

REVIEW ARTICLE

The legacy of kinesins in the pollen tube 30 years later

Giampiero Cai 

Dipartimento Scienze della Vita, Università di
Siena, Siena, Italy

Correspondence

Giampiero Cai, Dipartimento Scienze della
Vita, Università di Siena, via Mattioli 4, 53100
Siena, Italy.
Email: giampiero.cai@unisi.it

Abstract

The pollen tube is fundamental in the reproduction of seed plants. Particularly in angiosperms, we now have much information about how it grows, how it senses extracellular signals, and how it converts them into a directional growth mechanism. The expansion of the pollen tube is also related to dynamic cytoplasmic processes based on the cytoskeleton (such as polymerization/depolymerization of microtubules and actin filaments) or motor activity along with the two cytoskeletal systems and is dependent on motor proteins. While a considerable amount of information is available for the actomyosin system in the pollen tube, the role of microtubules in the transport of organelles or macromolecular structures is still quite uncertain despite that 30 years ago the first work on the presence of kinesins in the pollen tube was published. Since then, progress has been made in elucidating the role of kinesins in plant cells. However, their role within the pollen tube is still enigmatic. In this review, I will postulate some roles of kinesins in the pollen tube 30 years after their initial discovery based on information obtained in other plant cells in the meantime. The most concrete hypotheses predict that kinesins in the pollen tube enable the short movement of specific organelles or contribute to generative cell or sperm cell transport, as well as mediate specific steps in the process of endocytosis.

KEYWORDS

kinesin, microtubule, organelle movement, plant cell growth, pollen tube

1 | INTRODUCTION

Exactly 30 years ago, an article on kinesin and the pollen tube (Tiezzi, Moscatelli, Cai, Bartalesi, & Cresti, 1992) showed the presence of a 105-kD protein recognized by an antibody against the mammalian kinesin heavy chain in the tobacco (*Nicotiana tabacum* L.) pollen tube. At that time, kinesins had only been characterized in animal cells and no antibodies specifically directed against plant kinesins were available. Through immunofluorescence investigations, the antibody selectively labeled small point structures located at the pollen tube apex. I am not mistaken in saying that this was the first article on the presence of kinesin in plant cells even if the article was about an

immunoreactive homolog of kinesin. Proteins of the kinesin family had been identified a few years earlier in animal systems and had been characterized biochemically and cytologically, but until then had not been described in plants. That work marks the first evidence of microtubule motor proteins in plant cells and opens the front to the idea that microtubules may also participate in dynamic transport processes within plant cells. It must be recalled that, then as now, actin filaments are assumed to be primarily responsible for intracellular transport in plant cells. Therefore, the article had to overcome an initial justified skepticism before being published. Moreover, kinesin had been identified in particularly complex animal systems, such as nerve cells (Vale, Reese, & Sheetz, 1985), that have no counterparts in plants.

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Therefore, it might have seemed illogical that a microtubule motor protein such as kinesin would be present in plants. Since then, an impressive and continuous body of evidence has accumulated over time supporting the presence and function of kinesins in plant cells. Today, following analysis of several genomes, kinesins are found to be a highly represented gene superfamily in plant systems (Li, Xu, & Chong, 2012), where they perform a wide range of functions.

What remains of kinesin in the pollen tube after 30 years? Paradoxically, the first plant cell in which the kinesin was identified is still a cell in which the function of this motor protein is unfortunately vague. Although additional work was published later (Cai et al., 1993; Liu, Cai, Del Casino, Tiezzi, & Cresti, 1994) by providing more clues to the role of these motor proteins in the pollen tube, to date their role is not actually clear. Kinesins in the pollen tube are perhaps involved in the selective accumulation of secretory vesicles at the apex (Cai et al., 2000; Romagnoli, Cai, & Cresti, 2003) or even in the slow and direct transport of mitochondria (Romagnoli et al., 2007) or perhaps in other roles not yet clear; unfortunately, for now, these are only hypotheses. In contrast, many experimental data have been obtained over the years to support the role of kinesins in other plant cells and in various functions, such as cell division (Vanstraelen, Inze, & Geelen, 2006). The perplexity about the role of kinesins in the pollen tube also relates to the uncertainty about the role of microtubules within this plant cell. Although microtubules play a well-defined role in processes such as cell division and cell wall deposition, their exact function in the pollen tube is still a puzzle (Onelli, Idilli, & Moscatelli, 2015). In the absence of further experimental data, we can ask whether knowledge gained over time in other plant cells can now help us better understand the role of these microtubule motor proteins in the first plant cell (the pollen tube) in which they were identified.

2 | A QUICK SUMMARY OF THE KINESIN FAMILY

Kinesins are a family of microtubule motors that fuel the movement of organelles and macromolecules in eukaryotic cells. They were identified in the mid-1980s through pioneering studies that led to the first characterization of these motors in specialized cells, such as nerve cells (Schnapp, Vale, Sheetz, & Reese, 1985; Vale, Reese, & Sheetz, 1985; Vale, Schnapp, Reese, & Sheetz, 1985a; Vale, Schnapp, Reese, & Sheetz, 1985b), and then later in all eukaryotic cells. Kinesins were initially identified as microtubule motors capable of moving toward the plus end of microtubules but subsequently a broader analysis revealed that the kinesin superfamily includes members capable of moving in both directions. Therefore, the kinesin initially identified in nerve cells is just one member of a kinesin family present in eukaryotic cells. To date, there are ~14 to 16 classes (families) of kinesins, characterized by a distinct structure; some are dimeric, some are monomeric, some are heteromeric, and some move toward the plus end, some toward the minus end of microtubules. Not necessarily all members of the kinesin family are present within a given cell type.

Very often there is a functional specialization, although not extremely selective.

A globular domain of ~360 residues is a distinctive feature of proteins belonging to the kinesin superfamily. This domain contains an active site for adenosine triphosphate (ATP) hydrolysis and the binding site for microtubules. Most kinesins have an elongated shape with the globular domain at one end, whereas the other end may eventually interact with light chains or associate with the cargo to be delivered. Consistent with the characteristics of kinesin-1, the catalytic domain is also referred to as the “head,” followed by the stalk region and then the “tail” domain. The “head” domain is responsible for the movement boosted by ATP hydrolysis while the “stalk/tail” domain is important for interacting with other subunits or cargo molecules such as proteins, lipids, or nucleic acids. A detailed description of the general structure of kinesins can be found in various reviews (Friel, 2020; Hirokawa & Takemura, 2004; Lawrence et al., 2004; Miki, Okada, & Hirokawa, 2005). Here, I propose only a summary of the various kinesin families. Alignment of all amino acid sequences, especially catalytic sequences, obtained over several years allowed a classification that covers almost all kinesins and divides them into 14 distinct families. This made it possible to compare the complete sequences within each family, creating family-specific trees and providing insight into the specific features of each family. Briefly, the kinesin-1 group is present in plants and fungi as well as in animals (where they are most studied); in the latter, kinesin-1 members are motor proteins primarily involved in organelle transport. Kinesins-2 are still involved in intracellular movement but also in intraflagellar movement and therefore in the assembly of cilia and flagella; such a group of kinesins is to be considered absent in plants. The kinesin-3 group contains members that probably function as monomers or homodimers. They are most likely involved in organelle transport but appear to be absent in plants. The kinesin-4 group is considered a hybrid in that it contains members whose function is quite diverse and not particularly well-known. Their function ranges from organelle transport to chromosome movement and they can be found both in the nucleus and in the cytoplasm. The exact correspondence in the role of kinesin-4 between animal and plant systems is not known. Kinesins-5 are a highly conserved group. They are characterized by a motif that can be phosphorylated and is also found in plants. They most likely form homotetramers and are involved in cell division processes. In contrast, the presence of members of the kinesin-6 family in plants is debated. In other systems kinesins-6 are related to cytokinesis or microtubule transport; since plants have evolved a completely different mechanism of cell division the characterization of kinesins-6 in plants is difficult. The kinesin-7 family is characterized by a very long, family-specific neck. Members play a role in mitosis, microtubule capture at the kinetochore, and nuclear migration. Kinesins-7 are quite expanded in plants with several members in *Arabidopsis thaliana* and rice (*Oryza sativa* L.). Their role in plants is most likely different from that played in other organisms, as it relates to microtubule capture at phragmoplasts, the preprophase band, or cortical arrays of microtubules. Members of the kinesin-8 family are involved in various functions ranging from nucleus to cytoplasm, from mitochondria

transport to chromosome segregation. The kinesin-9 group contains no plant-specific members. The absence in plants suggests a role in the assembly of cilia and flagella. The kinesin-10 family probably binds directly to chromosomes, so much so that some members have been referred to more specifically as chromokinesins. This kinesin family could have various functions in cell division, especially in chromosome movement. The kinesin-11 group is quite peculiar because it has a very divergent catalytic core. Currently, this family consists of only one component, probably participating in signal transduction processes, that may not be mobility-activated along microtubules. The kinesin-12 family contains three subfamilies that are apparently related to organelle transport or nerve tissue development. Some members probably also have a role in cell division. The kinesin-13 family has often been associated with kinesins having an internal motor domain. While this is not always correct, it represents a somewhat characteristic trait. In animals, members of this family are involved in the transport of membranous structures and are also characterized by microtubule depolymerizing activity. Functionally, these kinesins are related to cell division and neuronal development. In the kinesin-14 family, the atypical position of the motor domain provides these kinesins with reverse motility and thus a different direction of movement. There are two subfamilies, kinesin-14A and 14B. The first subfamily consists of members present in all kingdoms and probably involved in mitotic activities. The kinesin-14B subfamily contains members involved in organelle transport. Specific subgroups, such as katD, contain plant members with a calponin homology domain. This domain allows interaction with actin filaments leading to the hypothesis that katD kinesins may function in transport processes associated with both actin filaments and microtubules. The kinesin-like calmodulin-binding protein (KCBP) subgroup is highly conserved in plants and is likely related to the absence of retrograde motors such as dyneins. The last two families of kinesins are kinesins-15 and kinesins-16. These are two relatively recent groups on which little information is available; attempting to grasp the role of the corresponding plant members on the basis of sequences is difficult because members of the same kinesin family very often have different roles between animals and plants.

3 | STRUCTURE AND FUNCTIONS OF KINESINS IN PLANT CELLS

The abundance of kinesin in plant cells is surprising. *Arabidopsis thaliana*, the model plant par excellence, contains 61 genes for kinesins (Lee & Liu, 2004), rice contains 52 genes (Lee, Qiu, & Liu, 2015), and even very simple organisms such as *Physcomitrella* contain an even higher number (Shen, Collatos, Bibeau, Furt, & Vidali, 2012). Analysis of gene sequences has revealed that some genes may be specific to angiosperms and others to lower plant organisms, thus suggesting an evolutionary pathway of kinesins. This hypothesis finds a basis for the case of kinesins-6 and kinesins-10 (Lee et al., 2015). Other members, such as kinesins-14, are also characterized by higher numbers of gene sequences. The abundance of kinesins in plants can also be attributed

to the absence of dyneins (Lawrence, Morris, Meagher, & Dawe, 2001); it is not to be excluded that kinesins had to functionally replace their motor counterparts whose genes were lost in plants. Many of the plant kinesins are involved in cell division processes (the so-called mitotic kinesins) and are therefore upregulated during cell division (Miki, Naito, Nishina, & Goshima, 2014). Members of kinesins-5 were localized to anaphase and correlated with the movement of sister chromatids and phragmoplast organization (Bannigan et al., 2007). Others such as kinesins-12 have conversely been linked to the organization and role of the preprophase band (thus to premitotic events) and of the phragmoplast (Müller & Livanos, 2019). Within the kinesins-14 cluster, the so-called KCBPs constitute a particular group that localizes to the preprophase band of microtubules and most likely interacts with the plasma membrane and actin filaments through specific domains (Preuss, Delmer, & Liu, 2003). Some members of the kinesin-3 group (such as KINUa/ARK3) (Malcos & Cyr, 2011) also localize at the level of the preprophase band, suggesting that the organization of this cytoskeletal ring requires the simultaneous action of molecular motors of different classes. Most likely the different kinesins act synergistically in a temporally and spatially controlled way.

The dynamic microtubule activity that takes place at the animal kinetochore most likely requires the action of several groups of kinesins, such as kinesin-4, -7, -8, -10, and -13 (Cross & McAinsh, 2014). Unfortunately, there are no firm data on the localization of these kinesins at the plant kinetochore and therefore their exact role is still to be clarified. Some of these kinesins may be involved in depolymerization of microtubules, thus allowing the microtubules to pull sister chromatids to their respective poles. Currently, only members of the kinesin-7 group have been localized in association with the kinetochore of *Physcomitrella* (Miki et al., 2014).

Several kinesin members localized to the phragmoplast, either in the distal or medial region. For example, kinesins of the KCBP group were localized to the distal end of phragmoplast (Preuss et al., 2003), whereas members of the kinesin-5 group decorate the phragmoplast more uniformly (Bannigan et al., 2007). So do some members of the kinesin-14 group (Gicking, Swentowsky, Dawe, & Qiu, 2018). Members of the kinesin-12 group were localized at the median level of the phragmoplast and are probably responsible for the overlapping of microtubules (Lipka et al., 2014). In addition to the kinesins cataloged within the 14 canonical groups, some orphan kinesins, such as KINID1a and KINID1b, appear to be equally important for the interdigitation of antiparallel microtubules in the phragmoplast (Hiwatashi, Sato, & Doonan, 2014). In addition to the role of microtubule organizers at the phragmoplast level, it is believed that members of the kinesin superfamily may also contribute to the transport of vesicular material for building the cell plate. The kinesin named AtPAKRP2 has been proposed to be involved in this process and therefore represents a potential motor capable of transporting secretory vesicles (Lee, Giang, & Liu, 2001).

In addition to their role during cell division, plant kinesins are most likely involved in additional varieties of functions. For example, plant kinesins can establish cross-talk between microtubules and actin

filaments. This is the case for the kinesins GhKCH1 and GhKCH2, which belong to the family of kinesins-14 (Xu et al., 2009). These have a calponin homology domain that allows them to interact with actin filaments. As discussed later, members of this family may also play a key role in the pollen tube. Members of the kinesin-13 and -14 family have been found in association with Golgi bodies or mitochondria. For example, kinesin-13A is localized to Golgi bodies (Wei, Zhang, Liu, & Li, 2009), thus is most likely involved in Golgi vesicle dynamics. In contrast, a member of the kinesin-14 family (AtKP1) is associated with mitochondria, especially with an outer membrane protein (voltage-dependent anion channel 3; Ni, Wang, Xu, Qu, & Liu, 2005).

An important concept is that a specific subfamily of kinesin does not uniquely identify a function. It is the classic example of the kinesin-4 subfamily, whose members associate with the chromosome arms, the spindle, and the midbody, so they most likely work at multiple stages of cell division (Li et al., 2012; Zhang et al., 2010). However, some members of the kinesin-4 subfamily (e.g., AtFRA1) are involved in functions unrelated to cell division, such as cell wall organization and composition (Zhong, Burk, Morrison III, & Ye, 2002). This has been shown in both *Arabidopsis* and rice (Zhang, Zhang, et al., 2010). FRA1 is associated with vesicles that most likely contain non-cellulosic material, suggesting that FRA1 carries cell wall components other than cellulose (Kong et al., 2015; Zhu et al., 2015). Recent studies have shown that FRA1 is also involved in the lateral stability of cortical microtubules and that binds to cellulose synthase-microtubule uncoupling protein (CMU; Ganguly, Zhu, Chen, & Dixit, 2020). FRA1 has, therefore, a dual role: on the one hand, it can guide the movement of polysaccharide-containing vesicles, on the other, it regulates the organization of cortical microtubules thereby affecting the movement of cellulose synthase.

4 | WHY MIGHT KINESINS BE IMPORTANT IN THE POLLEN TUBE?

The hypothetical role of kinesins in the pollen tube must necessarily be integrated into the mechanism of tip growth and cell elongation. The growth process of the pollen tube is designed to allow the cell to elongate while also maintaining a cylindrical shape with a hemispherical apex. This process is critical to allow for rapid, polarized, and directional growth and thus for the pollen tube's task of delivering sperm cells to the ovule. It is now known that several components and molecules, including calcium gradient (Kroeger, Geitmann, & Grant, 2008; Winship, Rounds, & Hepler, 2017), levels of reactive oxygen species (Kaya et al., 2014; Potocky, Jones, Bezvoda, Smirnov, & Zarsky, 2007), and nitric oxide (Reichler et al., 2009), as well as differential pH values (Cortal et al., 2008), different organization of the cytoskeleton (Cai, Parrotta, & Cresti, 2015; Foissner, Grolig, & Obermeyer, 2002; Fu, 2015), the exact balance between exocytosis and endocytosis (Idilli et al., 2013; Ketelaar, Galway, Mulder, & Emons, 2008), the accurate structure of the cell wall (Dardelle et al., 2010; Geitmann & Steer, 2006; Hepler, Rounds, & Winship, 2013; Mollet, Leroux, Dardelle, & Lehner, 2013) and the turgor pressure play a critical and

interconnected role in maintaining the typical shape of the pollen tube. The core of the mechanism of cell shape maintenance is the close relationship between all these components. This central mechanism is in turn controlled by a series of inputs sensed by a signal transduction system, which consists of receptors (Boisson-Demier et al., 2013; Muschietti & Wengier, 2018; Takeuchi & Higashiyama, 2016; Zou, Aggarwal, Zheng, Wu, & Cheung, 2011), small GTPases (Chen, Cheung, Wu, & ming, 2003; de Graaf et al., 2005; Hwang, Gu, Lee, & Yang, 2005), and membrane phospholipids (Monteiro et al., 2005; Potocký et al., 2012; Zhang & McCormick, 2010). The complex network of interrelationships between all these components regulates and drives vesicular traffic along actin filaments in the first place. Secretory vesicles contain cell wall building blocks, mainly pectins, as well as, new plasma membrane. Vesicles are transported to the extreme apex of the pollen tube where, through exocytosis, they release components in such a way as to maintain the pollen tube shape. Relative changes in calcium ions, reactive oxygen species, and intracellular pH are thought to create appropriate conditions for the vesicles to move to a specific point and then be secreted. In the cell wall, a progressive deposition and modification of specific polysaccharides occur in a highly controlled manner (Bosch, Cheung, & Hepler, 2005; Bosch & Hepler, 2005; Zhang, Feng, Wu, & Wang, 2010) such that turgor pressure can act in an extremely targeted way.

In all, the role of microtubules and microtubule-based motors is unclear. Although studying the distribution of microtubules was quite straightforward using specific antibodies (Del Casino et al., 1993; Derksen, Pierson, & Traas, 1985; Raudaskoski, Aström, Perttälä, Virtanen, & Louhelainen, 1987), it was quite complicated to know and interpret their dynamics. Moreover, the polarity of microtubules in the pollen tube is not known, and to date, we do not know whether microtubules are overall oriented with the plus end toward the tube apex or toward the grain or whether microtubules exist with both polarities (although this seems unlikely). Little data exist on microtubule assembly centers. This uncertainty makes it even more difficult to hypothesize the role of kinesins and most importantly makes it difficult to understand whether kinesins are required to move toward the plus end or toward the minus end of microtubules. Together with the evidence that microtubule inhibitors have no marked effect on pollen tube growth (Laitinen, Nieminen, Vihinen, & Raudaskoski, 2002), this has always raised perplexities and doubts about the actual role of microtubules within this cell. If we compare the few data with those of somatic cells, microtubules could conceivably take part in processes such as cell wall deposition, movement of membrane components, targeting of mRNAs, or tension sensing (in the latter case thus being part of the cell-cell communication mechanism) (Hamant, Inoue, Bouchez, Dumais, & Mjolsness, 2019; Wang, Sadeghnezhad, Guan, & Gong, 2021). However, it is unclear whether molecular motors such as kinesins are necessary for one or more of the processes described above.

Below I will attempt to discuss the potential involvement of kinesins in several pollen tube processes, including organelle movement, cell wall deposition, and generative cell or sperm cell transport. For each of these points, I will describe the potential involvement of kinesins but also data that may exclude their role.

5 | DO KINESINS MOVE ORGANELLES IN THE POLLEN TUBE?

It is relatively easy to observe an intense movement of organelles and vesicles in the pollen tube; this has often been the subject of several publications (Cai et al., 2015; de Win, Pierson, & Derksen, 1999; Fujiwara et al., 2012; Heslop-Harrison & Heslop-Harrison, 1988). Large organelles, such as plastids, mitochondria, Golgi bodies, can move back and forth along the main growth axis of the pollen tube, as normally occurs in other plant cells. In the apical growth zone, the movement of larger organelles stops a few micrometers from the pollen tube apex to give way to a small region at the hemispherical apex where larger organelles are absent and where secretory vesicles accumulate abundantly. Therefore, to simplify radically the movement of membranous organelles in the pollen tube, it is possible to distinguish between secretory vesicles that accumulate at the pollen tube apex (Wang, Sheng, Tian, Zhang, & Li, 2020) and larger organelles that are excluded. The latter can exhibit a back-and-forth movement along the pollen tube axis or can focus in a more restricted area as in the case of mitochondria (Lovy-Wheeler, Cardenas, Kunkel, & Hepler, 2007). It is worth specifying that this concept applies to those organelles that are traceable, for which there is a chemical probe or transgenic plants expressing organelle-specific fluorescent proteins. Due to the above methods, we know that organelles actively move along the pollen tube but are not homogeneously distributed. For example, mitochondria accumulate preferentially in the subapical region to provide ATP necessary for growth (Colaco, Moreno, & Feijo, 2012). Golgi bodies are perhaps more broadly distributed but are hardly or less found in the apical region rich in secretory vesicles (Rui, Wang, Li, Tan, & Bao, 2020). The endosome system is used to recycle vesicular material or remove excess apical plasma membrane and is basically distributed in the apical and subapical region of the pollen tube (Liao, Wang, Yang, Peng, & Sun, 2010). The vacuolar compartment, as well as the endoplasmic reticulum, are more evenly distributed in the pollen tube, except for the apical and (partially) subapical region (Lovy-Wheeler et al., 2007). To be fair, there is a third major type of movement in the pollen tube, namely the slow but steady movement of sperm cells and the vegetative nucleus.

It is now commonly accepted that the movement of pollen tube organelles occurs along actin filaments. In support of this hypothesis, there are pharmacological data with actin filament inhibitors showing that proper organization of this cytoskeletal system is strictly necessary (Heslop-Harrison & Heslop-Harrison, 1989). The pollen tube is characterized by at least three different arrays of actin filaments, which correspond approximately to the apex, subapex, and shank. In the apical region actin is shaped as short and highly dynamic filaments, while in the subapical region it is possible to observe longer actin filaments that give rise to distinct organizations described either as collars or meshes or fringes. On the other hand, actin filaments in the flank are long, thick, and run parallel to the direction of pollen tube growth. These actin bundles are responsible for the long-distance transport of various organelles (Zhang et al., 2018). The role of actin filaments in the subapical and apical region has been more difficult to

decipher but it is a common assumption that they may serve to direct vesicle traffic to the fusion site. This role appears to be primarily supported by structures such as actin collars or fringes (Dong, Pei, & Haiyun, 2012; Li et al., 2017; Rounds, Hepler, & Winship, 2014).

Since kinesins were initially identified as motors capable of transporting organelles and vesicles, it was rational to assume that they could also play a similar role in plant cells and thus in the pollen tube, albeit with peculiar differences. However, considering what we know today, does it still make sense to hypothesize that kinesins carry organelles or vesicles in the pollen tube and more generally in plant cells? It is now well established that the transport of organelles and vesicles in plant cells is dependent on actin filaments, which allow movement over long distances and at very high speeds. Thus, excluding this role, what function remains for kinesins in transport along microtubules? Theoretically, there can be two possibilities. The first involves microtubules being specialized in unique and distinct transports of specific classes of organelles or vesicles, regardless of actin filaments. The second hypothesis, on the other hand, predicts that there is a sort of functional cooperation between the two cytoskeletal tracks, as proposed several years ago for animal systems (Goode, Drubin, & Barnes, 2000).

Evidence that plant kinesins are involved in the transport of organelles and vesicles is few and often indirect. Members of the kinesin-4 subfamily have been observed in association with vesicle-like structures in the cortical region of plant cells. This suggested that they might be somehow involved in controlling the deposition and synthesis of cell wall polysaccharides (Kong et al., 2015). However, homologs of the same group are actually involved in the organization of cortical microtubules to favor the directional movement of cellulose synthase in the plasma membrane (Ganguly et al., 2020). In plant cells, microtubules probably contribute to the expansion and organization of the endoplasmic reticulum thus supporting the central role played by actin filaments. It should be noted that there is no evidence to indicate the involvement of kinesins in this process (Hamada, Ueda, Kawase, & Hara-Nishimura, 2014). In addition to the theoretical association with the endoplasmic reticulum, members of the kinesin-13 subfamily have also been localized in association with Golgi bodies both in the pollen tube (Wei, Liu, & Li, 2005) and in other plant cells (Lu, Lee, Pan, Maloof, & Liu, 2005; Wei et al., 2009). However, the exact role played by these kinesins is not known, and it is unclear whether they actually contribute to moving Golgi bodies along microtubules or whether they help anchoring Golgi bodies at specific points in the cell. The transient anchoring of Golgi bodies in plant cells is reminiscent of the process called “stop and go” by which Golgi bodies move quickly along actin filaments and then stop when they capture or release transport vesicles (Nebenführ et al., 1999); It is not known whether anchoring is dependent on (motor-based) microtubules but it is an interesting hypothesis. In all the cases described above, there is no evidence of actual movement of membranous cell compartments along the microtubules. Golgi vesicles isolated from the pollen tube of tobacco move along the microtubules in *in vitro* assays at much lower speeds than the cytoplasmic streaming observed in these cells (Romagnoli et al., 2003). These data suggest that microtubules may

not be critical in long-distance transport of Golgi bodies, but contribute to localized, shorter transport at specific points in the cell. As an indirect support of this hypothesis, Golgi-derived structures isolated from hazel (*Corylus avellana* L.) pollen were observed to co-precipitate with microtubules in *in vitro* assays (Liu et al., 1994). However, to date there is no clear vision of the spatial relationship between a hypothetical “stop and go” of the Golgi bodies, the organization of the endoplasmic reticulum, and the movement of Golgi vesicles toward the pollen tube apex. Microtubules are also probably involved in another type of membrane movement in the pollen tube, namely the internalization of the plasma membrane that occurs during endocytosis (Idilli et al., 2013). However, it is unclear whether microtubules play a role in directing endocytotic vesicles to the endosomal system of the pollen tube, or whether they facilitate the budding of endocytotic vesicles or whether mark the endocytosis zone without having a direct role in the movement of endocytotic membranes. In relation to the involvement of microtubules in endocytosis processes, an intriguing role of kinesins is the internalization of S-RNase during the gametophytic self-incompatibility reaction. Meng et al. (2014) have shown that S-RNase is imported into the pollen tube and co-localizes with Golgi vesicles during internalization. Furthermore, S-RNase is prevented from entering the pollen tube if pollen is treated with actin filament or microtubule inhibitors, as well as microtubule stabilizing agents. Therefore, cytoskeleton antagonists can prevent S-RNase-mediated inhibition of pollen tubes *in vivo* by blocking S-RNase internalization and suggesting that an intact and dynamic cytoskeleton is required. According to the authors, the internalization mechanism of S-RNase occurs through the interaction between Golgi vesicles and cytoskeleton in which kinesins could take part.

To cite a further type of kinesin-dependent movement, in cell systems other than the pollen tube members of the kinesin-14 family, namely KAC1 and KAC2, are involved in the light-dependent movement of chloroplasts (Suetsugu et al., 2010). This movement is dependent on short actin filaments to which the kinesins are probably associated. It is not yet clear what the exact role of these motors is, whether they actually contribute to the direct movement of chloroplasts. Similar evidence has not been found in the pollen tube regarding the movement of plastids.

6 | WHETHER AND HOW MICROTUBULES AND MICROTUBULE MOTORS HELP DEPOSIT THE CELL WALL

The cell wall of the pollen tube is organized according to a gradient, from the apex backward toward the grain. A matrix enriched in methyl-esterified pectins is present in the very apical region, followed by a region of acid pectins, sometimes deposited periodically. The succession of methyl-esterified pectins and acid pectins is the result of a progressive demethylation of pectins by pectin-methyl esterase and is necessary to progressively strengthen the cell wall thus forcing the turgor pressure to act mainly at the pollen tube apex (Hepler et al., 2013). To ensure that the pollen tube maintains a cylindrical

shape, a continuous layer of callose and cellulose is deposited from the subapex onwards with the callose normally more abundant than cellulose (Chebli, Kaneda, Zerzour, & Geitmann, 2012). This results in the formation of a very resistant sheath that contributes to the geometric shape of the pollen tube as well as to the high growth rate observed in angiosperms. If the role of callose in the pollen tube is now known, our knowledge about the role of cellulose in pollen tube growth is quite different. Unlike other plant cells, cellulose microfibrils are predominantly oriented in the direction of pollen tube elongation and not transversely (Aouar, Chebli, & Geitmann, 2010). Therefore, the traditional model describing the extension of plant cells is not fully applicable to the pollen tube. In plant cells, cellulose microfibrils are usually aligned with microtubules positioned below the plasma membrane. The interaction between microtubules and cellulose synthase, orchestrated by many proteins, allows plant cells to precisely orient cellulose microfibrils by preventive organization of microtubules. Among the various proteins that mediate the interaction between cellulose synthase and microtubules, the kinesin FRA1 was originally proposed as a possible linker between microtubules and cellulose synthase (Zhong et al., 2002). However, the role of this motor protein is unclear; later studies of kinesin-4A in cotton (*Gossypium hirsutum* L.) and its FRA1 homolog from Arabidopsis have shown that the motor protein localizes to vesicle-like structures that align and move along microtubules. Vesicles most likely carry non-cellulosic material (Kong et al., 2015; Zhu et al., 2015). Other studies have suggested that FRA1 is also used to stabilize cortical microtubules or to regulate the interaction between cellulose synthase and microtubules by binding to the CMU protein (Ganguly et al., 2020). Thus, it appears that the dynamic activity of FRA1 is related to several facets of cell wall deposition. Since FRA1 has been characterized in diffuse-growing cells (such as cotton fibers), the comparison between cotton fibers and the pollen tube (a tip-growing cell) may seem hazardous given the different patterns of cell expansion; nevertheless, this is important evidence that kinesins can indeed transport vesicular material in plant cells.

The delivery of cellulose synthase into the plasma membrane can also be ascribed to the dynamic role of microtubules. In fact, cellulose synthase is targeted to the plasma membrane through vesicular structures such as SmaCC/MASCs (small compartments/microtubule associated cellulose synthase compartment (CESA)), which are intermediate membranous compartments between the Golgi and the plasma membrane (Crowell et al., 2009; Gutierrez, Lindeboom, Paredes, Emons, & Ehrhardt, 2009). SmaCC/MASCs actively move along actin filaments but are most likely targeted to specific sites by means of microtubules; the interaction with microtubules is dynamic but probably dependent on polymerization/depolymerization of microtubules rather than on motor proteins.

The relationship between motor proteins and callose deposition is even less clear. The few available data suggest that proper microtubule dynamics are necessary for appropriate distribution of callose synthase in the pollen tube (Cai, Falieri, Casino, Emons, & Cresti, 2011). More recently, callose synthase has been shown not to interact with microtubules but, on the contrary, tubulin monomers may be part of the callose synthase complex. The data lead to the

hypothesis of a connection between callose synthases and microtubules in the pollen tube (Parrotta et al., 2022). In the same article, it was also pointed out that the MMR44 antibody-detected kinesin is not part of the callose synthase complex. This suggests no direct involvement of this specific kinesin in callose synthase movement or activity. However, considering the high number of kinesins putatively expressed in plant cells, we cannot exclude a priori that kinesins may participate in callose synthesis. Therefore, at the current state of knowledge, few hypotheses can be made about the involvement of kinesins in the synthesis of callose and cellulose in the pollen tube cell wall. (a) Kinesins may move secretory vesicles containing callose synthase or cellulose synthase; this seems an unlikely hypothesis and is not supported by the available data. (b) Kinesins could anchor plasma membrane glucan synthases to microtubules and help regulate their activity; again, there is no evidence to support it. (c) Kinesins could stabilize or organize cortical microtubules and thus indirectly regulate the activity of cellulose synthase and callose synthase. The latter hypothesis, although supported by few experimental data, seems to be the most plausible.

7 | IS THE TRANSPORT OF SPERM CELLS IN THE POLLEN TUBE DEPENDENT ON MICROTUBULES AND MICROTUBULE-BASED MOTORS?

The biological function of the pollen tube is to transport the male gametes to the ovule. To date, the mechanism by which this movement takes place is, to say the least, unclear. Although various hypotheses have been proposed over time, a precise idea of the mechanism that determines the active movement of sperm cells is not available. Typically, the two sperm cells and the vegetative nucleus move as a single entity referred to as the male germ unit (Kliwer & Dresselhaus, 2010). The biological rationale behind this association also relates to the control of transcription and gene silencing in sperm cells by the vegetative nucleus (Jiang et al., 2015). It is not known whether this association is somewhat related to their movement in the pollen tube. However, dissociation of the male germ unit blocks or slows down sperm movement suggesting that the transport mechanism requires the three units to be constantly associated (Ge et al., 2011). Although the process is of fundamental biological importance, few proteins are known to be involved in this process; for example, proteins of the tryptophan-proline-proline domain-interacting protein (WIP) and WPP domain-interacting tail-anchored protein (WIT) families are considered essential. These are proteins of the nuclear membrane, perhaps also capable of interacting with the myosin motor proteins (Zhou & Meier, 2014). The order by which the vegetative nucleus and the two sperm cells enter the pollen tube appears to be related to the shape of the vegetative nucleus. This is revealed by analyses of mutants in the KAKU4 protein showing an irregular shape of the vegetative nucleus and an altered order of entry into the pollen tube (Goto, Tamura, Nishimaki, Maruyama, & Hara-Nishimura, 2020). Small GTPase Rop1 proteins could also play a role in the movement of the generative cell and sperm cells, although this is currently only

hypothetically (Lin, Wang, Zhu, & Yang, 1996). Recent hypotheses suggest that the pollen tube may have evolved independently of the transport function of sperm cells. Indeed, the absence of sperm cells in mutant lines does not affect pollen tube development hypothetically suggesting that the two processes (i.e., pollen tube development and sperm cell transport) are decoupled and that the function of sperm cell transport was implemented later (Zhang et al., 2017).

Further hypotheses over time have predicted an interaction with actin filaments, thus a transport based on the dynamic activity of myosins (Tirlapur, Faleri, & Cresti, 1996). The periodic and regular deposition of callose plugs in the pollen tube also appears to be related to generative cell movement, at least in tobacco (Laitinen et al., 2002). Evidence, albeit indirect, that microtubules could be involved in the transport of generative or sperm cells was provided by experiments with microtubule inhibitors (Astrom, Sorri, & Raudaskoski, 1995; Laitinen et al., 2002). These observations showed that microtubules play a role in generative cell transport without providing precise indications on the mechanism. As mentioned above, the evidence that the pollen tube can function properly even in the absence of sperm cells (Zhang et al., 2017) suggests that a nuclear motility mechanism was already present in an ancestral pollen tube and that this mechanism was then adapted to the movement of sperm cells. The fact remains that a clear mechanism is still missing.

More recently, a model has been proposed by which sperm cell movement occurs because of the activity of the so-called kinesins with calponin homology domain (KCH), a group of kinesins that connect actin filaments with microtubules (Figure 1). According to this model, sperm cells are enclosed within a microtubule cage and the sperm cell-microtubule complex is actively transported by KCH activity along actin filaments (Schattner, Schattner, Munder, Höpfe, & Walter, 2021). On the other hand, the involvement of KCH in the nuclear transport in *Physcomitrella* has already been suggested (Yamada & Goshima, 2018). Given that the binding of KCH to cortical microtubules appears to depend on the dephosphorylation of tubulin (Schneider, Ludwig, & Nick, 2015), this may imply a possible mechanism of regulation of sperm cell movement. It is worth mentioning that the distribution of tyrosinated tubulin in the tobacco pollen tube is not uniform along microtubule arrays, but sometimes focused on specific areas (Del Casino et al., 1993). The model, although still to be finalized, is certainly interesting and proposes the direct involvement of cytoskeletal motors.

It is equally interesting to ask whether KCH can also be important in the transport of organelles in the pollen tube. This would assume that organelles to be transported are associated with short actin filaments and that the latter are dragged along the microtubules (Dixit, 2012). Direct evidence of this role is not yet available, but the hypothesis is just as suggestive as the involvement of KCH in the movement of the generative cell.

8 | FINAL REMARKS

After 30 years from the initial discovery and characterization of a kinesin-like protein in the pollen tube, the role that such proteins

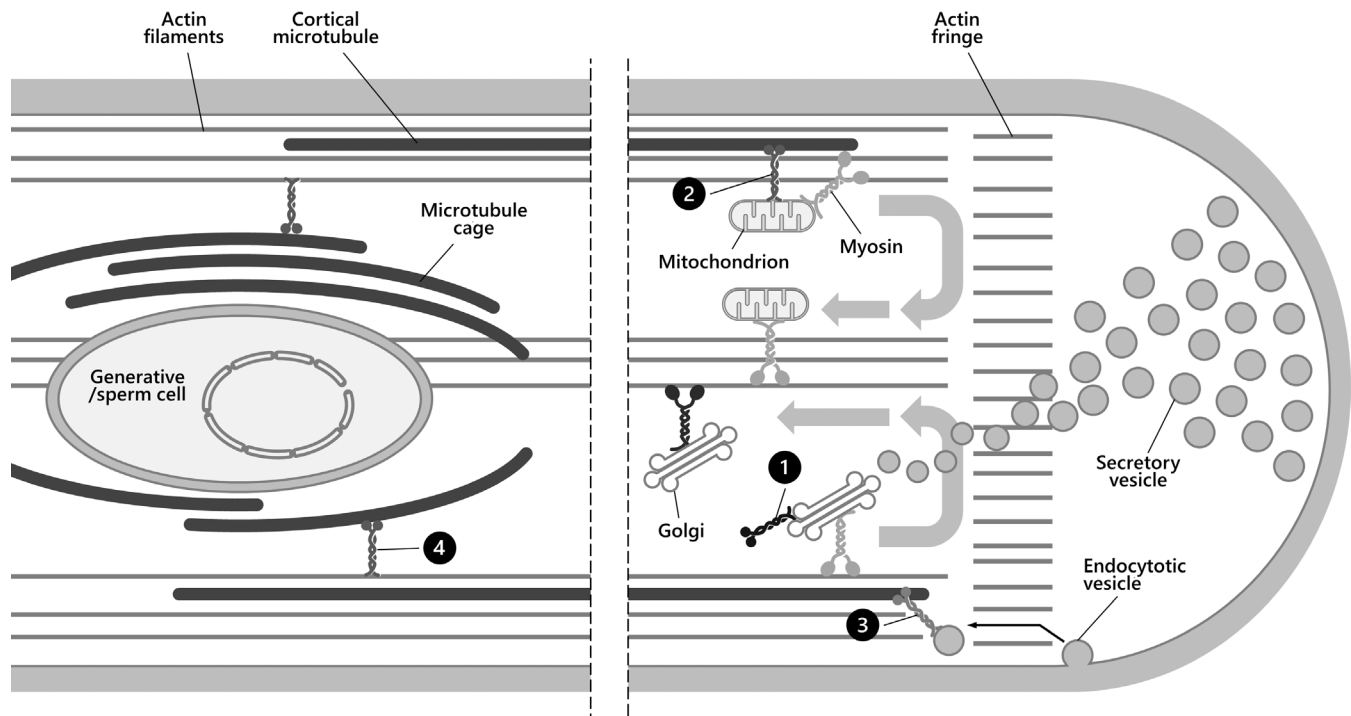


FIGURE 1 Illustration of the main hypothetical functions that kinesins (indicated by numbers) could perform in the pollen tube. Currently available data suggest that they could regulate the movement of Golgi bodies (1) perhaps by temporarily anchoring them to microtubules and decelerating their movement. Golgi bodies most likely move back and forth in the pollen tube but must release secretory vesicles that accumulate at the apex. A similar role could be played in the case of mitochondria (2) which could move rapidly along the actin filaments and slow down when contacting the microtubules. Mitochondria accumulate mainly in the subapical region, just behind the actin fringe, thus contributing to the high production of ATP, which is necessary for pollen tube growth. In both cases, the role of kinesins and microtubules is likely to help focus the organelles in specific areas. Other data suggest that kinesins may be involved in endocytosis (3) although the data are quite hypothetical. Recent data indicate a particular family of kinesins (kinesins with calponin homology) to be involved in the transport of generative or sperm cells along the pollen tube, perhaps by favoring the dynamic interaction between microtubule cages and actin filaments (4). Since a possible role of kinesins in cell wall deposition in the pollen tube is not supported by current data in the literature, it has not been included in the drawing.

could play inside the male gametophyte remains to be understood. Unfortunately, although these proteins have long since been identified and characterized, a lack of information at the gene level does not facilitate either interpretation or hypothesizing their actual role within the pollen tube. On the contrary, most of the information that we have today on myosins comes from the analysis of mutants or from the analysis of chimeric proteins fused with fluorescent proteins, which allowed to assemble a model of myosins functioning in pollen, as well as in other cells. In contrast, in the case of microtubular motors, none or a few kinesin sequences have been uniquely identified in the pollen tube and associated with specific functions; this obviously leaves open several (too many) possibilities and hypotheses (movement of organelles and vesicles? cell wall assembly? sperm cell transport?). Another weak point is represented by the long-standing lack of knowledge about the actual role of microtubules in the pollen tube. It is paradoxical that after so many years of research it is still unclear what their role is and in which processes of the pollen tube they are involved. Perhaps this is because most of the studies were performed *in vitro* and not *in vivo*, which can hide some critical functions; however, it does not justify the lack of information. What is most striking is that in

recent years the pollen tube has become a model cell for extracellular signaling processes and for regulation of tip growth. Numerous advances have been made in interpreting the dynamics of actin filaments in the apical and subapical region, thus in a very confined space. Therefore, most research has focused on the actin filament system, while neglecting the microtubule system, which is considered minimally involved in pollen tube function. On the contrary, I believe that the evidence available in the literature and summarized in this review indicates the need for further investigation on this topic to thoroughly understand the role of microtubules in the pollen tube, their dynamics, and the dynamic processes in which they are involved. As a result, this could lead to a revisiting of the role of kinesins in the pollen tube (with the hope that it will not take another 30 years...).

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Giampiero Cai  <https://orcid.org/0000-0002-4511-3557>

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