ca^{2+}]_c$ influx is regulated by capacitative entry. Trewavas and Malhó (1997) suggested that the ion channels in tip-growing cells might have their activity regulated by the degree of emptiness of intracellular Ca^{2+} stores; when these stores are full, plasma membrane channels would close, and vice versa.

Calmodulin, Ca^{2+} sensor and cross-regulator

Calmodulin (CaM) is a Ca^{2+} sensor known to modulate the activity of many proteins. Experiments with fluorescently labelled CaM microinjected into living PTs suggested that the protein distributes evenly (Moutinho et al. 1998b). The same authors observed also that the binding capacity of labelled CaM appears to be more intense in the subapical region, suggesting a higher concentration of CaM target molecules, possibly cytoskeletal elements. The MF distribution observed in the subapical region of PTs (Miller et al. 1996) resembles the V-shaped collar reported for CaM binding (Moutinho et al. 1998b) and thus a direct interaction between CaM and actin has been hypothesised. For *Saccharomyces cerevisiae*, it has been shown that CaM controls organisation of the actin cytoskeleton via regulation of phosphatidylinositol 4,5-bisphosphate (PIP_2) synthesis (Desrivières et al. 2002), thus linking CaM to the phosphoinositide signalling pathway.

An even distribution of CaM does not necessarily mean an even functionality. CaM activity is dependent on binding of Ca^{2+} ions, thus, on $[Ca^{2+}]_c$. High levels of $[Ca^{2+}]_c$, such as those found in the PT apex, may result in localised activation of CaM. Rato et al. (2004) showed that CaM activity is higher in the apex of growing PTs and the area of higher activity superimposes to a considerable degree with the tip-focused $[Ca^{2+}]_c$ gradient, suggesting that the latter might be involved in the control of CaM activity. Furthermore, it was found that CaM activity oscillates with a period similar to $[Ca^{2+}]_c$ (40–80 s). We have also shown, as with the manipulation of $[Ca^{2+}]_c$ in the apex (Malhó and Trewavas 1996), that a decrease in CaM levels in one side of the apical dome led to growth axis reorientation to the opposite side. This clearly involves CaM in the molecular events that control PT guidance. CaM might also participate in a feedback regulation of Ca^{2+} stores. In PTs, $[Ca^{2+}]_c$ drops within a few micrometers from about 1–3 μM in the apex to 200–220 nM in the subapical region (Messerli and Robinson 1997). Thus, a tight control between Ca^{2+} channels and pumps in this latter region must exist. CaM regulation of Ca^{2+} stores and D-*myo*-inositol-1,4,5-trisphosphate (IP_3) receptors (Ainger et al. 1993, Patel et al. 1997) suggests that CaM may allow both feedback control of membrane receptors and integration of inputs from other signalling pathways.

In addition to a role for Ca^{2+} in the control of CaM activation, Rato et al. (2004) provided evidence that a cyclic AMP (cAMP) signalling pathway is involved. A cAMP-dependent signalling pathway in pollen was recently shown (Moutinho et al. 2001) and cAMP levels were found to be approximately uniform in the pollen tube cytosol but showing transient increases in the apical region upon reorientation and apical perturbations. CaM thus emerges as a strong candidate to integrate signals between

Ca^{2+} and cAMP signalling pathways. Rato et al. (2004) found that treatment with forskolin (which increases cAMP levels [Moutinho et al. 2001]) and with a cAMP analog, 8-Br-cAMP, results also in a transient increase in CaM activity. Similarly, treatment with 2′,5′-dideoxyadenosine, an inhibitor of adenylyl cyclase, induced a transient decrease in CaM activity suggesting that the activation of downstream targets of cAMP is involved in the regulation of CaM activity, possibly through $[Ca^{2+}]_c$.

The actin cytoskeleton and the secretory apparatus are putative candidates for a cross-regulation between CaM and cAMP signalling pathways. A growing body of evidence implicates CaM as an important receptor linking changes in Ca^{2+} with cytoskeletal function (Zielinski 1998), for example, villin (P-135-ABP) (Yokota et al. 2000) and myosin (Yokota 2000). Also, Ca^{2+}-CaM is known to play a key role in the control of secretory activity in plant cells (Schuurink et al. 1996), and Rato et al. (2004) found that a decrease in CaM levels on one side of the apical dome results in a decrease of secretory activity and reorientation. Diminishing cAMP levels mimicked this effect, while an increase of cAMP (which augments CaM activity) promoted secretion. These data further support our claim for a close relationship between Ca^{2+}-CaM and intracellular cAMP in the control of pollen tube secretion.

Phosphoinositides and phospholipids – intersecting signalling pathways

Phosphoinositides are a vast family of molecules playing a major role in signalling, but their contribution for polar growth is still largely unknown (Malhó and Camacho 2004). PIP_2 has been shown to act in a common pathway with Rac GTPases (Kost et al. 1999) and IP_3 is known to modulate Ca^{2+} levels (Franklin-Tong et al. 1996, Malhó 1998). Inositol 3,4,5,6-tetra*kis*phosphate [Ins(3,4,5,6)P_4] was found to disrupt Cl^- efflux (Zonia et al. 2002) and Potocký et al. (2003) showed that apical growth depends on phospholipase D (PLD) activity. Therefore, it seems possible that phosphoinositides intersect multiple signalling pathways and are key regulators of polarity.

The intact PIP_2 molecule is a central player in actin dynamics, vesicle trafficking, and ion transport (Cremona et al. 1999, Stevenson et al. 2000) due to its ability to bind and regulate many proteins containing PIP_2 recognition domains, such as pleckstrin homology domains, basic patches, and epsin N-terminal homology domains (Martin 1998, Cockcroft and De Matteis 2001). Through phospholipase C (PLC), PIP_2 generates IP_3 and diacylglycerol, which can be converted to phosphatidic acid (PA) through diacylglycerol kinase (Munnik 2001). PIP_2 is also known to govern PLD activity leading to elevated PA formation (Powner et al. 2002). Multiple PLD genes have been identified in plants and the proteins they code for seem to be regulated by Ca^{2+} and G proteins (Zheng et al. 2000, Munnik 2001). Activation of plant PLDs is triggered by various cues, namely, pathogen elicitation (Young et al. 1996), and we recently described a pollen signalling protein (PsiP) involved in cAMP production sharing great homology with defense proteins (Moutinho et al. 2001).

IP_3 is possibly the most studied signalling phosphoinositide and is a potent mobilizer of Ca^{2+} from intracellular stores (Martin 1998). In pollen tubes, an IP_3-induced Ca^{2+} release seems to be required for transduction of signals from the apex to further regions of the cell (Malhó 1998). It was further suggested that IP_3 receptors may have an asymmetric activity depending on their spatial localisation: in the apex, where Ca^{2+} is elevated, the receptor undergoes an intrinsic inactivation when IP_3 is bound; in subapical regions, where Ca^{2+} is in the nanomolar range, increasing $[Ca^{2+}]_c$ potentiates Ca^{2+} release by IP_3 to the extent that Ca^{2+} and IP_3 can be regarded as coagonists for Ca^{2+} release (Dawson 1997). In animal cells, IP_3 receptor-like proteins were shown to be linked to actin filaments (Fujimoto et al. 1995), thus linking phosphoinositides to the cytoskeleton organisation.

In a recent work (Monteiro et al. 2005), we found that changes in the intracellular concentration of both PIP_2 and IP_3 (induced by photolysis of caged probes) modified tube growth and caused reorientation of the growth axis. However, measurements of $[Ca^{2+}]_c$ and the apical secretion revealed significant differences between the photorelease of PIP_2 and IP_3. When released within the first 50 μm of the pollen tube, PIP_2 led to transient growth perturbation, increase of $[Ca^{2+}]_c$, and inhibition of apical secretion. In contrast, a concentration of IP_3 which caused a transient $[Ca^{2+}]_c$ of similar magnitude, stimulated apical secretion and caused severe growth perturbation. Furthermore, the transient $[Ca^{2+}]_c$ induced by IP_3 was spatially different, causing a pronounced elevation in the subapical region. These observations suggest the existence of different targets for the two phosphoinositides. One of the targets was suggested to be PA, since this phospholipid is an end-product of PIP_2 hydrolysis via PLC or promotion of PLD activity. This phospholipid promotes membrane curvature and formation of secretory vesicles along with a crucial role in the structural integrity of the Golgi apparatus (Sweeny et al. 2002). It has been further demonstrated that continual production of PA is essential for cytoskeleton reorganisation (O'Luanaigh et al.

2002). As part of a feedback loop, PA can promote PIP_2 formation by phosphatidylinositol 4-phosphate 5-kinase (Anderson et al. 1999).

We found that antagonists of PA accumulation (e.g., butan-1-ol) and inhibitors of PLC and PLD reversibly halted polarity (Monteiro et al. 2005). Reduction of PA levels caused dissipation of the $[Ca^{2+}]_c$ gradient and inhibited apical plasma membrane recycling. Also, it was found to cause an extensive bundling of actin and abolition of the apical zonation. These data suggest that phosphoinositides and phospholipids regulate tip growth through a multiple-pathway system involving regulation of $[Ca^{2+}]_c$ levels, endo- and exocytosis, and MFs rearrangement.

Protein kinases and phosphorylation cascades

Phosphorylation cascades regulated by protein kinases and phosphatases represent primary, downstream transduction routes so it is tempting to speculate that their presence is associated with intensely signalling cells such as PTs. Many protein kinases have been identified in pollen but mainly associated with plasma membrane receptors or to cell cycle regulation. These will not be discussed here and we refer to other references (Rudd and Franklin-Tong 2001, Kachroo et al. 2002).

In plants, many of the kinases identified belong to the Ca^{2+}-dependent protein kinase or CaM-like domain protein kinase (CDPK) superfamily (Harmon et al. 2000). Among other targets, CDPKs have been suggested to be involved in the regulation of tension. Myosin and/or actin cross-linking proteins are likely to be the principal regulator(s) of tension within the actin network, and thus the principal targets of CDPKs. Results from experiments utilising CaM inhibitors suggest that the effect of Ca^{2+} on the tension within the actin network is CaM-dependent and involves the coordinate regulation of a CDPK and/or CaMK and a CaM-dependent phosphatase (Grabski et al. 1998). In PTs, a CDPK antibody was found to colocalise with F-actin (Putnam-Evans et al. 1989), while Moutinho et al. (1998a) reported the presence of a CDPK with a higher activity in the apical region of growing cells. When apical Ca^{2+} values were modulated (using caged probes), the activity of this CDPK changed accordingly and induced reorientation of the growth axis. This suggests that the protein kinase activity measured by Moutinho and co-workers and the one reported by Putnam-Evans et al. (1989) correspond to different members of this family. However, Putnam-Evans et al. (1989) did not show data of how the antibody distributes in the apical region and, therefore, a direct comparison between the different results cannot be established. More definitive conclusions require further results.

The role of cyclic nucleotides – an open question

The role of cyclic nucleotides in plant cell signalling has been clouded by many uncertainties and much controversy. In pollen, cloning of a putative adenylyl cyclase (AC) revealed motifs common with its fungal counterpart (Moutinho et al. 2001) but also with proteins involved in disease responses. cAMP is believed to be involved in such responses (Cooke et al. 1994) and parallels between PT growth in the style and fungal hyphal infection are frequently drawn. The transformation with antisense oligos directed against the pollen AC cDNA or treatment with antagonists of AC caused disruption of PT growth, suggesting a requirement for continued cAMP synthesis. This was supported by the imaging of cAMP in growing PTs, by which it was observed that forskolin, an AC activator, transiently increased cAMP, whilst dideoxyadenosine, an inhibitor of ACs, caused a temporary decline (Moutinho et al. 2001).

The targets of the cAMP signalling pathway are still largely unknown. cAMP may be involved in the regulation of Ca^{2+} levels (Volotovski et al. 1998). PTs submitted to different treatments that putatively affect the levels of cAMP (photolysis of caged cAMP, external addition of membrane-permeant cAMP, external addition of forskolin), experienced $[Ca^{2+}]_c$ transients. After caged release of cAMP at a concentration estimated to be about 1–2 μM, growth rates temporally declined and reorientation of the tube growth axis occurred. This was accompanied by a transient $[Ca^{2+}]_c$ elevation in the apex but not in the subapical region. In contrast, the addition of external cAMP led to a complete growth arrest and a $[Ca^{2+}]_c$ increase in the apical and subapical regions. When compared to the release of a small concentration of cAMP after flash photolysis, this suggests a toxic effect and a drastic perturbation of ion fluxes. Thus, a cAMP pathway might be involved in PT reorientation which most likely acts together with Ca^{2+} to control tube reorientation. Interestingly, experiments with a membrane-permeant version of cGMP diffusing from a microneedle placed near the tip of growing PTs failed to cause a visible response (Moutinho et al. 2001). Prado et al. (2004) suggested that cGMP could participate in the signaling cascade that affects growth regulation. However, the authors acknowledged that the drugs used in this work clearly disrupted growth regulation, so the

pleiotropic responses made it difficult to isolate or test its specificity. Further experimentation is required to evaluate the role of other cyclic nucleotides in PT growth and reorientation.

GTPases, the signaling switches

Small GTP-binding proteins (GTPases) are versatile signalling switches responsible for an extensive communication and cooperation between signal transduction pathways (Bar-Sagi and Hall 2000). They all belong to the Ras superfamily which includes 5 families: Ras, Rab, Arf, Ran, and Rho. Rab is the largest family of small GTPases that control the transport and docking of specific vesicles. A Rab2 homologue, necessary for membrane traffic between endoplasmic reticulum and Golgi apparatus in mammalian cells, is present in *Arabidopsis thaliana* pollen grains (Moore et al. 1997) and was recently shown to be important for PT growth (Cheung et al. 2002). The authors found that a pollen-predominant Rab2 functions in the secretory pathway between the endoplasmic reticulum and the Golgi apparatus in elongating PTs. Green-fluorescent protein (GFP)–NtRab2 fusion protein localised to Golgi bodies and dominant-negative mutations in NtRab2 proteins inhibited this localisation, blocked the delivery of Golgi-resident as well as plasmalemma and secreted proteins to their normal locations, and inhibited PT growth. These observations indicate that NtRab2 is important for trafficking between the endoplasmic reticulum and the Golgi bodies in PTs and may be specialised to optimally support the high secretory demands in these tip growth cells.

Rho GTPases regulate the actin cytoskeleton organisation and cell polarity development, control also gene expression, cell wall synthesis, H_2O_2 production, endocytosis, exocytosis, cytokinesis, cell cycle progression, and cell differentiation in various eukaryotic organisms (Yang 2002). In plants, these GTPases are named ROP (for Rho-related GTPase from plants) (Yang 2002) and they have been shown to play a significant role in PT elongation in *A. thaliana*, pea, and tobacco (Yang and Watson 1993, Lin and Yang 1997, Kost et al. 1999). In both *A. thaliana* and tobacco, ROPs were found to accumulate at the tube tip and similar observations were made in root hairs (Molendjik et al. 2001). In pollen, the constitutively active, GTP-bound form led to swollen tip growth, whereas the dominant-negative, GDP-bound form led to cessation of tube growth, which proves that the molecule has a central role in the regulation of tube extension. At the PT tip, ROPs seem to activate phosphatidylinositol kinase leading to the formation of PIP_2, which could function in release of Ca^{2+}. Transient expression of the mutant GTP- and GDP-bound proteins in tobacco PTs led to the formation of extensive and reduced actin cables, respectively, which shows that ROPs play a role in PT actin dynamics (Kost et al. 1999).

GTP-binding proteins have also been claimed to regulate endo- and exocytosis (Li et al. 1999, Lin et al. 1996) and the relationships between exocytosis, endocytosis, and the actin cytoskeleton in the growing PT tip make an interesting question to which no clear answer can yet be provided. The nonhydrolysable analogues of guanine nucleotide interfere in biological processes and it was found that GDPβS decreases growth rate, while GTPγS has the opposite effect (Ma et al. 1999, Camacho and Malhó 2003), thus confirming the importance of GTPases for apical growth. The release of GTPγS was also found to promote exocytosis, a process which seems to be more dependent on the active state of GTPases rather than on the cycling between the GTP- and the GDP-bound state (Zheng and Yang 2000). Selective exposure to elevated Ca^{2+} alone is not sufficient to explain the selectivity of membrane retrieval (Smith et al. 2000). It is therefore possible that GTPases play an active role in endo- and exocytosis by coupling the actin cytoskeleton to the sequential steps underlying membrane trafficking at the site of exocytosis.

Which signals activate the ROP signalling pathway and confine its activity to the tube apex is not clear. Li et al. (1999) previously reported an interaction between Ca^{2+} and the expression of a ROP protein. These authors demonstrated that inhibition of AtRop1 proteins modifies Ca^{2+} influx but could not determine the inhibition of the Ca^{2+} influx altogether. It was actually found that growth inhibition was reversed by higher extracellular Ca^{2+}, which means that influx was still possible. Camacho and Malhó (2003) made similar observations supporting a model in which Ca^{2+} and Rop GTPases act differentially, but in a concerted form, in the sequential regulation of PT secretion and membrane retrieval. However, inhibition (or knockout) of any protein crucial for tip growth will certainly affect the Ca^{2+} gradient either directly or through a signalling loop. Although there may be several different interpretations to these data, we suggest that Ca^{2+} plays a major role in the secretion of cell wall components, while Rop GTPases play a key role in the fusion of docked vesicles and endocytosis. This hypothesis is supported by the recent identification of two structurally distinct putative ROP1 targets in the control of polar growth in PTs, RIC3 and RIC4 (Wu et al. 2001). It is clear, though, that the exact position of these elements in the signalling cascade controlling PT growth and orientation is still dubious and requires further investigation.

Signalling pathways converge in the actin cytoskeleton

The actin cytoskeleton, along with the secretory apparatus, is a structural component in which most PT signalling pathways seem to converge. In the shank of the PT, thick bundles of MFs are seen axially or sometimes helically oriented throughout the cortical and central cytoplasm of the tube cell (Miller et al. 1996). Towards the apex, these bundles of MFs branch into thinner bundles, and in the subapical region, a dense network of fine, labile actin filaments is formed. The latter often appears diffuse but in some PTs it was reported to form a cortical ring (Kost et al. 1998) or a funnel-shaped structure (Vidali et al. 2001). In growing PTs expressing GFP-talin (Kost et al. 1998) the labelling at the tube apex revealed little or no MFs. Fu et al. (2001) demonstrated that very dynamic short actin bundles, correlating with the oscillatory growth rate, transiently penetrate into the extreme tip of tobacco pollen tubes. The scarcity of MFs and/or the lesser degree of MFs organisation at the tip of the growing tube was suggested to be due to the tip-focused Ca^{2+} gradient indispensable for the growth of the PT (Malhó et al. 1995, Pierson et al. 1996).

MFs have been reported to act as the main tracks along which organelles are transported by means of myosin molecules (Taylor and Hepler 1997). This hypothesis is based on motility assays with isolated organelles and treatment with pharmacological agents (e.g., cytochalasin and latrunculin) (Vidali et al. 2001). These data demonstrate that MFs, together with myosins, are essential for cytoplasmic streaming and the polarised growth of the PT. However, we cannot exclude the possibility of some organelle movement through an actin-polymerisation-based mechanism, as was demonstrated for peroxisomes and mitochondria (Mathur et al. 2002). In this model, the movement is independent of the motor protein and is probably generated by an asymmetric actin polymerisation at the surface of the organelle (Vantard and Blanchoin 2002). Recently, Wasteneys and Galaway (2003) suggested that, while the subcortical MFs bundles function in cytoplasmic streaming, the cortical actin could play a role both in biomechanical sensing during growth and in anchoring the subcortical MF bundles. It is noteworthy that wall membrane adhesions involving cortical F-actin were reported to occur along the length of pollen tubes grown in vivo (Lord and Sanders 1992). The role of these adhesions is still unknown, but in animal cells, adhesions to the extracellular matrix are known to be involved in signal transduction (Palanivelu and Preuss 2000). Recent data from plants also support the concept whereby the dynamic actin cytoskeleton is closely linked to the signalling cascades initiated at the plasma membrane (Baluška et al. 2003). Thus, it is likely that in in vivo grown PTs specific arrangements of the cortical F-actin may be induced in response to stimuli from the transmitting tissue.

The typical F-actin array in the subapical region was hypothesised to serve as a buffer to retain vesicles in this area and to deliver the vesicles onwards to the apical dome. In addition, it may also play a role in the positioning of the endocytotic machinery to a precise microdomain of the PT plasma membrane and to define the tip region by restricting the exit of calcium channels, secretory vesicles, and other components necessary for regulated exocytosis (Staiger 2000). A recent study comparing the cytomechanical properties of growing PTs with the precise configuration of the actin cytoskeleton suggest that the actin in the subapex may also have a role in the control of tube diameter (Geitmann and Parre 2004). According to Vidali et al. (2001), it is in this subapical region that the rapidly formed MFs are presumably incorporated into bundles extending through the shank of the PT. Vantard and Blanchoin (2002) suggest that the elongation of actin filaments near the tip may create part of the force necessary to push forward the apical region and generate PT growth.

Proteins homologous to those that regulate the structure and function of the actin cytoskeleton in animal and yeast cells have been identified in PTs (Vidali and Hepler 2001). In *Lilium longiflorum*, antibodies raised against myosin I, II, and VII labelled different structures (Miller et al. 1995) but analysis of the myosins in the *A. thaliana* genome sequence has revealed that plant myosins fall into two groups – class VIII and class XI (Reddy and Day 2001). Myosins XII are closely related to class V. Studies on the localisation and function of the pollen myosins are thus in demand. Several genes encoding small actin-binding proteins have also been characterised from pollen (Staiger et al. 1993, Mittermann et al. 1995, Lopez et al. 1996, Gibbon et al. 1998). These proteins are potent sensors of the pollen tube ionic and signalling environment and they may contribute to the regulation of actin dynamics. For example, cofilin and profilin are small polypeptides that have been suggested to have similar and opposite effects on MF dynamics (Bamburg 1999). Cofilin belongs to the ADF (actin depolymerising factor) protein family, whose members interact with actin monomers and filaments in a pH-sensitive manner. The properties of plant cofilin have been analysed with recombinant proteins from the maize pollen-specific cofilin gene *ZmADF1* (Hussey et al. 1998) and from *ZmADF3*, which is suppressed in pollen but expressed in all other maize tissues.

ZmADF3 has the ability to bind both the monomeric (globular) actin and F-actin and to decrease the viscosity of polymerised actin solutions, indicating an ability to depolymerise actin filaments (Lopez et al. 1996). ZmADF3 is phosphorylated on Ser6 by a CDPK in plant extracts (Smertenko et al. 1998), which suggests that phosphorylation and dephosphorylation could regulate the actin-binding ability of cofilin and thus affect actin cytoskeleton stability. ADF has also been shown to mediate Rop GTPase-regulated PT growth (Chen et al. 2003). Profilin is a globular-actin binding protein that is known to interact with proline-rich motifs of other proteins and with phosphoinositides. Pollen profilins act as actin-sequestering proteins that promote actin depolymerisation (Staiger et al. 1994). It has been shown that one of the profilins expressed in PTs shows higher proline-rich–motif binding capacity than others (Gibbon et al. 1998), and that the actin-sequestering activity of profilins is dependent on Ca^{2+}concentration (Staiger 2000). More recently, Cheung and Wu (2004) reported the presence of formins, which are actin-nucleating proteins that stimulate actin cable formation. A pollen villin was also isolated from *L. longiflorum* PTs as a 135 kDa actin-bundling protein (Yokota and Shimmen 1999) which colocalises to MFs bundles (Vidali et al. 1999). Ca^{2+} and CaM together inhibit the actin-bundling activity of this PT villin (Yokota et al. 2000), adding villin to the group of Ca^{2+}-regulated actin-binding proteins. The pollen villin also includes a phosphoinositide-binding domain (Vidali et al. 1999) thus making this protein a putative target of the phosphoinositide signalling pathway.

Microtubules – just a structural role?

Compared with MFs, the role and dynamics of microtubules (MTs) in PTs have received less attention. MTs were shown to be present within both the vegetative and the generative cells and sperm cells of PTs (Lancelle and Hepler 1991). The reports dealing with the cytoskeleton in the generative and sperm cells of angiosperms agree in showing a prominent system of MT bundles aligned helically or longitudinally to the long axis of the cell (Palevitz and Tiezzi 1992, Raudaskoski et al. 2001). These MT bundles, in which single MTs are often interconnected by abundant cross-bridges, are organised as a slightly twisted basket or cage under the plasma membrane and often prolong into the proximal end of the cell forming a tail-like structure. Their presence within the generative and sperm cells has been correlated to the establishment and/or maintenance of the typical shape of these cells and to the reshaping process that occurs during generative and sperm cell movements along the PT. This is probably mediated by intermicrotubule sliding (Palevitz and Tiezzi 1992). In the tube cell, MTs are largely absent from the apical region, while in the subapical region they are short and randomly oriented. In the shank of the tube, MTs are relatively long and thick and are localised primarily in the cell cortex, forming either net-axial or helical arrays (Del Casino et al. 1993, Cai et al. 2000, Derksen et al. 2002). Normally, the uniformity, number, and bundling of MTs increase around and behind the vegetative nucleus–generative cell complex (Raudaskoski et al. 2001). On the basis of data on the MTs distribution it was hypothesised that in long pollen tubes MTs could be nucleated by putative MT-organising centers at the apex and subsequently translocated to the subapical regions of the tube, where they appeared more organized (Moscatelli et al. 1999). The stability of the MT cytoskeleton is also dependent on Ca^{2+} concentration in a CaM-dependent manner (Fisher et al. 1996). The regulation by Ca^{2+} could explain the absence of MTs from the apex, the appearance of short MTs in the subapical region, and prominent MT tracks and spirals in the basal region of the tube behind the nuclei.

The role of MTs in PT growth is still a matter of debate, mainly because the studies to elucidate this role are mostly based on the disruption of the MTs. We now have indications that MTs play a role in controlling the apical transport of secretory vesicles (Cai et al. 1993), in the positioning of nuclei and organelles (Joos et al. 1994), and in pulsed growth (Geitmann et al. 1995). Furthermore, evidence that MTs and actin filaments cooperate functionally to move vesicles and organelles in different types of cells (Goode et al. 2000) suggests that the role of PT MTs in organelle transport could be complementary to actin-based transport. However, the only evidence of interactions between MFs and MTs in the PT is coalignment (Pierson et al. 1986, Lancelle and Hepler 1991); there is no indication that the two systems cooperate functionally during vesicle and organelle transport. A recent study examining the effects of the reversible hyperphosphorylation on the cytoskeleton of *L. longiflorum* tube cells (Foissner et al. 2002) provided evidence that the organisation of MTs may be regulated by MFs.

The involvement of MTs in the translocation of organelles along the tube remains obscure. A recent study based on in vitro motility assays using video-enhanced techniques provived evidence that membrane-bound organelles of pollen tubes move along MTs using kinesin-related motor proteins (Romagnoli et al. 2003). This in vitro organelle movement occurred in the absence of cytosol indicating that

the motor proteins are attached firmly to the surface of the isolated organelles and that no cytosolic factors are required. Noteworthy, MT-based motors of both the kinesin and dynein families have been identified in the PT in association with membrane-bound organelles, which suggests that these proteins could translocate organelles or vesicles along MTs under in vivo conditions as well. A pollen kinesin homolog (Tiezzi et al. 1992) and dynein-related polypeptides (Moscatelli et al. 1995) have been identified in the cytoplasm of PTs and localised in association with membrane structures (Liu et al. 1994, Moscatelli et al. 1998). The pollen kinesin homolog label occurred mainly in the apex and flanks of the tube (Tiezzi et al. 1992, Cai et al. 1993), whereas the 90 kDa ATP-MAP-labelled organelles follow the MT tracks in the basal part of the tube (Cai et al. 2000). The two 400 kDa dynein-related polypeptides localised to membrane structures throughout the PT (Moscatelli et al. 1998). In tobacco, two cDNAs for kinesin-like proteins have been isolated (Wang et al. 1996) and the product of one of these cDNAs, TCK1, binds CaM in a Ca^{2+}-dependent manner. Kinesins with comparable structure are known in *A. thaliana* (Reddy and Day 2001). This indicates a possible communication between microtubular-based movement and the Ca^{2+}-CaM signalling pathway.

Acknowledgments

The work was supported by a UE TMR grant (TIPNET) and Fundação Ciência e Tecnologia, Lisboa, Portugal (grants nr. BCI/37555/2001, BCI/44148/2002; FEDER).

References

Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, Barbarese E, Carson JH (1993) Transport and localisation of exogenous myelin basic protein mRNA microinjected into oligodendrocytes. J Cell Biol 123: 431–441

Anderson RA, Boronenkov IV, Doughman SD, Kunz J, Loijens JC (1999) Phosphoinositol phosphate kinases, a multifaceted family of signalling enzymes. J Biol Chem 274: 9907–9910

Baluška F, Šamaj J, Wojtaszek P, Volkmann D, Menzel D (2003) Cytoskeleton-plasma membrane-cell wall continuum in plants: emerging links revisited. Plant Physiol 133: 482–491

Bamburg JR (1999) Proteins of the ADF/cofilin family: essential regulators of actin dynamics. Annu Rev Cell Dev Biol 15: 185–230

Bar-Sagi D, Hall A (2000) Ras and Rho GTPases: a family reunion. Cell 103: 227–238

Cai G, Bartalesi A, Del Casino C, Moscatelli A, Tiezzi A, Cresti M (1993) The kinesin-immunoreactive homologue from *Nicotiana tabacum* pollen tube: biochemical properties and subcellular localization. Planta 191: 496–506

Cai G, Romagnoli S, Moscatelli A, Ovidi E, Gambellini G, Tiezzi A, Cresti M (2000) Identification and characterization of novel microtubule-based motor associated with membranous organelles in tobacco pollen tubes. Plant Cell 12: 1719–1736

Camacho L, Malhó R (2003) Endo-exocytosis in the pollen tube apex is differentially regulated by Ca^{2+} and GTPases. J Exp Bot 54: 83–92

Chen CY-h, Cheung AY, Wu H-m (2003) Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth. Plant Cell 15: 237–249

Cheung AY, Wu H-m (2004) Overexpression of an *Arabidopsis* formin stimulates supernumerary actin cable formation from pollen tube cell membrane. Plant Cell 16: 257–269

Cheung AY, Chen CY-h, Glaven RH, de Graaf BHJ, Vidali L, Hepler PK, Wu H-m (2002) Rab2 GTPase regulates vesicle trafficking between the endoplasmic reticulum and Golgi bodies and is important to pollen tube growth. Plant Cell 14: 945–962

Cockcroft S, De Matteis MA (2001) Inositol lipids as spatial regulators of membrane traffic. J Membr Biol 180: 187–194

Cooke CJ, Smith CJ, Walton TJ, Newton RP (1994) Evidence that cyclic AMP is involved in the hypersensitive response of *Medicago sativa* to a fungal elicitor. Phytochemistry 35: 889–895

Cremona O, Di Paolo G, Wenk MR, Lüthi A, Kim WT, Takei K, Daniell L, Nemoto Y, Shears SB, Flavell RA, McCormick DA, De Camilli P (1999) Essential role of phophoinositol metabolism in synaptic vesicle recycling. Cell 99: 179–188

Dawson AP (1997) Calcium signaling: How do IP_3 receptors work? Curr Biol 7: R544–R547

Del Casino C, Li Y, Moscatelli A, Scali M, Tiezzi A, Cresti M (1993) Distribution of microtubules during the growth of tobacco pollen tubes. Biol Cell 79: 125–132

Derksen J, Knuiman B, Hoedemaekers K, Anouchka G, Bonhomme S, Pierson ES (2002) Growth and cellular organization of *Arabidopsis* pollen tubes in vitro. Sex Plant Reprod 15: 133–139

Desrivières S, Cooke FT, Morales-Johansson H, Parker PJ, Hall MN (2002) Calmodulin controls organization of the actin cytoskeleton regulation of phosphatidylinositol (4,5)-bisphosphate synthesis in *Saccharomyces cerevisiae*. Biochem J 366: 945–951

Fisher DD, Gilroy S, Cyr JR (1996) Evidence for opposing effects of calmodulin on cortical microtubules. Plant Physiol 112: 1079–1087

Foissner I, Grolig F, Obermeyer G (2002) Reversible protein phosphorylation regulates the dynamic organization of the pollen tube cytoskeleton: effects of calyculin A and okadaic acid. Protoplasma 220: 1–15

Franklin-Tong VE, Drøbak BK, Allan AC, Watkins PAC, Trewavas AJ (1996) Growth of pollen tubes of *Papaver rhoeas* is regulated by a slow moving calcium wave propagated by inositol triphosphate. Plant Cell 8: 1305–1321

Fu Y, Wu G, Yang Z (2001) Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. J Cell Biol 152: 1019–1032

Fujimoto T, Miyawaki A, Mikoshiba K (1995) Inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae is linked to actin filaments. J Cell Sci 108: 7–15

Geitmann A, Parre E (2004) The local cytomechanical properties of growing pollen tubes correspond to the axial distribution of structural cellular elements. Sex Plant Reprod 17: 9–16

Geitmann A, Li Y-Q, Cresti M (1995) The role of cytoskeleton and dictyosome activity in the pulsatory growth of *Nicotiana tabacum* and *Petunia hybrida* pollen tubes. Bot Acta 109: 102–109

Gibbon BC, Zonia LE, Kovar DR, Hussey PJ, Staiger CJ (1998) Pollen profilin function depends on interaction with proline-rich motifs. Plant Cell 10: 981–993

Goode BL, Drubin DG, Barnes G (2000) Functional cooperation between the microtubule and actin cytoskeletons. Curr Opin Cell Biol 12: 63–71

Grabski S, Arnoys E, Busch B, Schindler M (1998) Regulation of actin tension in plant cells by kinases and phosphatases. Plant Physiol 116: 279–290

Harmon AC, Gribskov M, Harper JF (2000) CDPKs – a kinase for every Ca^{2+} signal? Trends Plant Sci 5: 154–159

Holdaway-Clarke TL, Hepler PK (2003) Control of pollen tube growth: role of ion gradients and fluxes. New Phytol 159: 539–563

Holdaway-Clarke TL, Feijó JA, Hackett GR, Kunkel JG, Hepler PK (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. Plant Cell 9: 1999–2010

Hussey PJ, Yuan M, Calder G, Khan S, Lloyd CW (1998) Microinjection of pollen-specific actin-depolymerizing factor, ZmADF1, reorientates F-actin strands in *Tradescantia* stamen hair cells. Plant J 14: 353–357

Joos U, van Aken J, Kristen U (1994) Microtubules are involved in maintaining the cellular polarity in pollen tubes of *Nicotiana sylvetris*. Protoplasma 179: 5–15

Kachroo A, Nasrallah ME, Nasrallah JB (2002) Self-incompatibility, receptor-ligand signaling, and cell–cell communication in plant reproduction. Plant Cell 14: S227–S238

Kost B, Spielhofer P, Chua N-H (1998) A GFP-mouse talin fusion protein labels plant actin filaments in vivo and visualizes the actin cytoskeleton in growing pollen tubes. Plant J 16: 393–401

Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua M-H (1999) Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. J Cell Biol 145: 317–330

Lancelle SA, Hepler PK (1991) Association of actin with cortical microtubules revealed by immunogold localization in *Nicotiana* pollen tubes. Protoplasma 165: 167–172

Li H, Lin Y, Heath RM, Zhu MX, Yang Z (1999) Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. Plant Cell 11: 1731–1742

Lin Y, Yang Z (1997) Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth. Plant Cell 9: 1647–1659

Lin Y, Wang Y, Zhu J-K, Yang Z (1996) Localization of a Rho GTPase implies a role in tip growth and movement of the generative cell in pollen tubes. Plant Cell 8: 293–303

Liu GQ, Cai G, Del Casino C, Tiezzi A, Cresti M (1994) Kinesin-related polypeptide is associated with vesicles from *Corylus avellana* pollen. Cell Motil Cytoskeleton 29: 155–166

Lopez I, Anthony RG, Maciver SK, Jiang C-J, Khan S, Weeds AG, Hussey PJ (1996) Pollen specific expression of maize genes encoding actin depolymerizing factor-like proteins. Proc Natl Acad Sci USA 93: 7415–7420

Lord EM, Sanders LC (1992) Roles for the extracellular matrix in plant development and pollination: a special case of cell movement in plants. Dev Biol 153: 16–28

Ma L, Xu X, Cui S, Sun D (1999) The presence of a heterotrimeric G protein and its role in signal transduction of extracellular calmodulin in pollen germination and tube growth. Plant Cell 11: 1351–1364

Malhó R (1998) The role of inositol(1,4,5)triphosphate in pollen tube growth and orientation. Sex Plant Reprod 11: 231–235

Malhó R, Camacho L (2004) Signalling the cytoskeleton in pollen tube germination and growth. In: Hussey PJ (ed) The plant cytoskeleton in cell differentiation and development. Annual Plant Reviews, vol 10. Blackwell, Oxford, pp 240–264

Malhó R, Trewavas AJ (1996) Localized apical increases of cytosolic free calcium control pollen tube orientation. Plant Cell 8: 1935–1949

Malhó R, Read ND, Trewavas AJ, Pais MS (1995) Calcium channel activity during pollen tube growth and reorientation. Plant Cell 7: 1173–1184

Malhó R, Camacho L, Moutinho A (2000) Signalling pathways in pollen tube growth and reorientation. Ann Bot 85 Suppl A: 59–68

Martin TFJ (1998) Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation, and membrane trafficking. Annu Rev Cell Dev Biol 14: 231–264

Mathur J, Mathur N, Hülskamp M (2002) Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. Plant Physiol 128: 1031–1045

Messerli M, Robinson KR (1997) Tip localized Ca^{2+} pulses are coincident with peak pulsatile growth rates in pollen tubes of *Lilium longiflorum*. J Cell Sci 110: 1269–1278

Messerli MA, Créton R, Jaffe LF, Robinson KR (2000) Periodic increases in elongation rate precede increases in cytosolic Ca^{2+} during pollen tube growth. Dev Biol 222: 84–98

Miller DD, Scordilis SP, Hepler PK (1995) Identification and localization of three classes of myosins in pollen tubes of *Lilium longiflorum* and *Nicotiana alata*. J Cell Sci 108: 2549–2563

Miller DD, Lancelle SA, Hepler PK (1996) Actin filaments do not form a dense meshwork in *Lilium longiflorum* pollen tube tips. Protoplasma 195: 123–132

Mittermann I, Swoboda I, Pierson E, Eller N, Kraft D, Valenta R, Heberle-Bors E (1995) Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. Plant Mol Biol 27: 137–146

Molendjik AJ, Bischoff F, Rajendrakumar CSV, Friml J, Braun M, Gilroy S, Palme K (2001) *Arabidopsis thaliana* Rop GTPases are localized to tips of root hairs and control polar growth. EMBO J 20: 2779–2788

Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malhó R (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$, and membrane secretion. J Exp Bot 56: 1665–1674

Moore I, Diefenthal T, Zarsky V, Shell J, Palme K (1997) A homolog of the mammalian GTPase Rab2 is present in *Arabidopsis* and is expressed predominantly in pollen grains and seedlings. Proc Natl Acad Sci USA 94: 762–767

Moscatelli A, Del Casino C, Lozzi L, Cai G, Scali M, Tiezzi A, Cresti M (1995) High molecular weight polypeptides related to dynein heavy chains in *Nicotiana tabacum* pollen tubes. J Cell Sci 108: 1117–1125

Moscatelli A, Cai G, Ciampolini F, Cresti M (1998) Dynein heavy chain-related polypeptides are associated with organelles in pollen tubes of *Nicotiana tabacum*. Sex Plant Reprod 11: 31–40

Moscatelli A, Cai G, Cresti M (1999) Actin filament- and microtubule-based motor systems: their concerted action during pollen tube growth. In: Cresti M, Cai G, Moscatelli A (eds) Fertilization in higher plants: molecular and cytological aspects. Springer, Berlin Heidelberg New York, pp 301–315

Moutinho A, Trewavas AJ, Malhó R (1998a) Relocation of a Ca^{2+}-dependent protein kinase activity during pollen tube reorientation. Plant Cell 10: 1499–1510

Moutinho A, Love J, Trewavas AJ, Malhó R (1998b) Distribution of calmodulin protein and mRNA in growing pollen tubes. Sex Plant Reprod 11: 131–139

Moutinho A, Hussey PJ, Trewavas AJ, Malhó R (2001) cAMP acts as a second messenger in pollen tube growth and reorientation. Proc Natl Acad Sci USA 98: 10481–10486

Munnik T (2001) Phosphatidic acid: an emerging plant lipid second messenger. Trends Plant Sci 6: 227–233

O'Luanaigh N, Pardo R, Fensome A, Allen-Baume V, Jones D, Holt MR, Cockcroft S (2002) Continual production of phosphatidic acid by phospholipase D is essential for antigen-stimulated membrane ruffling in cultured mast cells. Mol Biol Cell 13: 3730–3746

Palanivelu R, Preuss D (2000) Pollen tube targeting and axon guidance: parallels in tip growth mechanisms. Trends Cell Biol 10: 517–524

Palevitz BA, Tiezzi A (1992) Organization, composition, and function of the generative cell and sperm cytoskeleton. Int Rev Cytol 140: 149–185

Patel S, Morris SA, Adkins CE, O'Beirne G, Taylor CW (1997) Ca^{2+}-independent inhibition of inositol trisphosphate receptors by calmodulin: redistribution of calmodulin as a possible means of regulating Ca^{2+} mobilization. Proc Natl Acad Sci USA 94: 11627–11632

Pierson ES, Derksen J, Traas JA (1986) Organization of microfilaments and microtubules in pollen tubes grown in vitro or in vivo in various angiosperms. Eur J Cell Biol: 41 14–18

Pierson ES, Miller DD, Callaham DA, van Aken J, Hackett G, Hepler PK (1996) Tip-localized calcium entry fluctuates during pollen tube growth. Dev Biol 174: 160–173

Prado AM, Porterfield M, Feijó JA (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 131: 2707–2714

Potocký M, Elias M, Profotova B, Novotna Z, Valentova O, Zarsky V (2003) Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. Planta 217: 122–130

Powner DJ, Wakelam MJO (2002) The regulation of phospholipase D by inositol phospholipids and small GTPases. FEBS Lett 531: 62–64

Putnam-Evans C, Harmon AC, Palevitz BA, Fechheimer M, Cormier MJ (1989) Calcium-dependent protein kinase is localized with F-actin in plant cells. Cell Motil Cytoskeleton 12: 12–22

Rato C, Monteiro D, Hepler PK, Malhó R (2004) Calmodulin activity and cAMP signalling modulate growth and apical secretion in pollen tubes. Plant J 38: 887–897

Raudaskoski M, Åström H, Laitiainen E (2001) Pollen tube cytoskeleton: structure and function. J Plant Growth Regul 20: 113–130

Reddy ASN, Day IS (2001) Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence. Genome Biol 2(7): research0024.1–0024.17

Romagnoli S, Cai G, Cresti M (2003) In vitro assays demonstrate that pollen tube organelles use kinesin-related motor proteins to move along microtubules. Plant Cell 15: 251–269

Roy SJ, Holdaway-Clarke TL, Hackett GR, Kunkel JG, Lord EM, Hepler PK (1999) Uncoupling secretion and tip growth in lily pollen tubes: evidence for the role of calcium in exocytosis. Plant J 19: 379–386

Rudd JJ, Franklin-Tong VE (2001) Unravelling response-specificity in Ca^{2+} signalling pathways in plant cells. New Phytol 151: 7–33

Šamaj J, Baluška F, Menzel D (2004) New signalling molecules regulating root hair tip growth. Trends Plant Sci 9: 217–220

Schuurink RC, Chan PV, Jones RL (1996) Modulation of calmodulin mRNA and protein levels in barley aleurone. Plant Physiol 111: 371–380

Smertenko AP, Jiang C-J, Simmons NJ, Weeds AG, Davies DR, Hussey PJ (1998) Ser6 in the maize actin-depolymerizing factor, ZmADF3, is phosphorylated by a calcium-stimulated protein kinase and is essential for the control of functional activity. Plant J 14: 187–193

Smith RM, Baibakov B, Ikebuchi Y, White BH, Lambert NA, Kaczmarek LK, Vogel SS (2000) Exocytotic insertion of calcium channels constrains compensatory endocytosis to sites of exocytosis. J Cell Biol 148: 755–767

Staiger CJ (2000) Signaling to the actin cytoskeleton in plants. Annu Rev Plant Physiol Plant Mol Biol 51: 257–288

Staiger CJ, Goodbody KC, Hussey PJ, Valenta R, Drøbak BK, Lloyd CW (1993) The profilin multigene family of maize: differential expression of three isoforms. Plant J 4: 631–641

Staiger CJ, Yuan M, Valenta R, Shaw PJ, Warn RM, Lloyd CW (1994) Microinjected profilin affects cytoplasmic streaming in plant cells by rapidly depolymerizing actin microfilaments. Curr Biol 4: 215–219

Stevenson JM, Perera IY, Heilmann I, Persson S, Boss WF (2000) Inositol signaling and plant growth. Trends Plant Sci 5: 252–258

Steer MW, Steer JL (1989) Pollen tube tip growth. New Phytol 111: 323–358

Sweeney DA, Siddhanta A, Shields D (2002) Fragmentation and re-assembly of the Golgi apparatus in vitro – a requirement for phosphatidic acid and phosphatidylinositol 4,5-bisphosphate synthesis. J Biol Chem 77: 3030–3039

Taylor LP, Hepler PK (1997) Pollen germination and tube growth. Annu Rev Plant Physiol Plant Mol Biol 48: 461–491

Tiezzi A, Moscatelli A, Cai G, Bartalesi, Cresti M (1992) An immunoreactive homolog of mammalian kinesin in *Nicotiana tabacum* pollen tubes. Cell Motil Cytoskeleton 21: 132–137

Trewavas AJ, Malhó R (1997) Signal perception and transduction: the origin of the phenotype. Plant Cell 9: 1181–1195

Vantard M, Blanchoin L (2002) Actin polymerization processes in plant cells. Curr Opin Plant Biol 5: 502–506

Vidali L, Hepler PK (2001) Actin and the pollen tube growth. Protoplasma 215: 64–76

Vidali L, Yokota E, Cheung AY, Shimmen T, Hepler PK (1999) The 135 kDa actin-bundling protein from *Lilium longiflorum* pollen is the plant homologue of villin. Protoplasma 209: 283–291

Vidali L, McKenna ST, Hepler PK (2001) Actin polymerization is essential for pollen tube growth. Mol Biol Cell 12: 2534–2545

Volotovski ID, Sokolovsky SG, Molchan OV, Knight MR (1998) Second messengers mediate increases in cytosolic calcium in tobacco protoplasts. Plant Physiol 117: 1023–1030

Wang W, Takezawa D, Narasimhulu SB, Reddy ASN, Poovaiah BW (1996) A novel kinesin-like protein with a calmodulin-binding domain. Plant Mol Biol 31: 87–100

Wasteneys GO, Galway ME (2003) Remodeling the cytoskeleton for growth and form: an overview with some new views. Annu Rev Plant Biol 54: 691–722

Wu G, Gu Y, Li S, Yang Z (2001) A genome-wide analysis of *Arabidopsis* Rop-interactive CRIB motif-containing proteins that act as Rop GTPase targets. Plant Cell 13: 2841–2856

Yang Z (2002) Small GTPases: versatile signaling switches in plants. Plant Cell 14: S375–388

Yang Z, Watson JC (1993) Molecular cloning and characterization of rho, a ras-related small GTP-binding protein from the garden pea. Proc Natl Acad Sci USA 90: 8732–8736

Yokota E (2000) Identification and characterization of higher plant myosins responsible for cytoplasmic streaming. J Plant Res 113: 511–519

Yokota E, Shimmen T (1999) The 135-kDa actin-bundling protein from lily pollen tubes arranges F-actin into bundles with uniform polarity. Planta 209: 264–266

Yokota E, Muto S, Shimmen T (1999) Inhibitory regulation of higher-plant myosin by Ca^{2+} ions. Plant Physiol 119: 231–239

Yokota E, Takahara K, Shimmen T (1998) Actin-bundling protein isolated from pollen tubes of lily: biochemical and immunocytochemical characterization. Plant Physiol 116: 1421–1429

Yokota E, Muto S, Shimmen T (2000) Calcium-calmodulin suppresses the filamentous actin-binding activity of a 135-kilodalton actin-bundling protein isolated from lily pollen tubes. Plant Physiol 123: 645–654

Young SA, Wang X, Leach JE (1996) Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with *Xanthomonas oryzae* pv oryzae. Plant Cell 8: 1079–1090

Zheng L, Krishnamoorthi R, Zolkiewski M, Wang X (2000) Distinct Ca^{2+} binding properties of novel C2 domains of plant phospholipase D alpha and beta. J Biol Chem 275: 19700–19706

Zheng Z-L, Yang Z (2000) The Rop GTPase switch turns on polar growth in pollen. Trends Plant Sci 5: 298–303

Zielinski RE (1998) Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 49: 697–725

Zonia L, Cordeiro S, Tupý J, Feijó JA (2002) Oscillatory chloride efflux at the pollen tube apex has a role in growth and cell volume regulation and is targeted by inositol 3,4,5,6-tetrakisphosphate. Plant Cell 14: 2233–2249