Navigating the plant cell: intracellular transport logistics in the green kingdom

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ABSTRACT Intracellular transport in plant cells occurs on microtubular and actin arrays. Cytoplasmic streaming, the rapid motion of plant cell organelles, is mostly driven by an actin–myosin mechanism, whereas specialized functions, such as the transport of large cargo or the assembly of a new cell wall during cell division, are performed by the microtubules. Different modes of transport are used, fast and slow, to either haul cargo over long distances or ascertain high-precision targeting, respectively. Various forms of the actin-specific motor protein myosin XI exist in plant cells and might be involved in different cellular functions.

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INTRODUCTION

Cells must move molecules and organelles between different locations within the cytoplasm in order to function. In mammalian cells, some of the fastest transport processes occur in neuronal axons, in which cargo vesicles move at rates up to few micrometers per second along the microtubule array (Hill *et al.*, 2004). However, this velocity is dwarfed by intracellular movement rates that occur in plant cells. Among the fastest rates are those observed in the giant internodal cells of the green alga *Nitella*, which are upward of 50 µm/s (Mustacich and Ware, 1977; Kuroda, 1990). This *Perspective* provides a snapshot of our current understanding of the functions, mechanisms, and implications of intracellular transport along the plant cytoskeleton.

BIOLOGICAL FUNCTIONS OF TRANSPORT IN PLANT CELLS

The principal functions of intracellular motility in plants are thought to include cargo delivery, strategic repositioning of organelles, and mechanical stirring of the cytosol. Cargo delivery can comprise, for example, the transport of molecules from their location of synthesis to their destination or the uptake of substances from the outside and their subsequent transfer to the cytoplasmic organelles responsible for their use or recycling. In plant cells, such transport cargo includes polysaccharides that are synthesized in the Golgi and used in the assembly of the cell wall outside the plasma membrane (Nebenführ and Staehelin, 2001). Even foreign bodies such as viruses are transported by recruiting the cellular motility machinery (Harries and Ding, 2011). Strategic repositioning of organelles is typically used to optimize metabolic functioning under certain environmental conditions, such as variations in light intensity, or in response to external stimuli, such as a pathogenic attack. For example, the translocation of chloroplasts within the cellular space is carried out depending on the direction and intensity of sunlight (Sato *et al.*, 2001) with the aim of optimizing photosynthetic activity at the cellular level. Finally, organelle translocation might not be related to the organelles' metabolic functions, but their motion may have a physical purpose. Stirring the cytosol is a mechanical process that increases the probability of dissolved molecules interacting. This solute mixing may be particularly important in plant cells, in which the cytoplasm often consists of a thin, two-dimensional layer sandwiched between the plasma membrane and the vacuole (Verchot-Lubicz and Goldstein, 2010). The shear forces generated by the cytoplasmic streaming might even be transmitted through the tonoplast to the vacuolar contents (Goldstein *et al.*, 2008).

Intracellular movements in general occur by free diffusion or along the filaments of the cytoskeleton. Despite the somewhat misleading name, the cytoskeletal arrays are highly dynamic. Actin filaments are continuously polymerized and depolymerized, they bundle and separate—processes that are regulated by proteins interacting with the filaments and their monomers (Blanchoin *et al.*, 2010). Microtubules are similarly dynamic (Shaw *et al.*, 2003), and different types of motor proteins are responsible for moving cargo on this array (Lee and Liu, 2004). Although actin filaments are

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Abbreviations used: CESA, cellulose synthase; ER, endoplasmic reticulum.
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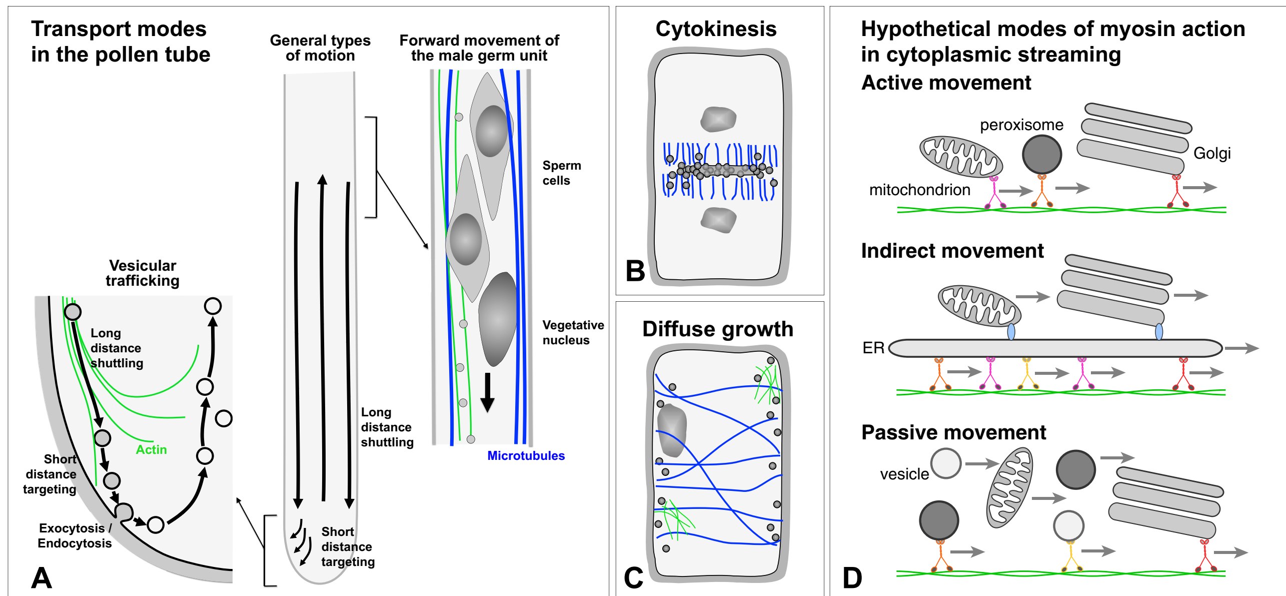

FIGURE 1: (A) Different modes of transport in the pollen tube, a rapidly growing cell with very active intracellular transport. (B) Cytokinesis in plant cells involves the assembly of new cell wall from vesicles targeted along microtubules (blue) precisely to the location of the future dividing cell wall. (C) In diffusely growing cells, targeting is less obvious but involves different modes of transport along actin filaments (green), fast and slow. The microtubules (blue) are primarily cortical and are responsible for in the guidance of cellulose synthases located in the plasma membrane. (D) Hypothetical models of myosin action in cytoplasmic streaming. The active-movement model predicts that every moving organelle associates with at least one myosin motor, presumably with different isoforms (different colors) specific for different organelles. In the indirect-movement model, different myosin isoforms are assumed to cooperate in moving the ER along actin filaments (green). Other organelles would physically associate with the ER (pale blue attachments) and get carried along. The passive-movement model assumes that active movement of some organelles generates a local hydrodynamic flow in the cytosol that drags other organelles along. The nature of the myosin-associated organelles is not known at this time.

generally considered to be the dominant actor in plant intracellular mobility, microtubules play an important role in a variety of situations. The well-studied function of the cortical microtubule array in guiding cellulose synthases during cell wall assembly or in separating chromosomes during mitosis will not be discussed here (Ehrhardt and Shaw, 2006; Nick, 2008); instead, the focus will be on microtubular transport of organelles through the volume of the cell. In pollen tubes, for example, a clear division of labor between the actin and microtubular arrays seems to take place. Rapid organelle movements of upward of 4 μm/s (Heslop-Harrison and Heslop-Harrison, 1987; de Win *et al.*, 1999; Bove *et al.*, 2008) occur along the actin cytoskeleton and correlate with cellular growth (Geitmann and Emons, 2000). The situation is different for the mobility of the largest intracellular cargo in pollen tubes, the male sperm unit comprising the vegetative nucleus and the sperm cells (Figure 1A). The movement of this large unit is in fact significantly hampered by the pharmacological interference with microtubule functioning, indicating that microtubules rather than actin filaments are responsible for its motion (Laitiainen *et al.*, 2002). Moreover, unlike the much faster, smaller organelles, the male germ unit moves relatively slowly, as it usually keeps the same speed as that of the growing pollen tube tip—between 10 and 20 μm/min in vitro for lily or camellia (Rounds *et al.*, 2010; Bou Daher and Geitmann, 2011)—but possibly faster in vivo, since growth conditions provided by the pistil are generally better compared with those in the Petri dish. Interestingly, the speed of the male germ unit is adjusted depending on the growth rate of the tube tip, since the distance between the two is typically kept constant, thus suggesting the existence of a regulatory feedback mechanism. This notion is corroborated by observations showing that when the male germ unit is temporarily prevented from moving forward by a mechanical obstacle constraining the width of the tube, it accelerates once the obstacle is passed. It seems as if the nucleus attempts to "catch up" with the growing tube tip and makes an effort to return to its set distance from the tip (Sanati Nezhad *et al.*, 2013). How the actual distance of the male germ unit from the tip is perceived and which role the microtubules may play in the potential sensing mechanism, other than providing the means of transport, is unknown.

Another important transport process coordinated by the microtubule cytoskeleton occurs during plant cytokinesis (Figure 1B). Following the separation of the chromosomes, the dividing plant cell must construct a new wall that divides the cytoplasm of the two daughter cells. This dividing wall is built starting from the center of the cytoplasmic volume and relies on the delivery of cell wall material containing vesicles that aggregate and fuse. The targeted delivery of these vesicles is orchestrated by the phragmoplast, an array of microtubules oriented toward the site of the future dividing wall. This array dynamically adjusts its spatial configuration to accommodate the increasing diameter of the cell plate until the latter encounters the parental plasma membrane and fuses with it to completely separate the daughter cells (Smith, 2002). Cargo vesicles are therefore delivered exactly where they are needed for construction purposes, and the presence of kinesin on these vesicles supports the

notion that the primarily responsible cytoskeletal array is indeed composed of microtubules (Lee *et al*., 2001; Jürgens, 2005).

MODES OF TRANSPORT

Transport processes on the plant actin network occur in a variety of different modes. Cargo can be hauled over long distances at high velocity, or the motions occur in short, seemingly erratic spurts resembling Brownian motion that may serve to target the cargo toward its destination (Bove *et al*., 2008). Precise cargo targeting has been found to be associated with fanned-out arrays of individual actin filaments or thin cables, whereas long-distance transport seems to be mediated by thicker actin cables (Geitmann and Emons, 2000). For example, in root hairs and pollen tubes, long-distance transport of most organelles occurs rapidly and efficiently on actin cables that traverse the entire length of these longitudinal cells (Chebli *et al*., 2013). Typically, this long-distance transport occurs in well-defined lanes, but the spatial arrangement of the lanes differs depending on the cell type. In angiosperm pollen tubes, the motion from the pollen grain toward the tip of the tube occurs in the periphery of the cytoplasm, whereas rearward traffic is typically focused in the center (de Win *et al*., 1999). In gymnosperm pollen tubes, this pattern is reversed. In root hairs, on the other hand, peripheral lanes are present in both directions (Miller *et al*., 1999). The regulatory mechanisms that lead to these different arrangements of actin cables are currently unknown. Precise targeting of vesicles toward the site of exocytosis in tip-growing cells occurs on actin filaments arranged in a finely fanned-out array; in pollen tubes, this array is called the "apical fringe" (Vidali and Hepler, 2001; Ketelaar *et al*., 2003). This fringe must be constantly renewed by polymerization of additional actin filaments to ascertain its presence in the growing region at all times as the cell expands forward (Kroeger *et al*., 2009). The important role of the subapical actin fringe in morphogenetic control has been demonstrated in pollen tubes upon artificially triggered changes in the growth direction (Bou Daher and Geitmann, 2011). The externally visible morphogenetic change in cell shape is consistently preceded by an internal reorganization of the actin fringe. Similarly, in root hairs triggered to initiate an infection thread by the application of *Rhizobium* nodulation factor, rearrangements in the actin cytoskeleton occur before the morphogenetic event (De Ruijter *et al*., 1999).

In diffusely growing cells, the role of the actin cytoskeleton in guiding secretory cargo to the proper location on the cell surface could be inferred only indirectly by using actin inhibitors. Specifically, it was shown that even delivery to the plasma membrane of cellulose synthase (CESA) complexes, the enzymes that synthesize cellulose at the surface of plant cells, requires a functional actin cytoskeleton. CESA was still delivered in the absence of actin, but the distribution was less uniform and, interestingly, matched that of Golgi stacks inside the cells (Gutierrez *et al*., 2009). This suggests that the actin cytoskeleton regulates Golgi body positioning for accurate CESA delivery but that secretion is not dependent on actin filaments. Unlike CESA delivery to the cell surface in hypocotyl cells, the positioning of the Golgi body seems to be less important for the delivery of polysaccharides, as observed in *Arabidopsis* seed coat cells producing pectin mucilage (Young *et al*., 2008). This is also consistent with the motion pattern of Golgi stacks in pollen tubes, which never reach the actual site of pectin exocytosis at the very tip of the cell (Cresti and Tiezzi, 1990). It should be noted, however, that actin filaments are still present in the latter two examples and could serve as tracks for longer-distance delivery of post-Golgi vesicles.

SPEED OF TRANSPORT

The movement speeds and patterns of organelles being shuttled on different actin arrays vary significantly and seem to depend on the actin configuration rather than the organelle or type of motor (Akkerman *et al*., 2011). In pollen tubes, the speed of vesicles varies between 1 and 4 μm/s in the shank and <1 μm/s in the tip region (Heslop-Harrison and Heslop-Harrison, 1987; de Win *et al*., 1999; Bove *et al*., 2008). Movement within the tip region has been proposed to either occur on highly dynamic and therefore ephemeral actin filaments or to not be based on motor-driven transport at all but instead on diffusion (Kroeger *et al*., 2009) and possibly bulk flow. How the speed of actively transported organelles is regulated is largely elusive. Plant myosins are among the fastest mechanochemical enzymes in any living being (Tominaga *et al*., 2003). Aside from direct regulation of enzymatic activity of the motors (Yokota *et al*., 1999), the number of motor molecules attached to an individual organelle linking it to the actin array may be one of the determining factors of organelle speeds. Also, thicker actin cables may offer more opportunities for motor-mediated connection between a given organelle and the cytoskeletal array, hence more efficient organelle motion against the drag forces of the cytosol may be ascertained on thicker actin cables.

In tip growing cells, transport is essentially confined to two directions, thus simplifying its quantification (Figure 1A). In cells with a less polar growth mechanism, our understanding of the role of actin is more vague, but the function is seemingly analogous: In the cylindrical and diffusely growing cells of *Arabidopsis* root epidermal cells, Golgi bodies and other organelles were observed to display different modes of mobility in different cellular regions: fast, directed motion at 2–7 μm/s; and "wiggling," a seemingly nondirectional movement with speeds below 2 μm/s (Akkerman *et al*., 2011; Figure 1C). Fast movement was found to be associated with thicker actin cables, whereas wiggling occurred at locations with fanned-out, individual filaments. Similar distinct motion patterns have been found in other plant cell types, such as BY-2 cell cultures (Nebenführ *et al*., 1999). Individual organelles typically switch between wiggling and rapid long-distance movement by unknown mechanisms.

MECHANISMS OF FAST MYOSIN-DEPENDENT MOVEMENTS

Fast, actin-driven organelle movements in plant cells are driven by class XI myosin motor proteins. These motors are homologous to myosin V in animals and fungi and contain, besides the typical motor domain, a long neck with six IQ motifs, a coiled-coil region, and a C-terminal globular tail domain that encompasses the so-called dilute domain (Kinkema and Schiefelbein, 1994). The long neck region allows both myosin V and XI dimers to take 35-nm-long steps along actin filaments (Tominaga *et al*., 2003) while at the same time mediating regulation by calcium via the attached calmodulin-like light chains (Tominaga *et al*., 2012). The relatively short coiled-coil region of myosin XI provides only weak dimerization that is stabilized by interaction with the cargo (Li and Nebenführ, 2008a). Cargo binding occurs in the globular tail domain that has the same fold as the equivalent domain in myosin V but has little sequence conservation of the surface residues (Li and Nebenführ, 2007). Unlike myosin V, plant myosin XI can reach high velocities of up to 7 μm/s in flowering plants (Tominaga *et al*., 2003) and 60 μm/s in algae like *Chara* (Ito *et al*., 2003, 2007). These remarkable speeds seem to be possible because of subtle changes in the otherwise highly conserved myosin motor (Ito *et al*., 2009; Henn and Sadot, 2014; Diensthuber *et al*., 2015).

While the basic biochemical action of myosin XI as an actin-based motor is well established, the cell biological function of these motors is less clear. In particular, the apparent need for several different myosin XI motors (Mühlhausen and Kollmar, 2013) in individual cells of flowering plants is still not resolved. A simple first hypothesis is to assume that each of the different myosin XI subtypes associates with a different organelle (Figure 1D, "Active movement"). This was tested by transient expression of fluorescently tagged tail constructs, since it can be assumed, in analogy with myosin V, that the globular tail domain functions as the cargo-binding domain (Li and Nebenführ, 2008b). These experiments did reveal a variety of localizations within plant cells (Li and Nebenführ, 2007; Reisen and Hanson, 2007; Sparkes *et al.*, 2008; Avisar *et al.*, 2009; Sattarzadeh *et al.*, 2011), supporting the notion that the various myosin XI isoforms are responsible for the movement of different organelles.

This conclusion, however, is called into question by functional studies involving dominant-negative and knockout experiments. Specifically, loss of single myosin motors in insertional knockout mutants resulted in reduced mobility of several organelles (Peremyslov *et al.*, 2008) that these particular myosins do not seem to localize to (Li and Nebenführ, 2007; Reisen and Hanson, 2007; Sparkes *et al.*, 2008; Avisar *et al.*, 2009; Sattarzadeh *et al.*, 2011). These movements were further reduced in higher-order mutants that were missing two or more myosin motor genes (Prokhnevsky *et al.*, 2008; Peremyslov *et al.*, 2010; Ueda *et al.*, 2010). Combined with the progressively reduced organelle movements, the phenotypic defects in the higher-order mutants became more and more severe, with the quadruple mutant resulting in much smaller plants with smaller cells (Peremyslov *et al.*, 2010), demonstrating that myosin activity is necessary for cell expansion. At face value, these results suggest a high level of redundancy among myosin XI motors (Peremyslov *et al.*, 2010) that is difficult to reconcile with the concept of different functions based on the observed different localizations of the respective tail constructs (Li and Nebenführ, 2007; Reisen and Hanson, 2007; Sparkes *et al.*, 2008; Avisar *et al.*, 2009; Sattarzadeh *et al.*, 2011).

Similarly confusing results were obtained with the previously discussed tagged tail constructs that were found to reduce the movement of various organelles in the transformed cells. This dominant-negative effect was also seen in animal systems (e.g., Wu *et al.*, 1998) and is usually explained by a saturation of motor-binding sites on the surface of the target organelles. Curiously, this simple one-to-one match of labeled organelle and reduced speed did not hold in plant cells. For example, tail constructs from several myosin isoforms were found to reduce Golgi stack movements, but none of those studied seemed to localize to the Golgi (Avisar *et al.*, 2009). In fact, one particular tail construct was found to inhibit the movement of all organelles tested (Avisar *et al.*, 2009, 2012), implying some kind of universal function for this motor, which is also supported by the strong defects associated with the loss of this gene in single and multiple mutants (Ojangu *et al.*, 2007, 2012; Peremyslov *et al.*, 2008, 2010; Prokhnevsky *et al.*, 2008; Park and Nebenführ, 2013). Interestingly, this dominant-negative effect was found to depend on two positively charged residues on the surface of the globular tail domain (Avisar *et al.*, 2012) that are known to mediate head-to-tail interactions in the related animal myosin V motors (Li *et al.*, 2008). It is therefore possible that this particular myosin tail construct exerts its dominant-negative function by interacting with the motor itself rather than the cargo.

Another possible explanation for the observation of broad, seemingly nonspecific effects of knockout mutants or dominant-negative constructs is that organelle movements might occur indirectly rather than by direct association of motors with individual organelles (Buchnik *et al.*, 2015). For example, it is possible that organelles associate with the ER surface (Figure 1D, "Indirect movement"), as has been proposed for Golgi stacks (daSilva *et al.*, 2004). Active movement of myosins associated with the surface of the endoplasmic reticulum (ER) could then result in passive displacement of the ER-associated organelles (Stefano *et al.*, 2014). Loss of motors that have a strong influence on ER movements would lead to reduced movements of many organelles simultaneously. A similar scenario could result if organelles attached directly to actin filaments, since it was shown that myosin motors are responsible for dynamic rearrangements of these filaments (Park and Nebenführ, 2013; Cai *et al.*, 2014). An alternative explanation for the indirect effects of myosin mutants could be that active movement of some organelles leads to hydrodynamic flow (Esseling-Ozdoba *et al.*, 2008) that then passively drags other organelles through the cell (Figure 1D, "Passive movement"). In this model, indirect effects of myosin motors would also be highly confined in space, since the physics of liquid flow at the low Reynolds numbers that dominate fluid behavior in small volumes such as cells would likely prevent large-scale effects (Pickard, 2003). At the same time, it could be expected that inhibition of different myosin isoforms by mutation or dominant-negative interference would lead to at least some preferential effect on the different organelles.

It is not possible to distinguish between these different scenarios with our current knowledge. Identification of the direct targets of myosin motors, that is, immediate cargo that binds to their globular tails, will allow us to distinguish between the different models. A number of interacting proteins have been identified in recent years (Hashimoto *et al.*, 2008; Peremyslov *et al.*, 2013; Tamura *et al.*, 2013; Stephan *et al.*, 2014), but in several cases, their biological relevance or precise intracellular localization has not yet been identified. Better characterization of these and possibly additional myosin-binding partners, as well as direct manipulation of their interactions, should allow us to better understand how the biochemical action of myosin motors is translated into complex cellular behaviors. This will also require major advances in our ability to track and analyze the complex intracellular movements displayed by organelles in plants (Nebenführ *et al.*, 1999). Current analysis methods fall short of the ideal of complete analysis (Danuser, 2011; Chenouard *et al.*, 2014) and fail to capture more subtle changes in organelle behavior in mutants or under different environmental conditions.

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