Plant Cell Monogr (3)
R. Malhó: The Pollen Tube
DOI 10.1007/7089_048/Published online: 26 January 2006

The Microtubular Cytoskeleton in Pollen Tubes: Structure and Role in Organelle Trafficking

Giampiero Cai (✉) · Mauro Cresti

Dipartimento Scienze Ambientali "G. Sarfatti" – Università degli Studi di Siena,
Via Mattioli 4, 53100 Siena, Italy
cai@unisi.it, cresti@unisi.it

Abstract Microtubules are a fundamental component of plant cells, in which they achieve many critical functions. In pollen tubes, however, their specific role remains unsolved and ambiguous. Microtubules are extremely abundant in the pollen tube and are undoubtedly important in critical processes like the transport of sperm cells. Recent advances have also shown a dynamic interaction with pollen tube organelles and a low speed translocation suggesting that microtubules are not strictly essential in the cytoplasmic streaming but rather in the regulation of such process. Here we focus on the organization of microtubules and on their putative role in the transport of pollen tube organelles. We will discuss the model of functional cooperation between microtubules and actin filaments and adapt it to the pollen tube system.

1 Introduction

Microtubules are a fundamental component of the cytoskeleton in all eukaryotic cells operating in the intracellular positioning and dynamics of organelles and molecules. Microtubules are the main scaffold along which chromosomes separate during cell division, representing the tracks for the precise transport of organelles and vesicles and constituting the central part of specialized structures like cilia and flagella. The organization of microtubules in plant cells is rather different from the one typically found in an animal cells, as four distinct arrays of microtubules are characteristically present throughout the cell cycle (Goddard et al. 1994). Unlike somatic cells, the pollen tube is a cell that continuously grows but does not divide. Consequently, we can only distinguish the interphase microtubule array (Fig. 1), which shares some parallelism but also exhibits a number of differences compared with somatic cells. In pollen tubes, microtubules are organized as thick bundles in the base region of the tube, which progressively shift into thin bundles or likely into single microtubules in the subapical region (Del Casino et al. 1993; Derksen et al. 1985; Lancelle et al. 1987; Pierson et al. 1986; Raudaskoski et al. 1987). Their presence in the apical domain is still questionable. Detection of microtubules in the tube apex may be consequential to fixation artifacts, but it may also reflect the dynamicity that characterizes the growth

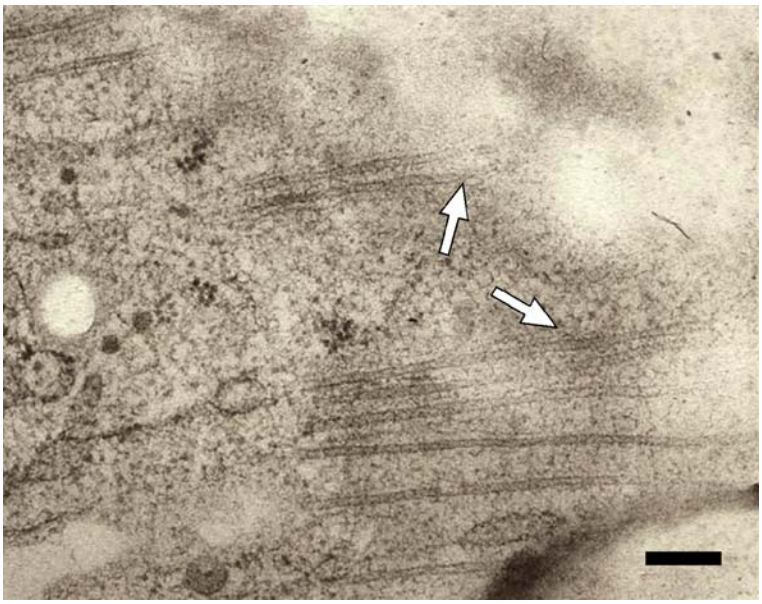

Fig. 1 Microtubules in the pollen tube of tobacco. The electron micrograph after freeze-fixation shows the organization of microtubules in the cortical region of tobacco pollen tubes. Arrows indicate microtubules that are positioned parallel to each other and according to the elongation axis of the cell. Bar: 200 nm. Courtesy of Fabrizio Ciampolini/Claudio Milanesi

region. In addition, microtubules were reported to be present in the apex only when the pollen tube changes the growth direction (Foissner et al. 2002), suggesting a role in guidance and emphasizing a significant contribution under in vivo conditions. Although microtubules are likely involved in many functions during tube growth, this chapter will mainly focus on a specific aspect of the pollen tube microtubules, namely their involvement in the transport of organelles and vesicles.

2
The Structure of Microtubules in Pollen Tubes

2.1
Molecular Composition

Microtubules are composed of α- and β-tubulin subunits, two polypeptides with nearly identical molecular weight and amino acid sequence (McKean et al. 2001). Each subunit is represented by different isotypes, which are consequence of the expression of different genes or of selective posttranslational

modifications. The *Arabidopsis* genome contains several tubulin genes, at least six different α-tubulin genes and nine β-tubulin genes (Kopczak et al. 1992; Snustad et al. 1992). Consequently, the molecular composition of microtubules may differ according to specific tissues. For example, one α-tubulin isotype of *Arabidopsis* is preferentially expressed in pollen (Carpenter et al. 1992). The same concept can be applied to β-tubulins. In carrot cells, at least six β-tubulin isotypes can be discriminated by 2D-electrophoresis. The isotype β4 appears in immature and mature stamens and becomes the predominant isotype in mature pollen. Other isotypes (β1 and β3) are conversely absent from pollen (Hussey et al. 1988). In rice, seven β-tubulin genes are mainly expressed in leaves, while TUB8 is preferentially expressed in anthers and in mature pollen (Yoshikawa et al. 2003). The presence of one anther-specific β-tubulin gene suggests its importance during anther and pollen development or at some stage of pollen tube growth. The TUB9 (β-tubulin) gene of *Arabidopsis* is equally expressed in floral tissues, with the highest levels of expression observed in pollen, pollen tubes and ovules (Cheng et al. 2001). A characteristic α-tubulin gene is restricted to the male gametophyte of sunflowers and few other species (Evrard et al. 2002). Pollen tubes are also characterized by high levels of tubulin (and actin) transcripts, which could be generated during pollen tube growth (Sorri et al. 1996). These results consequently suggest that the development and functioning of the male gametophyte may require the presence of specific α- and β-tubulin genes and proteins, although the differential expression does not always provide definite functions.

Tubulin is also posttranslationally modified to achieve complete functioning. Both acetylated and tyrosinated forms of α-tubulin have been detected in tobacco pollen tubes using specific antibodies. Tyrosinated α-tubulin was found in the apical domain of pollen tubes and, following elongation, along the base domain (Del Casino et al. 1993). In both cases, the staining pattern was distinct from the classical filamentous organization, suggesting that the tyrosinated form of α-tubulin does not distribute uniformly along microtubules. Tyrosinated α-tubulin may be used to mark specific domains of the microtubule population. However, whether such domains correspond to more or less stable microtubules is not determined. Acetylated α-tubulin was detected in the generative cell at different developmental stages (in the kinetochore fibers, in the polar regions and in the phragmoplast) (Astrom 1992). As the generative cell microtubules have been suggested to be more stable that the vegetative microtubules, acetylated α-tubulin may correlate with and consequently mark the arrays of more stable microtubules. The posttranslational acetylation seems to concern with the most abundant isoform of pollen tubulin, the α3, which is also considerably polyglutamylated. In addition, tyrosination of the tubulin isoform α6 seems to be pollen-specific (Wang et al. 2004). Tubulin from *Malus domestica* pollen is also modified by transglutaminases through the addition of polyamines (Del Duca et al. 1997). As

the transglutaminase-catalyzed reactions can interfere with the binding of molecular motors to cytoskeletal filaments (Kim et al. 1998), the posttranslational addition of polyamines may be used to inactivate the cytoskeletal-based transport of organelles in pollen.

All together, these evidences suggest that the assembly of microtubules in pollen requires the expression of specific tubulin genes (in addition to those ubiquitously expressed) as well as the posttranslational modifications of both subunits. Why all these differentially-expressed genes and their relative post-translational alterations are necessary and how those different isotypes interact with other cell components is actually unknown. One simple hypothesis is that the growth of pollen tubes proceeds through the assembly of different microtubule subsets, such as those around the generative cell or at the tube apex.

2.2 Sites of Origin

In plant cells, the exact places from which microtubules originate have always been a matter of discussion. The absence of centrioles (a specific marker of microtubule-organizing centers in animal cells) never allowed researchers to identify precisely where microtubules initiate in plant cells. In interphase cells, microtubules are mainly cortical, suggesting that the plasma membrane should be involved in the organization of microtubules. Clearer evidence came from the discovery of γ-tubulin (a critical component of the microtubule-organizing complex) in plants and its association with membranes (Bo et al. 1994; Drykova et al. 2003). The current model predicts that plant microtubules are nucleated by distinct but flexible centrosome-like structures located in different cell regions, such as the nuclear surface and the plasma membrane (Chan et al. 2003). Unlikely somatic cells, pollen tubes grow by local expansion at the tube apex and microtubules are arranged along the growth axis. Furthermore, γ-tubulin is found all along the microtubules with no predominant distribution in specific cell regions (Palevitz et al. 1994). Thus, in pollen tubes the putative microtubule-nucleation sites may be uniformly distributed. On the contrary, another putative centrosomal protein (p77) seems to accumulate in the tube apex (Cai et al. 1996), suggesting that the tip domain may be important in the assembly of new microtubules. When pollen tube microtubules are depolymerized by drug or cold treatment, their recovery initiate at the cell periphery (Åström et al. 1991; Heslop-Harrison and Heslop-Harrison 1988) but recovery from treatment with calyculin A indicated no preferential sites for the newly growing microtubules (Foissner et al. 2002). A hypothesis to explain these data is that the tip domain of pollen tubes may function similarly to the nuclear surface of somatic cells, which is considered the main plant microtubule-organizing centre. As plant γ-tubulin is distributed all along the microtubule length, it

may be more directly involved in microtubule dynamics rather than in their nucleation (Schmit 2002). Consequently, pollen tube microtubules could be initially assembled as single short (and possibly highly dynamic) structures in the tip domain by protein complexes containing p77; if the tube apex is converted into a more stable domain during growth, microtubules could be then stabilized and further elongated in the cell cortex through the incorporation of γ-tubulin subunits. The stabilization of microtubules could involve their assembly into bundles by putative microtubule-associated proteins and their lateral binding to the cell membrane through microtubule-membrane proteins, like p161 (Cai et al. 2005). The role of actin filaments and of the cell wall in the stabilization of microtubules is unidentified. This model is summarized in Fig. 2.

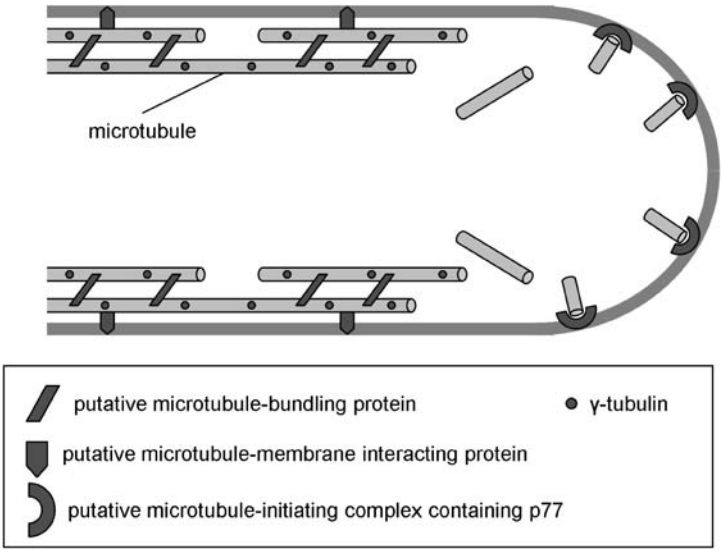

Fig. 2 Sites of origin and the organization of microtubules in the pollen tube. Microtubules may originate in the apical and subapical region of the pollen tube through the activity of nucleating complexes containing the centrosome-like protein p77 and γ-tubulin. In this region, microtubules may be single, short and highly dynamic structures, a precondition for a cell region characterized by rapid changes in ion and molecule composition. The stabilization of nascent microtubules may occur in the subapical region and be dependent on the activity of different molecular complexes containing γ-tubulin. Further stabilization and bundling of microtubules may be then generated by microtubule-associated proteins that connect microtubules to the plasma membrane (like p161) or to each other. Association of microtubules with actin filaments may occur through the activity of proteins like p190 (Igarashi et al. 2000) but it is speculative and simply based on the co-localization of the two cytoskeletal systems. How microtubules may eventually influence the organization of actin filaments (and vice versa) is unknown. Objects are not drawn to scale

3
Proteins that Interact with Microtubules

3.1
Microtubule-associated Proteins

Microtubules achieve most of their functions using a set of specialized proteins (microtubule-associated proteins, or MAPs), which change microtubules from a static filament into a dynamic structure. MAPs are a wide class of proteins that allow microtubules to control the rate of polymerization–depolymerization and to interact with each other and with other cell structures. The research in the plant MAP field is relatively recent in comparison with the animal counterpart. This is essentially due to the relatively low abundance of MAPs in the plant cell and to the lack of a model cell like neurons. Standard biochemical approaches have allowed the identification of a small number of proteins that fit the criteria for MAPs (Sedbrook 2004) but genetic and molecular studies have significantly extended the identification of plant MAPs. For example, MOR1 is a microtubule organizing protein originally identified in *Arabidopsis* using temperature-sensitive mutants. MOR1 is the plant version of an early family of MAPs that is essential for the organization of cortical microtubules (Whittington et al. 2001). Another example of genetically-characterized MAPs is the MAP65 family (Hussey et al. 2002).

Evidences for MAPs in pollen tubes are even scarcer. Using a method based on the taxol-dependent polymerization of endogenous microtubules, Tiezzi et al. (1987) have initially identified pollen tube proteins that interact with microtubules. Apart from this initial study, no further evidences have been obtained despite the good qualities of pollen tubes as a model system; microtubules show different organization levels relating to the different stages of pollen tube growth, as the distribution and structure of microtubules radically change during pollen hydration and germination (Tiwari and Polito 1990). All of these dynamic arrangements imply the presence of MAPs, which could control the assembly and interaction of microtubules with other cell components. Other structural evidences for the presence of MAPs are the spatial association of microtubules with actin filaments (Lancelle and Hepler 1991) and with the endoplasmic reticulum (Hepler et al. 1990). Recently, two proteins with MAP-like properties have been identified in the pollen tube of tobacco on the basis of the immunological cross-reactivity with antibodies to plant MAPs (Cai et al. 2005). These proteins interact with both animal and plant tubulin, can control the rate of tubulin assembly and localize both in the vegetative and generative cytoplasm. The most striking feature of those proteins is their interaction with the plasma membrane, which suggests their involvement in the association of microtubules with the cell membrane. In this context, the physical association between microtubules

and the plasma membrane would be a prerequisite for the correct modeling and shaping of the pollen tube. Microtubules would provide the scaffold for the cylindrical shape of the pollen tube, most likely in association with the cell wall components.

The identification of MAPs and the characterization of their activity are also important to understand how the assembly and dynamics of microtubules are regulated. The phosphorylation of both or either tubulins and microtubule-interacting proteins seems critical in the regulation of microtubule organization (Foissner et al. 2002) and could occur through the alteration of associated proteins like in other cell types (Gong et al. 2000).

3.2 Kinesin-like Proteins

Kinesins are microtubule-based motors that play different roles in cells and are coded by a large gene family whose sequence homology is sometimes restricted to small domains. The most important feature of kinesins is their ability to interact with and move along microtubules using the energy of ATP hydrolysis. The functions of kinesins are broad and include the transport of organelles and the relocation of mRNAs (Hirokawa and Takemura 2004). Kinesins are grouped into distinct subfamilies that have been recently reviewed and standardized on the basis of 14 family designations (Lawrence et al. 2004). Screening of the entire *Arabidopsis* genome has revealed more than sixty kinesin-like proteins (Lee and Liu 2004; Reddy and Day 2001) although it is not clear whether some of these genes are pseudogenes. The number is nevertheless huge if compared with the kinesin gene family in other genomes. It is difficult to assign a function to plant kinesins simply based on the specific subfamily to which they belong, but it is possible that plant kinesins perform different tasks from their animal counterparts belonging to the same subfamilies. However, most of the plant kinesins are expected to have a role in the assembly of the mitotic spindle and in the organization of the cortical microtubule array (Liu and Julie Lee 2001). Few indications support a role for plant kinesins (and generally for microtubule motors) in organelle transport and most of these evidences are indirect (Krishnakumar and Oppenheimer 1999; Sato et al. 2001; Van Gestel et al. 2002). Remarkably, the pollen tube is likely the plant cell on which we have more information on the interaction between kinesin-like proteins and cell organelles (Cai et al. 2001).

Kinesin-like proteins were first identified in the pollen tube of tobacco using a monoclonal antibody raised against the heavy chain of bovine brain kinesin (Tiezzi et al. 1992). The antibody allowed the identification of a $\sim$ 100 kD-polypeptide that binds to microtubules. Further biochemical analysis revealed that the polypeptide has a microtubule-dependent ATPase activity and a ATP-dependent microtubule-binding ability (Cai et al. 1993).

The polypeptide was localized by immunofluorescence microscopy in the apex and, more faintly, in the flanks of pollen tubes. The antibody also cross-reacted with one polypeptide of similar molecular mass in hazel pollen (Liu et al. 1994) that localized in association with Golgi vesicles. These preliminary evidences indicated that pollen vesicles could have a microtubule-dependent motor on their surface but did not provide support for the movement of vesicles along microtubules. Remarkably, a comparable localization pattern was found in the pollen tube of gymnosperms in which a different antibody to kinesins stained the tube apex (Terasaka and Niitsu 1994).

The use of a different pan-kinesin peptide antibody revealed the presence of kinesin-like proteins in the generative cell of tobacco and, partially, in the vegetative cytoplasm (Liu and Palevitz 1996). This motor was supposed to have a role in the formation of the sperm cells in tobacco.

A further step in our understanding of the role of kinesins in pollen tubes was indirect. One *Arabidopsis* gene, ZWICHEL (ZWI), is critical in the process of trichome morphogenesis and is a member of the kinesin superfamily. Double mutants in the ZWI and SUZ genes (SUZ is an extragenic suppressor of zwi mutations) exhibited a male-sterile phenotype because of defects in pollen tube germination and growth (Krishnakumar and Oppenheimer 1999). These observations indicated a role for the kinesin-like gene ZWI in pollen germination and pollen tube growth but did not demonstrate that pollen tube organelles move along microtubules. This came with the identification of 80 and 90 kD proteins called ATP-MAPs which were identified based on their selective binding to microtubules (Cai et al. 2000). In addition to be a microtubule-dependent ATPase, the 90-kD ATP-MAP is able to move microtubules on a glass surface, is recognized by a pan-kinesin antibody and localizes in the cortical region of pollen tubes (but not in the apical domain) in association with membrane-bounded organelles. The relatively low speed of microtubule gliding suggested that the 90-kD kinesin-like protein is not involved in the fast transport of pollen tube organelles. The relative distribution of these potential microtubule motors in the pollen tube is illustrated in Fig. 3. A second critical evidence was the observation that isolated pollen tube organelles move along microtubules (Romagnoli et al. 2003). The trafficking ability involves different classes of organelles, from relatively low-density vesicles to high-density mitochondrial fractions and is dependent on ATP but independent on soluble factors. These data suggest that pollen tube organelles have microtubule-dependent motors tightly associated with their surface. Again, the motility speed was much lower than the cytoplasmic streaming of pollen tubes. The organelle fraction showing the higher transport rate contains a membrane protein of ~ 105 kD that binds to microtubules in a ATP-dependent manner and is also recognized by anti-kinesin antibodies. This protein is consequently a likely candidate to move organelles in the pollen tube.

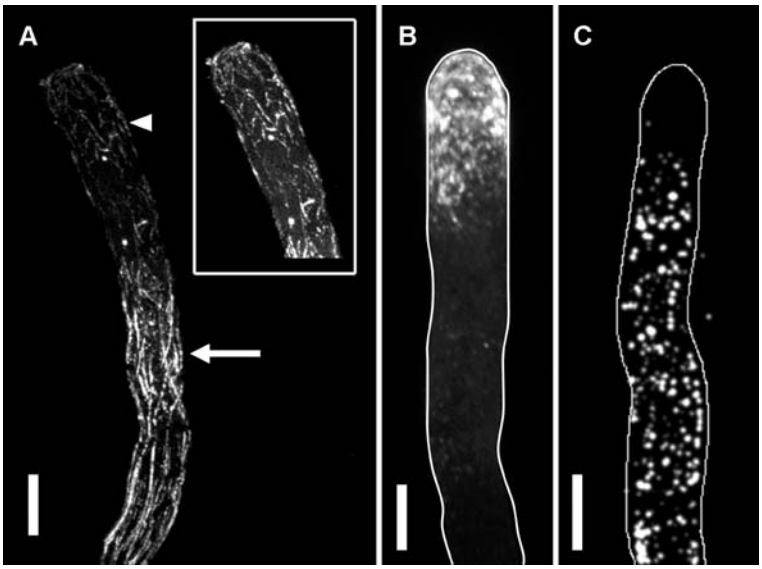

Fig. 3 Distribution of microtubule-dependent motors in the pollen tube of tobacco. Immunolocalization of microtubules (**A**) and of two microtubule-dependent motors (**B**, **C**). All images are from chemically-fixed materials. **A** Microtubules are organized as bundles positioned along the elongation axis of the pollen tube. The base region of the pollen tube contains thicker bundles (*arrow*), whereas the apical domain contains thin fibrillar elements or no microtubules at all (*arrowhead*). In the *inset* in A, the *grayscale levels* have been changed to emphasize the faint (and possibly dynamic) microtubule cytoskeleton in the apex (the presence of microtubules in the apical domain is still speculative). **B** The anti-kinesin k71s23 (Tiezzi et al. 1992) yields a faint but distinct staining pattern in the tube apex, suggesting the presence of kinesin-like proteins in association with the secretory vesicles. **C** The antibody MMR44 (Cai et al. 2000) stains punctuate structures in the pollen tube with the exception of the apical domain, suggesting the association of different kinesins with pollen tube organelles. Bars: 15 μm. **A** is a courtesy of Cecilia Del Casino and **C** is a courtesy of Antonio Tiezzi and Elisa Ovidi

3.3 Dynein-like Proteins

The presence of dynein or dynein-like proteins in plants is still debated. The last years have seen contradictory manuscripts on this topic. The analysis of the *Arabidopsis* genome has shown that dynein-like sequences are missing (Lawrence et al. 2001), suggesting that plants may have evolved a different or alternative mechanism for the minus end-directed movement of organelles along microtubules or that plants may simply not possess this type of transport. Conversely, examination of the whole genome shotgun sequence for rice indicated the presence of four dynein heavy chains (King 2002). This finding may indicate that the absence of dynein sequences in the *Arabidopsis* genome

is not a general trait and that plants may really use dynein-based mechanisms for the intracellular transport. Another interpretation for the missing of dynein-like sequences in *Arabidopsis* may arise from the large number of reported kinesin-like proteins; twenty-one genes coding for minus end-directed kinesins have been found in the *Arabidopsis* genome (Lee and Liu 2004), suggesting that the mechanochemical work performed by dynein may be as much efficiently done in plants by kinesins. Further data supporting the existence of plant dyneins is the biochemical identification of dynein-like polypeptides in tobacco pollen tubes (Moscatelli et al. 1995) and their apparent association with pollen tubes organelles (Moscatelli et al. 1998). In addition, a small fragment of a gene coding for the tobacco dynein heavy chain was isolated from genomic DNA and from a cDNA library of pollinated styles (Scali et al. 2003). The fragment has a very high homology with the *Chlamydomonas dhc*1 gene for 1-alpha dynein heavy chain and contains the first P-loop consensus motif, a region highly conserved in all dyneins.

4
One Choice or Two for Organelle Movement?

What can we learn from these few reports? The massive transport of organelles in plants (designated as "cytoplasmic streaming") is supposed to occur on actin filaments and to be dependent on myosins (Chapter VII). Recent advances on the study of organelle transport in plant cells have made use of modern technologies to dissect all the molecular components that take part into the process. The movement of plant cell organelles along actin filaments is now clearly established: the photorelocation of chloroplasts, the positioning of mitochondria, the transport of peroxisomes and the movement of Golgi bodies along networks of endoplasmic reticulum are all examples of how plant cell organelles interact dynamically with the actin cytoskeleton (Wada and Suetsugu 2004). In opposition, the role of microtubules and of microtubule-dependent motors in plant organelle trafficking has always been misleading. For example, the shaping and motility of the endoplasmic reticulum in plants is known to be affected by cytochalasin D but is relatively independent on microtubule-affecting drugs, such as colchicine or oryzalin (Knebel et al. 1990). However, microtubules have a critical role in cell morphogenesis and maintenance of cell polarity. Genetic and pharmacological studies indicated that the role of actin filaments and microtubules is more or less the same in tip-growing and diffuse-growing cells: while microtubules appear to be important for establishing and maintaining the cell polarity, actin filaments move the materials necessary for growth to particular sites. To sum up, microtubules say how to organize a cell and where to transport materials whereas actin filaments do the transport (Mathur and Hulskamp 2002). Examples of this putative function of microtubules are many. In rhizoids,

microtubules have a central role in determining the cell polarity while the actin cytoskeleton is responsible for the motile processes (Braun and Sievers 1994). In trichome morphogenesis, disruption of actin networks does not affect the establishment of polarity, while the depolymerization of microtubules inhibit cell polarization, causes trichome cells to swell and stops the focused growth (Szymanski et al. 1999). The depolymerization of microtubules in root hairs causes the loss of directionality of growth and the formation of many autonomous growth points (Bibikova et al. 1999). In *Medicago truncatula* root hairs, microtubules form a specific subset, called endoplasmic microtubules, which is present all through the subapical cytoplasmic region but progressively disappears during growth arrest, in full-grown root hairs and after treatment with oryzalin (Sieberer et al. 2002). The characterization of *Arabidopsis* mutants with growth defects in hypocotyls cells allowed the identification of a small protein (SPR1) that colocalizes with cortical microtubules (Nakajima et al. 2004). This protein is highly expressed in tissues showing rapid cell elongation, suggesting that plant-specific microtubule-associated proteins are necessary for the anisotropic growth of elongating cells. In pollen tubes of *Picea abies*, microtubules and actin microfilaments form a dense matrix corresponding to the direction of cell elongation; depolymerization of microtubules causes the pollen tube to stop elongation and to branch while disturbance of actin filaments stops tube growth and blocks germination (Anderhag et al. 2000). Consequently, microtubules are important in promoting tip extension in conifer pollen tubes, likely by controlling the positioning of organelles in the tip and by mediating the organization of actin filaments (Justus et al. 2004).

The role of microtubules in the angiosperm pollen tube is more difficult to decipher. The use of specific antagonists initially showed that microtubules are not important for organelle transport (specifically, for the cytoplasmic streaming) and for tube growth (Heslop-Harrison et al. 1988). However, comparable studies with different microtubule antagonists revealed that microtubules might take part in different aspects of the pollen tube growth. For examples, the in vivo growth (Joos et al. 1995), the cell polarity during in vitro growth (Joos et al. 1994), the pulsatory growth (Geitmann et al. 1995) and the positioning of organelles (He et al. 1995) are all affected by anti-microtubule drugs. The most dramatic effect of microtubule depolymerization in the pollen tube concerns the motility of both generative cell and vegetative nucleus. The application of oryzalin on tobacco pollen tubes significantly reduces the motility rate of the male germ unit, suggesting that microtubules have a critical though not complete role in this motor activity (Astrom et al. 1995). In addition, the movement of the generative cell is unrelated to the synthesis of the callose plugs, which indicates that microtubules may not take part in their production (Laitiainen et al. 2002). All of these data suggest that microtubules take part into distinct processes and, consequently, that they interact with several cellular compartments. The precise functions

they have are nevertheless uncertain, and uncertain is how they interact with each other and with other cell components and what proteins mediate such interactions. For example, the finding that oryzalin treatment reduces the motility rate of the generative cell may suggest that either microtubule-dependent motors are present at the generative cell surface or the correct organization of actin filaments around the generative cell is dependent on the proper architecture of microtubules. Alternatively, microtubules may simply indicate to the generative cell where to move, leaving the motion force to actin filaments. Consequently, a conclusion on the role of microtubules in the pollen tube needs supplementary methodical investigations with different techniques in addition to drug studies.

The relationships between microtubule-dependent motors and organelle transport are more difficult to understand. The cytoplasmic streaming depends essentially on actin filaments (Chapter VII) but the importance of the coordination between the actin- and microtubule-based transports must be emphasized. Although most reports state that organelle transport relies on actin filaments, data on the presence of microtubule-dependent motors and the results of in vitro motility assays (Romagnoli et al. 2003) suggest that some kind of microtubule-based movement should take place in the pollen tube. The challenge is now to make sense of this specific motility in the context of the pollen tube growth. For this purpose, we can compare what is already known in other cell types. Generally, the movement of cytoplasmic organelles in eukaryotic cells is recognized to be based on both microtubules and actin filaments and on their dependent motor proteins. This model is called "functional cooperation" and is supported by several data from different cell types (Goode et al. 2000). Briefly, the model assumes that cell organelles have motor proteins of different families associated with their surface; furthermore, the model suggests that the correct transport and positioning of organelles is the result of two distinct processes that do not exclude each other. The first process consists of the transport of organelles throughout the cell and is dependent on the sequential activity of microtubule- and actin-based motors. In nerve cells, for example, organelles are transported along the axon using microtubules and kinesins while the final delivery in the synapse is mediated by actin filaments and myosins (Brown et al. 2004). The second process consists of the correct transport and positioning of organelles in non-nerve cells and is dependent on the simultaneous activity of motors from different families. As an example, the proper positioning of organelles in melanophores is maintained by the steady activity of the motor forces of kinesin, dynein and myosin (Lambert et al. 1999). Although nerve cells and melanophores are distant from plant cells, a similar kind of cooperation has been recently suggested to occur in characean internodal cells (Foissner 2004), in which the transport of mitochondria could occur along both microtubules and actin filaments according to the different pH of specific cell regions.

The extension of this model to the pollen tube may be hazardous. However, if we sum up all the data on motility and motors, we can develop one model that takes into account the role of actin filaments, microtubules and their dependent motors (kinesin and myosin). The model is schematically summarized in Fig. 4. In theory, it is possible to distinguish at least two different regions of the pollen tube in which the functional cooperation could efficiently occur: the base region and the apical region. In the base region, transport of organelles occurs according to the standard scheme of cytoplasmic streaming and is likely to be mostly dependent on actin filaments and myosins. In this particular region, the slow motion mediated by

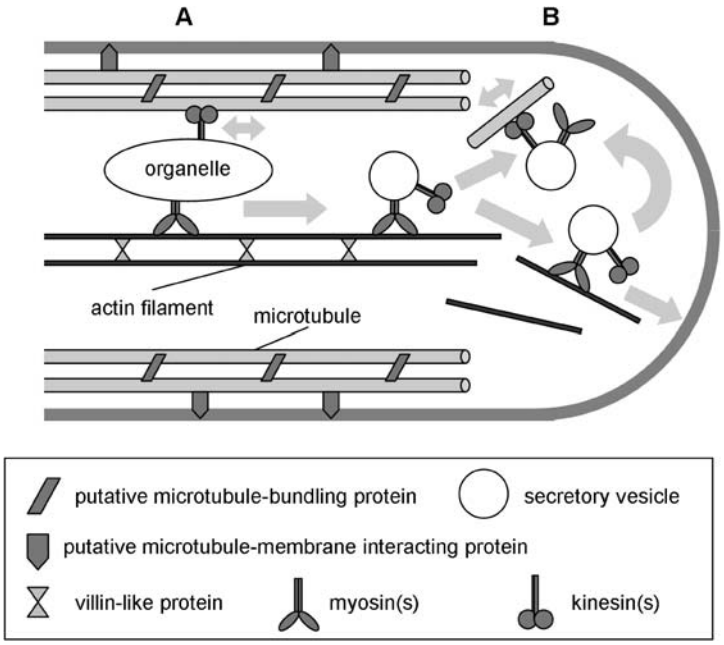

Fig. 4 Examples of functional cooperation between microtubule- and actin filament-dependent motors in the pollen tube. Microtubules may cooperate in two ways with actin filaments in promoting the correct positioning of organelles. In the first case, microtubule- and actin filament-dependent motors (kinesins and myosins) are used simultaneously **A** in order that the net movement of each organelle derives from the equilibrium of both motors' activity. The *long grey arrows* indicate that the organelle has a preferential movement along actin filaments, whereas the *short bidirectional arrows* indicate that microtubule-dependent motors may act as a regulator of the actin-based movement. In the second case (**B**), motors are used sequentially to promote the correct delivery or to maintain the position of vesicles in the tube apex. *Arrows* indicate the same type of movement described previously. In order to complete the scheme, other proteins have been drawn (e.g. the villin-like protein), which forms actin bundles, and some microtubule-binding proteins. Objects are not drawn to scale

microtubule-dependent motors could serve to regulate the trafficking of organelles. Consequently, motors from different families should work together to ensure a correct transport of organelles. In the apical region, accumulation of vesicles requires an intact (and dynamic) actin cytoskeleton, which serves as tracks for the myosin-dependent movement of vesicles. In this context, the role of microtubules and dependent motors may be consecutive or complementary to the one of actin filaments. Microtubules and kinesins may be used in processes related to tip growth, like exocytosis, endocytosis, the focusing of secretory vesicles (Bi et al. 1997), or the maintaining of specific proteins in the apical plasma membrane. Alternatively, their role could be critical when the pollen tube grows within the style and consequently undergoes regulation by the pistil.

5
The Organization of Microtubules in Relation with Pollen Tube Growth

There are two aspects of the pollen tube physiology where the function of microtubules is still elusive: cell shaping and cell signaling. The former is known to depend on the organization of cortical microtubules, which in turn influences the pattern of cellulose deposition and then how a cell expands (Wasteneys 2004). The stability of cortical microtubules is a function of their orientation, because divergent microtubules within the array depolymerize in few minutes. The dynamics of microtubules in the cortex is likely dependent on the activity of different proteins that regulate their stability, the formation of bundles, the interaction with other cell structures, the relative sliding to each other and their interaction with the cellulose-synthase complex (Sedbrook 2004). All these concepts are partially adaptable to the pollen tube. In fact, pollen tube microtubules are aligned along the growth axis of the tube, while they are usually disposed transversally to the expansion axis in somatic cells. This divergent way to organize microtubules weakens the similarity of their role between the pollen tube and somatic cells and thus questions if microtubules control the shape of the pollen tube. Cell morphogenesis is a biological puzzle known to depend on two important events: the accumulation of extracellular material and the mechanical deformation of the cell surface (see Chapter IX).

The second critical aspect is cell signaling. The pollen tube is a cell that constantly grows and changes its direction following the interactions with other cells; however, we ignore how external signals influence the organization of microtubules and if (or how) alterations in their structure may affect the movement of organelles and the organization of the cell wall. The cascade of intracellular signals following the interaction of the pollen tube surface with extracellular hints has effects on many of the cellular activities in the pollen tube. It is likely that the microtubule cytoskeleton of the pollen tube

responds to this signaling cascade and organizes accordingly. MAPs are good candidates to mediate such re-organization activity. In this context, proteins analogous to phospholipase D are candidates to convert the extracellular signals into signals that microtubules can recognize (Chapter VI). Activation of phospholipase D affects the organization of plant microtubules by releasing them from the plasma membrane and by partial depolymerization (Dhonukshe et al. 2003). Hypothetically, during the in vivo growth of the pollen tube, external signals (after conversion into intracellular messengers that control the organization of microtubules) may regulate the transport rate of the generative cell and vegetative nucleus and the "concentration" of organelles in specific domains of the pollen tube. A nicely and intriguing though speculative hypothesis is that microtubules and actin filaments may regulate and reorganize after receiving external information. The reorganization of the cytoskeleton may then influence the way organelles distribute in the pollen tube cytoplasm and, in turn, influence the growth direction and the polarity of the pollen tube. This hypothesis assumes that signaling molecules bind to microtubules and mediate the activity of different environmental triggers (such as cold, osmotic stress and pathogens) (Wasteneys 2004). The consequence of this theory is that actin filaments are the operative force that guides organelle movement in the pollen tube, while microtubules may appropriately contribute to dispose organelles at their right place.

Acknowledgements We are grateful to Professor Bo Liu (University of California at Davis, USA) for carefully reading the manuscript and provide important suggestions and criticisms.

References

Anderhag P, Hepler PK, Lazzaro MD (2000) Microtubules and microfilaments are both responsible for pollen tube elongation in the conifer Picea abies (Norway spruce). Protoplasma 214:141–157

Astrom H (1992) Acetylated α-tubulin in the pollen tube microtubules. Cell Biol Int Rep 16:871–881

Astrom H, Sorri O, Raudaskoski M (1995) Role of microtubules in the movement of the vegetative nucleus and generative cell in tobacco pollen tubes. Sex Plant Reprod 8:61–69

Åström H, Virtanen L, Raudaskoski M (1991) Cold-stability in the pollen tube cytoskeleton. Protoplasma 160:99–107

Bi G-Q, Morris RL, Liao G, Alderton JM, Scholey JM, Steinhardt RA (1997) Kinesin- and myosin-driven steps of vesicle recruitment for Ca^{2+}-regulated exocytosis. J Cell Biol 138:999–1008

Bibikova TN, Blancaflor EB, Gilroy S (1999) Microtubules regulate tip growth and orientation in root hairs of Arabidopsis thaliana. Plant J 17:657–665

Bo L, Joshi HC, Wilson TJ, Silflow CD, Palevitz BA, Snustad DP (1994) γ-tubulin in Arabidopsis: gene sequence, immunoblot, and immunofluorescence studies. Plant Cell 6:303–314

Braun M, Sievers A (1994) Role of the microtubule cytoskeleton in gravisensing Chara rhizoids. Eur J Cell Biol 63:289–298

Brown JR, Stafford P, Langford GM (2004) Short-range axonal/dendritic transport by myosin-V: A model for vesicle delivery to the synapse. J Neurobiol 58:175–188

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, Moscatelli A, Del Casino C, Chevrier V, Mazzi M, Tiezzi A, Cresti M (1996) The anti-centrosome mAb 6C6 reacts with a plasma membrane-associated polypeptide of 77-kDa from the Nicotiana tabacum pollen tubes. Protoplasma 190:68–78

Cai G, Romagnoli S, Cresti M (2001) Microtubule motor proteins and the organization of the pollen tube cytoplasm. Sex Plant Reprod 14:27–34

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

Cai G, Ovidi E, Romagnoli S, Vantard M, Cresti M, Tiezzi A (2005) Identification and characterization of plasma membrane proteins that bind to microtubules in pollen tubes and generative cells of tobacco. Plant Cell Physiol 46:563–578

Carpenter JL, Ploense SE, Snustad DP, Silflow CD (1992) Preferential expression of an α-tubulin gene of Arabidopsis in pollen. Plant Cell 4:557–571

Chan J, Calder GM, Doonan JH, Lloyd CW (2003) EB1 reveals mobile microtubule nucleation sites in Arabidopsis. Nature Cell Biol 5:967–971

Cheng Z, Snustad DP, Carter JV (2001) Temporal and spatial expression patterns of TUB9, a β-tubulin gene of Arabidopsis thaliana. Plant Mol Biol 47:389–398

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

Del Duca S, Bregoli AM, Bergamini C, Serafini-Fracassini D (1997) Transglutaminase-catalyzed modification of cytoskeletal proteins by polyamines during the germination of Malus domestica pollen. Sex Plant Reprod 10:89–95

Derksen J, Pierson ES, Traas JA (1985) Microtubules in vegetative and generative cells of pollen tubes. Eur J Cell Biol 38:142–148

Dhonukshe P, Laxalt AM, Goedhart J, Gadella TWJ, Munnik T (2003) Phospholipase D Activation Correlates with Microtubule Reorganization in Living Plant Cells. Plant Cell 15:2666–2679

Drykova D, Cenklova V, Sulimenko V, Volc J, Draber P, Binarova P (2003) Plant γ-tubulin interacts with $\alpha\beta$-tubulin dimers and forms membrane-associated complexes. Plant Cell 15:465–480

Evrard JL, Nguyen I, Bergdoll M, Mutterer J, Steinmetz A, Lambert AM (2002) A novel pollen-specific alpha-tubulin in sunflower: structure and characterization. Plant Mol Biol 49:611–620

Foissner I (2004) Microfilaments and microtubules control the shape, motility, and subcellular distribution of cortical mitochondria in characean internodal cells. Protoplasma 224:145–157

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

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

Goddard RH, Wick SM, Silflow CD, Snustad P (1994) Microtubule components of the plant cell cytoskeleton. Plant Physiol 104:1–6

Gong CX, Wegiel J, Lidsky T, Zuck L, Avila J, Wisniewski HM, Grundke-Iqbal I, Iqbal K (2000) Regulation of phosphorylation of neuronal microtubule-associated proteins MAP1b and MAP2 by protein phosphatase-2A and -2B in rat brain. Brain Res 853:299–309

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

He Y, Wetzstein HY, Palevitz BA (1995) The effect of a triazole fungicide, propiconazole, on pollen germination, the growth and cytoskeletal distribution in Tradescantia virginiana. Sex Plant Reprod 8:210–216

Hepler PK, Palevitz BA, Lancelle SA, McCauley MM, Lichtscheidl I (1990) Cortical endoplasmic reticulum in plants. J Cell Sci 96:355–373

Heslop-Harrison J, Heslop-Harrison Y (1988) Sites of origin of the peripheral microtubule system of the vegetative cell of the Angiosperm pollen tube. Ann Bot 62:455–461

Heslop-Harrison J, Heslop-Harrison Y, Cresti M, Tiezzi A, Moscatelli A (1988) Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. J Cell Sci 91:49–60

Hirokawa N, Takemura R (2004) Kinesin superfamily proteins and their various functions and dynamics. Exp Cell Res 301:50–59

Hussey PJ, Hawkins TJ, Igarashi H, Kaloriti D, Smertenko A (2002) The plant cytoskeleton: recent advances in the study of the plant microtubule-associated proteins MAP-65, MAP-190 and the Xenopus MAP215-like protein, MOR1. Plant Mol Biol 50:915–924

Hussey PJ, Lloyd CW, Gull K (1988) Differential and developmental expression of beta-tubulins in a higher plant. J Biol Chem 263:5474–5479

Igarashi H, Orii H, Mori H, Shimmen T, Sonobe S (2000) Isolation of a novel 190 kDa protein from tobacco BY-2 cells: possible involvement in the interaction between actin filaments and microtubules. Plant Cell Physiol 41:920–931

Joos U, van Aken J, Kristen U (1995) The anti-microtubule drug carbetamide stops Nicotiana sylvestris pollen tube growth in the style. Protoplasma 187:182–191

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

Justus CD, Anderhag P, Goins JL, Lazzaro MD (2004) Microtubules and microfilaments coordinate to direct a fountain streaming pattern in elongating conifer pollen tube tips. Planta 219:103–109

Kim E, Bobkova E, Miller CJ, Orlova A, Hegyi G, Egelman EH, Muhlrad A, Reisler E (1998) Intrastrand cross-linked actin between Gln-41 and Cys-374. III. Inhibition of motion and force generation with myosin. Biochemistry 37:17801–17809

King SM (2002) Dyneins motor on in plants. Traffic 3:930–931

Knebel W, Quader H, Schnepf E (1990) Mobile and immobile endoplasmic reticulum in onion bulb epidermis cells: short- and long-term observations with a confocal laser scanning microscope. Eur J Cell Biol 52:328–340

Kopczak SD, Haas NA, Hussey PJ, Silflow CD, Snustad DP (1992) The small genome of Arabidopsis contains at least six expressed α-tubulin genes. Plant Cell 4:539–547

Krishnakumar S, Oppenheimer DG (1999) Extragenic suppressors of the arabidopsis zwi-3 mutation identify new genes that function in trichome branch formation and pollen tube growth. Development 126:3079–3088

Laitiainen E, Nieminen KM, Vihinen H, Raudaskoski M (2002) Movement of generative cell and vegetative nucleus in tobacco pollen tubes is dependent on microtubule cytoskeleton but independent of the synthesis of callose plugs. Sex Plant Reprod 15:195–204

Lambert J, Vancoillie G, Naeyaert JM (1999) Molecular motors and their role in pigmentation. Cell Mol Biol 45:905–918

Lancelle SA, Cresti M, Hepler PK (1987) Ultrastructure of cytoskeleton in freeze-substituded pollen tubes of Nicotiana tabacum. Protoplasma 140:141–150

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

Lawrence CJ, Dawe RK, Christie KR, Cleveland DW, Dawson SC, Endow SA, Goldstein LS, Goodson HV, Hirokawa N, Howard J, Malmberg RL, McIntosh JR, Miki H, Mitchison TJ, Okada Y, Reddy AS, Saxton WM, Schliwa M, Scholey JM, Vale RD, Walczak CE, Wordeman L (2004) A standardized kinesin nomenclature. J Cell Biol 167:19–22

Lawrence CJ, Morris NR, Meagher RB, Dawe RK (2001) Dyneins have run their course in plant lineage. Traffic 2:362–363

Lee YR, Liu B (2004) Cytoskeletal motors in Arabidopsis. Sixty-one kinesins and seventeen myosins. Plant Physiol 136:3877–3883

Liu B, Julie Lee YR (2001) Kinesin-related proteins in plant cytokinesis. J Plant Growth Regul 20:141–150

Liu B, Palevitz BA (1996) Localization of a kinesin-like protein in the generative cells of tobacco. Protoplasma 195:78–89

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

Mathur J, Hulskamp M (2002) Microtubules and microfilaments in cell morphogenesis in higher plants. Curr Biol 12:R669-R676

McKean PG, Vaughan S, Gull K (2001) The extended tubulin superfamily. J Cell Sci 114:2723–2733

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, Del CC, 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(Pt 3):1117–1125

Nakajima K, Furutani I, Tachimoto H, Matsubara H, Hashimoto T (2004) SPIRAL1 encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding Arabidopsis cells. Plant Cell 16:1178–1190

Palevitz BA, Liu B, Joshi C (1994) γ-tubulin in tobacco pollen tubes: association with generative cell and vegetative microtubules. Sex Plant Reprod 7:209–214

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

Raudaskoski M, Åström H, Perttila K, Virtanen I, Louhelainen J (1987) Role of microtubule cytoskeleton in pollen tubes: an immunochemical and ultrastructural approach. Biol Cell 61:177–188

Reddy ASN, Day IS (2001) Kinesins in the Arabidopsis genome: a comparative analysis among eukaryotes. BMC Genomics 2:2

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

Sato Y, Wada M, Kadota A (2001) Choice of tracks, microtubules and/or actin filaments for chloroplast photo-movement is differentially controlled by phytochrome and a blue light receptor. J Cell Sci 114:269–279

Scali M, Vignani R, Moscatelli A, Jellbauer S, Cresti M (2003) Molecular evidence for a cytoplasmic dynein heavy chain from Nicotiana tabacum L. Cell Biol Int 27:261–262

Schmit AC (2002) Acentrosomal microtubule nucleation in higher plants. Int Rev Cytol 220:257–289
Sedbrook JC (2004) MAPs in plant cells: delineating microtubule growth dynamics and organization. Curr Opin Plant Biol 7:632–640
Sieberer BJ, Timmers ACJ, Lhuissier FGP, Emons AM (2002) Endoplasmic microtubules configure the subapical cytoplasm and are required for fast growth of Medicago truncatula root hairs. Plant Physiol 130:977–988
Snustad DP, Haas NA, Kopczak SD, Silflow CD (1992) The small genome of Arabidopsis contains at least nine expressed beta-tubulin genes. Plant Cell 4:549–556
Sorri O, Åström H, Raudaskoski M (1996) Actin and tubulin expression and isotype pattern during tobacco pollen tube growth. Sex Plant Reprod 9:255–263
Szymanski DB, Marks MD, Wick SM (1999) Organized F-actin is essential for normal trichome morphogenesis in Arabidopsis. Plant Cell 11:2331–2348
Terasaka O, Niitsu T (1994) Kinesin localized in the pollen tube tips of Pinus densiflora. Jpn J Palynol 40:1–6
Tiezzi A, Moscatelli A, Cai G, Bartalesi A, Cresti M (1992) An immunoreactive homolog of mammalian kinesin in Nicotiana tabacum pollen tubes. Cell Motil Cytoskeleton 21:132–137
Tiezzi A, Moscatelli A, Milanesi C, Ciampolini F, Cresti M (1987) Taxol-induced structures derived from cytoskeletal elements of the Nicotiana pollen tube. J Cell Sci 88:657–661
Tiwari SC, Polito VS (1990) The initiation and organization of microtubules in germinating pear (Pyrus communis L.) pollen. Eur J Cell Biol 53:384–389
Van Gestel K, Kohler RH, Verbelen JP (2002) Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. J Exp Bot 53:659–667
Wada M, Suetsugu N (2004) Plant organelle positioning. Curr Opin Plant Biol 7:626–631
Wang W, Vignani R, Scali M, Sensi E, Cresti M (2004) Post-translational modifications of α-tubulin in Zea mays L. are highly tissue specific. Planta 218:460–465
Wasteneys GO (2004) Progress in understanding the role of microtubules in plant cells. Curr Opin Plant Biol 7:651–660
Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO (2001) MOR1 is essential for organizing cortical microtubules in plants. Nature 411:610–613
Yoshikawa M, Yang G, Kawaguchi K, Komatsu S (2003) Expression analyses of beta-tubulin isotype genes in rice. Plant Cell Physiol 44:1202–1207