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Invited Expert Review

Putting On The Breaks: Regulating Organelle Movements in Plant Cells

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Abstract

A striking characteristic of plant cells is that their organelles can move rapidly through the cell. This movement, commonly referred to as cytoplasmic streaming, has been observed for over 200 years, but we are only now beginning to decipher the mechanisms responsible for it. The identification of the myosin motor proteins responsible for these movements allows us to probe the regulatory events that coordinate organelle displacement with normal cell physiology. This review will highlight several recent developments that have provided new insight into the regulation of organelle movement, both at the cellular level and at the molecular level.

Keywords: Cytoplasmic streaming; cytoskeleton; myosin; organelle.

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Introduction

Most organelles of eukaryotic cells are not rigidly anchored in place, but can move through the cell in various ways. Even in cases where an organelle appears immobile in a specific region of a cell, which is often the case with the nucleus, it is still able to migrate to that point and also can re-assume its position after perturbations (Williamson 1993). A classic example of organelle motility is the photorelocation movement of chloroplasts, that helps to maximize photosynthesis via the accumulation reaction at low light intensities, and minimize photodamage via the avoidance reaction at high light intensities (Kong and Wada 2011). The most striking form of organelle movement, however, is the rapid cytoplasmic streaming found in most plant cells (**Figure 1**). These movements are driven by the actomyosin system (Shimmen 2007), and result in a constant mixing of cytosol (Houtman et al. 2007). While it is generally assumed that these movements facilitate rapid distribution of organelles and solutes in large cells (Shimmen and Yokota 1994), we still know remarkably little about their specific function in normal cell physiology.

In recent years, several groups have concentrated on the myosin motor proteins of plants to gain more insight into the mechanisms underlying cytoplasmic streaming. Most activities have centered on the myosin XI family of genes, since they show remarkable structural similarity to myosin V in animals and fungi which are well-known for their role in organelle movements (Kinkema and Schiefelbein 1994; Hammer and Sellers 2012). In particular, myosin XI heavy chains consist of a motor domain that binds actin and hydrolyzes ATP (Tominaga et al. 2003), a neck region that binds calmodulin-like proteins and acts as a lever arm (Yokota et al. 1999; Tominaga et al. 2003), a coiled-coil region that mediates dimerization (Li and Nebenführ 2008a), and a globular tail that functions as the organelle binding domain (Li and Nebenführ 2007). The excellent experimental tool box available in the model species *Arabidopsis thaliana* has allowed rapid progress in several areas. For example, a reverse genetic approach has revealed

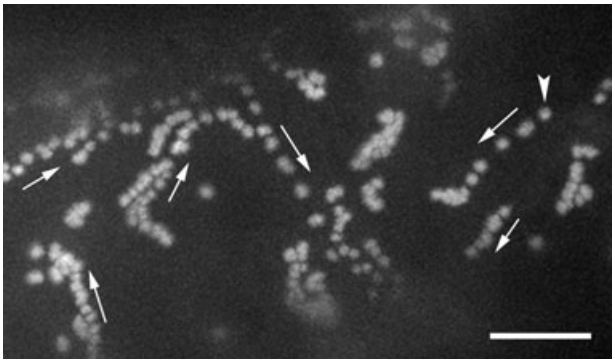

Figure 1. Movement of Golgi stacks in the cortical cytoplasm of an onion epidermal cell.

Superimposition of 11 consecutive images taken in 1 s intervals. Arrows indicate direction of movement of individual Golgi stacks. The stack indicated by the arrowhead changes its behavior from directed movement ("go") to random wiggling ("stop"). Size bar = 10 μm.

that loss of several myosin motor proteins leads to a cessation of organelle movements (Peremyslov et al. 2008; Prokhnevsky et al. 2008; Peremyslov et al. 2010), thus proving the long-held conjecture that myosin motor proteins propel organelles along actin filaments. This loss of organelle movement was accompanied by reduced growth (Peremyslov et al. 2010), suggesting that myosin activity is required for normal cell expansion. It is not clear, however, whether the reduced growth resulted from a general slow-down of cell metabolism due to reduced mixing, or whether myosin motors perform a specific function that is directly involved in growth, such as delivery of secretory vesicles to the plasma membrane (PM).

In order to gain a better understanding of the function of cytoplasmic streaming in plant cells, it is necessary to better characterize the movements and identify possible regulatory mechanisms. The advent of fluorescent live-cell markers for organelles has made it possible to follow their movement in exquisite detail. These studies have revealed that organelles such as Golgi stacks (Boevink et al. 1998; Nebenführ et al. 1999), mitochondria (van Gestel et al. 2002), and peroxisomes (Collings et al. 2002; Jedd and Chua 2002; Mathur et al. 2002) do not move continuously, but show saltatory, or stop-and-go, motions (**Figure 1**). It has been speculated that this irregular movement pattern does not simply reflect random events such as a motor losing its footing on the actin filaments, but actually represents different functional phases in the life of these organelles. In particular, the "stop" phases could be characterized by exchange of material with the local environment, whereas the "go" episodes would serve to deliver the organelle and its products to a different region of the cell. This model was first developed for the Golgi apparatus, which depends strongly on the efficient exchange of vesicles with other organelles of the secretory system (Nebenführ and Staehelin 2001). In this case, it was postulated that individual Golgi stacks would stop at endoplasmic reticulum (ER) export sites where vesicles destined for the Golgi are released. Similarly, Golgi stacks might stop in areas where their products are needed at the PM for cell growth (Nebenführ et al. 1999). Subsequent work has called this model into question, as it was found that ER export sites, when labeled with proteins of the COPII vesicular machinery, move together with Golgi stacks (daSilva et al. 2004), although some data are also inconsistent with this "secretory units" model (Kang and Staehelin 2008; Staehelin and Kang 2008). Recent work on the delivery of cellulose synthase to the PM, however, seems to support the original idea that organelles stop in places where their products are needed.

Spatial Regulation of Organelle Stops

Cellulose synthase is the enzyme responsible for assembly of the major load-bearing structures of the cell wall, the cellulose microfibers. Thus, these enzymes can be found in regions where cell expansion is taking place, and could therefore be a useful tool to test the predicted stopping of Golgi stacks in these areas. Such an experiment became possible with the cloning of *CesA* genes and their successful tagging with fluorescent proteins (Paredez et al. 2006). When such constructs are expressed under the control of the *IRX3* (*CESA7*) promoter, cellulose synthase complexes (CSCs) can be detected in developing tracheid cells (Wightman and Turner 2008). Since these cylindrical cells produce prominent secondary wall thickenings that wrap in rings around their circumference, it was expected that CSCs would accumulate in these regions. This prediction was confirmed by imaging the distribution of YFP-IRX3 in living seedlings (Wightman and Turner 2008). Conveniently, CSCs can also be visualized in Golgi stacks where they assemble prior to delivery to the PM (Paredez et al. 2006; Wightman and Turner 2008; Crowell et al. 2009). Interestingly, the YFP-IRX3 labeled Golgi stacks were found to pause preferentially at bands of CSC accumulation in the PM, for example, at the sites of secondary wall thickenings (Wightman and Turner 2008). Thus, these experiments are consistent with the concept of specific stops of Golgi stacks at sites of secretion to the PM. What was missing, however, were (a) the demonstration that these stops are accompanied by the release of secretory vesicles from the Golgi and their delivery to the PM, and (b) identification of the mechanism that is responsible for these organelle pauses.

Movement of CSCs in the PM follows microtubules (MTs) that are closely associated with the PM (Paredez et al. 2006). Indeed, accumulation of CSCs in bands around young tracheid

cells also matches and depends on cortical MTs (Wightman and Turner 2008). Thus, it might be possible that MTs mark the spots where Golgi stacks pause. This concept was further investigated in hypocotyl epidermis cells, which are more amenable to high-resolution microscopic analysis since they are found on the surface of the seedling. In epidermal cells, primary wall growth occurs diffusely over the entire surface, and microtubules form an evenly-distributed array (Paredez et al. 2006). Nevertheless, it was possible to identify new insertions of CSCs into the PM (Crowell et al. 2009; Gutierrez et al. 2009). These CSC insertion events occurred along oryzalin-sensitive lines (Crowell et al. 2009) and preferentially coincided with cortical MTs (Gutierrez et al. 2009). Thus, it appears that secretion of CSC, while not dependent on cortical MTs, is guided by these cytoskeletal elements. Disruption of actin filaments with latrunculin B, on the other hand, resulted in a clustering of Golgi stacks which was accompanied by delivery of CSCs to the PM only in the vicinity of the Golgi stack clusters (Crowell et al. 2009; Gutierrez et al. 2009). This demonstrates that CSC secretion does not depend on actin filaments, although CSC distribution over the cell surface does. More importantly, observation of the behavior of individual Golgi stacks revealed that pause events preceded the local insertion of CSCs into the PM (Crowell et al. 2009), directly supporting the hypothesis that interruption of organelle movements results in targeted delivery of products of the organelle (Nebenführ et al. 1999). The model that emerges from these observations is that actomyosin-driven movements of Golgi stacks lead to a broad distribution of CSCs over the cell surface, where their insertion into the PM is guided by the cortical MTs that also serve as their tracks during cellulose synthesis.

This model implies that Golgi stacks preferentially stop at MTs, which has also been borne out by direct observation of Golgi dynamics in relation to cortical MTs (Wightman and Turner 2008; Hamada et al. 2012). Interestingly, this behavior was also detected for other organelles that are not part of the secretory system. In particular, mitochondria, peroxisomes, and even RNA processing bodies (P-bodies), which are not bounded by a membrane, also preferentially interrupt their movements at MTs (Hamada et al. 2012). The generality of this behavior suggests that cortical MTs may constitute a general "stop" signal that prevents normal actin-dependent movements. In fact, the ER, which forms a rapidly anastomosing network of membrane tubules and sheets, also shows this behavior. Regions of the ER that are relatively static compared to the flexible tubular connections between them (Sparkes et al. 2009) coincided preferentially with cortical MTs (Hamada et al. 2012), again suggesting a role of these cytoskeletal elements in preventing actomyosin-based movements. It is not known how MTs would exert this function, but the presence of MT-binding proteins on organelle surfaces (Chuong et al. 2005) might suggest a mechanism by which organelles can be trapped at MTs. Of particular interest in this respect are organelle-based kinesin motors (Romagnoli et al. 2003; Lu et al. 2005; Ni et al. 2005; Romagnoli et al. 2007), since they could lead to a switching of tracks for the organelles that is accompanied by a dramatic slowdown in movement (Romagnoli et al. 2003; Romagnoli et al. 2007).

However, this potential regulation of organelle stop sites by cortical MTs is called into question by experiments that disrupted these MTs with oryzalin. In developing tracheids, Golgi stacks still displayed pauses in their movements, even though no distinct bands of secondary wall thickenings were formed (Wightman and Turner 2008). Similarly, the speed distribution of several organelles did not change in hypocotyl epidermis cells after oryzalin treatment (Hamada et al. 2012), again suggesting that MTs are not necessary to cause the stopping of these organelles. It is not clear how these pauses in organelle movement come about in the absence of MTs. It has been proposed that small populations of transverse actin filaments might disrupt normal longitudinal movement (Wightman and Turner 2008). Unfortunately, this hypothesis cannot easily be tested, since disruption of the transverse actin filaments would also remove longitudinal cables and hence prevent all movement. In another proposal, the stopping of organelles does not involve MTs directly, but instead depends on "cortical landmarks" that are normally associated with MTs (Hamada et al. 2012). In this model, disruption of MTs would leave these cortical landmarks behind, and organelle stops could still occur. Since nothing is known about these postulated landmarks, it will be difficult to test this hypothesis further. However, it may be possible to observe in living cells whether organelle stop sites remain intact during oryzalin treatment. Of course, this will only be successful if these cortical landmarks do not change their position after MT disruption. Another possible direction of research might be to examine the role of static ER patches more closely, as they may also play a role in regulating the motility of other organelles. More fundamentally, it would be interesting to see if the stop signal is a local diffusible signal such as calcium (Yokota et al. 1999), if it is dependent on specific protein-protein interactions such as the postulated membrane matrix interactions (Staehelin and Kang 2008), or if it is a structural feature such as a constriction of the cytosol between the PM and the tonoplast, or a physical obstruction that runs across the actin filaments at the stop sites.

Regulation of Organelle Movements at the Level of Motors

The difficulty in identifying the mechanism(s) that lead(s) to the stopping of organelles at MTs or other specific sites in the cell cortex points to the larger problem that we still know relatively little about the precise mechanism by which organelle

movement occurs in cells. The generally accepted model is that myosin motors bind to organelle surfaces and pull them along actin filaments through the cytoplasm (Shimmen 2007; Li and Nebenführ 2008b; Sparkes 2011). This concept is supported by the association of myosin tail constructs, which contain the coiled-coil and organelle binding domains, with various organelles in cells (Li and Nebenführ 2007; Reisen and Hanson 2007; Sparkes et al. 2008; Avisar et al. 2009). This colocalization, however, does not directly prove that these myosin isoforms are responsible for the movement of the respective organelles. In fact, it has been observed that the tail constructs can slow down the movement of organelles that they are not associated with (Sparkes et al. 2008; Avisar et al. 2009). For example, the tail of *Arabidopsis* myosin XIK did not localize to any clearly-identifiable structure, but was still able to slow down the movement of Golgi stacks, peroxisomes, and mitochondria (Avisar et al. 2008; Sparkes et al. 2008). This apparently non-specific dominant negative effect contradicts the expectation that saturation of motor binding sites on the organelle surface by the non-functional tail constructs would prevent movement of the thus decorated organelles (Sparkes 2010; **Figure 2B**).

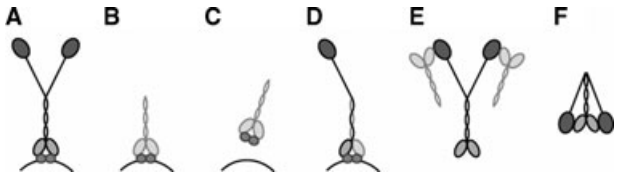

Figure 2. Possible dominant negative effects of over-expressed tail fragments on organelle movement.

Native myosin heavy chains are shown in black, introduced tail fragments are shown in grey.

(**A**) Predicted association of myosin XI with adaptor proteins on organelle surface.

(**B**) Tail fragments may saturate adaptor proteins on the organelle surface and prevent binding of functional motors (Sparkes 2010).

(**C**) Tail fragments may interact with adaptor proteins in the cytosol and prevent their association with organelles (Sparkes 2010).

(**D**) Tail fragments may dimerize with endogenous myosin heavy chains and lead to non-processive motors (Sparkes 2010).

(**E**) Tail fragments may bind to the motor domain of endogenous myosin heavy chains and block their activity (Avisar et al. 2012). It should be noted that the mechanisms depicted in panels **B** through **E** are not mutually-exclusive, and may occur in the same cell.

(**F**) Predicted head-to-tail interaction of endogenous myosin motors that may function in normal motor regulation (Li and Nebenführ 2007). This mechanism has been shown to exist in myosin V (Thirumurugan et al. 2006; Li et al. 2008), but is only hypothesized to exist in plant myosin XI.

A recent publication may have solved this apparent dilemma. In particular, it was found that two positively charged residues in the globular tail domain of myosin XIK in *Arabidopsis* are responsible for the dominant negative effect (Avisar et al. 2012). Replacing either of these arginines with alanine (R1386A or R1443A) abolished the dominant negative effect of the tail construct (Avisar et al. 2012). Interestingly, these two residues had previously been proposed to function in an intramolecular interaction between the globular tail and the motor domain (Li and Nebenführ 2007) that serves in the related animal myosin V to inactivate the motor (Thirumurugan et al. 2006; Li et al. 2008). Thus, it is possible that the dominant negative effect of myosin tails in plant cells is brought about by their binding to the motor domain of endogenous myosins, thereby blocking their activity (**Figure 2E**). This is an exciting novel idea that should be tested experimentally. It would be particularly interesting to see whether these predicted head-tail interactions show specificities for different isoforms, which could explain the different effects of the various tail constructs (Avisar et al. 2012). While this new discovery may explain some of the apparent off-target effects of myosin constructs, this new model does not rule out the older models. Indeed, all of the possibilities depicted in **Figure 2B–E** may occur simultaneously.

Another possible mechanism of regulating organelle movement is by controlling the association of motors with their cargo. This principle has been shown to apply to transport of the vacuole in the growing bud of *Saccharomyces cerevisiae*. In this case, an adaptor protein, Vac17p, that mediates binding of the myosin Myo2p to the vacuolar membrane is degraded in the bud, thereby preventing further movement of the vacuole (Weisman 2006). This differential motor attachment is also an attractive possibility for regulation of plant organelle movement; however, only three proteins have been identified as interactors of the organelle-binding domain of plant myosins so far. These proteins were isolated in yeast two-hybrid screens with the full tail (coiled-coil and globular tail) as bait proteins. In the first study (Hashimoto et al. 2008), two small G proteins, RabC2a and RabD1, were identified as interacting with *Arabidopsis* MYA2. Additional *in vitro* pull-down experiments in the presence or absence of GTP suggested that MYA2 functions as a classical Rab effector, as it preferentially bound to the active form of the G protein (Hashimoto et al. 2008). Interestingly, fluorescently tagged RabC2a appeared to localize to peroxisomes (Hashimoto et al. 2008), which matches one of the localizations of MYA2 previously identified by immunofluorescence (Hashimoto et al. 2005) and YFP-tagged tail constructs (Li and Nebenführ 2007; Reisen and Hanson 2007). It would be interesting to see whether active RabC2a recruits MYA2 to peroxisomes in living cells, and how this interaction is regulated.

Very recently, an additional myosin interacting protein was identified in maize (*Zea mays*). In this case, a putative

heat-shock protein interacting protein (HIP) was identified by screening a cDNA library with the tail of Opaque1, the maize homolog of *Arabidopsis* XI-I (Wang et al. 2012). Not much is known about HIP, except that it carries a tetratrico peptide repeat and a Phox/Bem1p domain in its amino-terminal half, two domains known to be involved in protein-protein interactions. At the same time, binding to the myosin tail occurred near its C-terminus (Wang et al. 2012). This protein could thus function as a scaffold protein that mediates binding of the motor to organelles, possibly the ER (Wang et al. 2012). Further characterization of this interaction, particularly in living cells, would be of great interest given the unusual phenotype of the *opaque1* mutant. In mutant endosperm, protein storage bodies are smaller and more irregular in shape, suggesting that motor-dependent movements of the ER membranes are required to obtain normal clustering of storage proteins in the ER lumen (Wang et al. 2012). If this interpretation can be corroborated, it would suggest that ER movements may be able to "pump" luminal proteins throughout the ER network. This would extend the predicted mixing function of cytoplasmic streaming from the cytosol, where myosin motors function, to the inside of organelles.

Summary and Outlook

The last five years have seen an explosion in research on organelle movements and myosin motors in plants. This work has firmly established the long-held conjecture that myosins are responsible for cytoplasmic streaming, and that these movements are necessary for normal cell growth. The combined genetic, molecular, and cellular approaches have demonstrated that different myosin isoforms associate with different organelles, and have pointed out new ways in which myosin function can be regulated. Despite this rapid progress, however, we still have significant gaps in our knowledge. For example, we know very little about the mechanisms that allow myosins to associate with their cargo. While three candidates that might meditate organelle binding have been identified (Hashimoto et al. 2008; Wang et al. 2012), we need a deeper characterization of these interactions, as well as an expansion to other myosin isoforms, to fully understand how myosins drive organelle movements. Furthermore, it is not clear how the genetic redundancy within the myosin XI gene family (Peremyslov et al. 2008; Prokhnevsky et al. 2008; Peremyslov et al. 2010) can be reconciled with the apparent isoform-specific distribution of myosins within a cell (Sparkes et al. 2008; Avisar et al. 2009). In other words, why do angiosperms encode about a dozen different myosin XI motors in their genomes? More detailed analysis of mutants combined with cellular approaches examining myosin localization and organelle movements are likely to provide new insights into this question. Finally, we have only a rudimentary understanding of how organelle movements are integrated with normal cell physiology. It is now firmly established that cytoplasmic streaming is needed for growth (Peremyslov et al. 2010), but exactly how these myosin-driven movements contribute to normal cell functioning is still not clear. A comprehensive analysis of cellular dynamics in wild-type and myosin mutants may be necessary to address this issue. At the same time, any progress on the two former questions is likely to contribute to our understanding of this ultimate question. The tools for the needed experiments have largely been developed, and, if the past several years are any indication, we can expect to see rapid advances in all areas.

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