Golgi Body Motility in the Plant Cell Cortex Correlates with Actin Cytoskeleton Organization

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The actin cytoskeleton is involved in the transport and positioning of Golgi bodies, but the actin-based processes that determine the positioning and motility behavior of Golgi bodies are not well understood. In this work, we have studied the relationship between Golgi body motility behavior and actin organization in intercalary growing root epidermal cells during different developmental stages. We show that in these cells two distinct actin configurations are present, depending on the developmental stage. In small cells of the early root elongation zone, fine filamentous actin (F-actin) occupies the whole cell, including the cortex. In larger cells in the late elongation zone that have almost completed cell elongation, actin filament bundles are interspersed with areas containing this fine F-actin and areas without F-actin. Golgi bodies in areas with the fine F-actin exhibit a non-directional, wiggling type of motility. Golgi bodies in areas containing actin filament bundles move up to 7 μm s⁻¹. Since the motility of Golgi bodies changes when they enter an area with a different actin configuration, we conclude that the type of movement depends on the actin organization and not on the individual organelle. Our results show that the positioning of Golgi bodies depends on the local actin organization.

Keywords: Actin filament bundles • Arabidopsis • Cell expansion • Fine F-actin • Golgi bodies • Plant cell.

Abbreviations: CESA, cellulose synthase; F-actin, filamentous actin; FABD2, fimbrin actin binding domain 2; GFP, green fluorescent protein; mRFP, monomeric red fluorescent protein; SmaCC, small cellulose synthase-containing compartment; STtmd, sialyltransferase transmembrane domain.

Introduction

Cell growth is the irreversible increase in cell volume (Ketelaar and Emons 2001). The basis of cell growth is the secretion of cell wall matrix material containing vesicles in a cell with a flexible wall under turgor pressure. Whilst the contents of vesicles are deposited against the existing cell wall, the membrane of these vesicles fuses with the plasma membrane, which increases the plasma membrane surface and inserts transmembrane proteins into the plasma membrane. One of the transmembrane protein complexes that is inserted into the plasma membrane is the cellulose microfibril-producing cellulose synthase (CESA) complex. The insertion of fluorescently tagged CESA complexes into the plasma membrane can be followed in living cells using fluorescence microscopy (Paredez et al. 2006) and serves as an excellent marker for (part of the) secretory events. Secretory vesicles are derived from Golgi bodies. Recently, it has been found that, while cortical microtubules position CESA complex delivery to the plasma membrane and interact with small cellulose synthase-containing compartments, SmaCCs (Gutierrez et al. 2009), the acto-myosin system traffics CESA-containing Golgi bodies (Crowell et al. 2009, Gutierrez et al. 2009, Kato et al. 2010). This finding implicates a role for the actin cytoskeleton in the secretion during cell wall formation. Experimental depolymerization of the actin cytoskeleton causes aggregation of Golgi bodies in specific areas of the cell cortex, whereas in other regions Golgi bodies are absent. The insertion of CESA complexes into the plasma membrane occurs predominantly at the locations where Golgi bodies aggregate during actin depolymerization, but also, in situations where the actin cytoskeleton is intact, more CESA complex insertion into the plasma membrane occurs at locations where Golgi bodies coincidentally aggregate (Crowell et al. 2009, Gutierrez et al. 2009). Thus, the actin-dependent positioning and motility of Golgi bodies determines where secretion of CESA complex-containing vesicles occurs. Since CESA complex insertion occurs predominantly close to Golgi bodies, it appears plausible that there could be an actin-based mechanism that positions Golgi bodies at locations where secretion is required.

In tip-growing root hairs, the role of the actin cytoskeleton in polarized secretion has been well studied. The actin cytoskeleton in growing root hairs consists of thick actin filament bundles (Miller et al. 1999, Ketelaar et al. 2003) which branch into finer bundles of actin filaments (fine F-actin) in the subapex (Arabidopsis: Ketelaar et al. 2003). The fine F-actin configuration is only present in growing cells, and experimental depolymerization of specifically the fine F-actin inhibits cell growth (Miller et al. 1999, Ketelaar et al. 2003), strongly suggesting that...
in the tips of growing root hairs the presence of fine F-actin is a prerequisite for cell growth. The correlation between fine F-actin and cell growth becomes even more evident from the response of the legume root hair actin cytoskeleton to *Rhizobium* nodulation factor during the early stages of symbiosis. This host-specific lipochito-oligosaccharide induces root hair curling and initiates the production of nitrogen-fixing root nodules. Its application affects the actin cytoskeleton (Cárdenas et al. 1999) such that fine F-actin and cell elongation are reinitiated in growth-terminating root hairs (De Ruijter et al. 1999). In tip-growing pollen tubes a configuration of fine F-actin that correlates with cell growth, similar to that in root hairs, has been reported (Gibbon et al. 1999, Geitmann et al. 2000, Lovy-Wheeler et al. 2005, Vidali et al. 2009). It has been suggested that, amongst others, the fine F-actin configuration plays a role in maintaining Golgi vesicles close to the growing root hair tip by locally inhibiting the cytoplasmic streaming (Miller et al. 1999).

Unlike tip-growing root hairs and pollen tubes, most plant cells grow by intercalary, also called diffuse, growth. This is a type of polar growth in which cells expand over their whole length in one direction. These cells have a highly dynamic cortical array of F-actin (Staiger et al. 2009, Smertenko et al. 2010). In the present work, we have studied the relationship between Golgi body motility and the configuration of the actin cytoskeleton in intercalary expanding cells. We compared the organization of the cortical actin cytoskeleton and the behavior of Golgi bodies in the cortex root epidermal cells of different developmental stages: the early elongation zone and the late elongation zone.

In the cortex of intercalary growing root atrichoblasts two actin configurations are present, fine F-actin in small cells of the early elongation zone and thick actin filament bundles interspersed with fine F-actin and areas devoid of filamentous actin in larger cells during later stages of cell elongation.

For the study of the actin cytoskeleton we used 35S::GFP:FABD2 (green fluorescent protein: fimbrin actin binding domain 2)-expressing Arabidopsis seedlings (Ketelaar et al. 2004) combined with spinning disk microscopy. We compared two developmental stages of atrichoblasts: young, small cells located just above the root meristem, which we defined as the early elongation zone, and large, still growing cells just below the area where root hairs emerge, which we defined as the late elongation zone (Fig. 1A; see Sugimoto et al. 2000). We used non-root hair-forming epidermal cells, atrichoblasts, to exclude possible changes in actin configuration due to root hair initiation and growth. Young atrichoblasts were identified by tracking a non-root hair-forming cell file from the root hair zone towards the root tip.

In cells in the early elongation zone the configuration of the cortical F-actin was comprised of a diffuse network of (bundles of) actin filaments (fine F-actin; Fig. 1A, C; Ketelaar et al. 2003). Collings et al. (2006) describe a similar F-actin configuration, which they call ‘weakly bundled F-actin’. In these cells no thick bundles of actin filaments, hereafter called actin filament

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**Fig. 1** The actin organization (GFP:FABD2) in the cortex of atrichoblasts in the early elongation zone and the late elongation zone. (A) Overview of a root in which the locations where B and C have been taken are indicated. (B) The cortex of a atrichoblast in the late elongation zone shows dispersed actin filament bundles. The areas without actin filament bundles either contain fine F-actin or are devoid of detectable actin filaments. (C) The cortex of atrichoblasts in the early elongation zone is occupied by fine F-actin, and no actin filament bundles are visible. Bars = 10 μm.
bundles, were present in the cell cortex. In cells of the late elongation zone, i.e. cells at the end of their rapid elongation (Baskin 2001), actin filament bundles, often interconnected and branching, were present in the cell cortex. The space between these bundles was occupied by fine F-actin or did not contain detectable actin filaments (Fig. 1B).

Golgi bodies wiggle in areas occupied by fine F-actin and move with velocities up to 7 \mu m s^{-1} along actin filament bundles

We studied the influence of the different actin configurations on organelle motility. For this, we used a line with fluorescently tagged Golgi bodies [expressing 35S::STtd:GFP (sialyltransferase transmembrane domain::GFP)]. Fig. 2 shows a comparison of the motility of Golgi bodies in the cortical cytoplasm of a typical atrichoblast in the early elongation zone (Fig. 2A) with that of a typical atrichoblast in the late elongation zone (Fig. 2B). To analyze the routes and velocities of Golgi bodies in the cortical cytoplasm, we manually tracked six Golgi bodies in time projections (cell from the early elongation zone, Fig. 2C; cell from the late elongation zone, Fig. 2D) for a total duration of 2 min. The Golgi bodies showed two types of motion: wiggling (Fig. 2E) and directed motion (Fig. 2F). Fig. 2I and J shows velocity graphs of the individual tracked Golgi bodies (colors correspond to the colors in Fig. 2E and F). The motility of Golgi bodies throughout the whole cortex of cells in the early elongation zone can best be described as ‘wiggling’: non-directional displacement of <2 \mu m s^{-1}. In the cortical cytoplasm of cells in the late elongation zone, two types of Golgi body motility were observed: fast directed motion with velocities ranging from 2 to 7 \mu m s^{-1} (yellow and blue Golgi bodies) and wiggling (green and purple Golgi bodies). This wiggling motility was similar to the Golgi body behavior in cells of the early elongation zone (Fig. 2E, F).

Golgi bodies switched between the two types of dynamic behavior when they moved from an area where directed, high velocity motility occurred to an area where wiggling occurred, and vice versa. The blue colored Golgi body in Fig. 2F illustrates this. In the first 60 s it traveled by fast, directed motion. Then, when it entered a different cortical area, its velocity dropped.

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Fig. 2 Distribution and movement of Golgi bodies in the cortical cytoplasm of atrichoblasts in the early elongation zone (left) and late elongation zone (right). (A and B) Confocal images of the outer layer of the cortical cytoplasm. (C and D) Time projections of Golgi bodies in the outer layer of the cortical cytoplasm over a period of 120 s; maximum projections of 301 images acquired at 0.4 s intervals. (E and F) The same time projections as C and D overlaid with colored routes of six manually tracked Golgi bodies. In G and H the same time projections are used as in C and D, however, slow (<2 \mu m s^{-1}) moving Golgi bodies are colored blue, and rapidly moving (\geq 2 \mu m s^{-1}) Golgi bodies are colored red. (I and J) Graphs of the velocity of tracked Golgi bodies plotted over time, I of the atrichoblast in the early elongation zone and J of the atrichoblast in the late elongation zone. Colors correspond to the colors used in E and F. Bar = 10 \mu m.
and it showed a typical wiggling behavior. Besides this organelle, many others behaved similarly. Thus, the kind of motility is not a property of the organelles, but of the area in which they reside. The positions of areas where fast, directed motion and wiggling occurred were flexible. During the 10 min time frame, a repositioning of these areas was clearly visible (data not shown).

A novel algorithm was developed to quantify the motility of large populations (approximately 10–80 per cell) of Golgi bodies simultaneously over time (see Supplementary data). The algorithm discriminates between fast, directed (>2 μm s⁻¹) and wiggling (<2 μm s⁻¹) motion by removing the fluorescence from Golgi bodies that move at velocities ≥2 μm s⁻¹ from the images. The output of this algorithm only shows Golgi bodies with a velocity <2 μm s⁻¹ (Fig. 3). In Fig. 4A, images of a cell in the early elongation zone over time are shown. Image processing with the algorithm revealed that Golgi bodies move with a velocity <2 μm s⁻¹ throughout the cortical cytoplasm of the cells in the early elongation zone (Fig. 4B). Figs. 4C and D show time projections of the time series from which output data of the algorithm (Fig. 4A, B) have been taken, before (Fig. 4C) and after (Fig. 4D) processing with the algorithm. Fig. 4E–H shows similar images of a cell in the late elongation zone. In this cell, more distal from the root tip, areas where Golgi bodies wiggle are interlaced with areas where Golgi bodies show fast, directed motion (Fig. 4F, H). To compare the Golgi body behavior in atrichoblasts of the early elongation zone and atrichoblasts of the late elongation zone, we determined the fraction of the total fluorescent signal that was still present after processing with the algorithm. In cells of the early elongation zone, the fraction of the total fluorescence produced by Golgi bodies was 0.90 ± 0.03 (n = 9 cells), while in cells in the late elongation zone it was 0.76 ± 0.08 (n = 9 cells). The difference is highly significant (Kolmogorov–Smirnov test, α < 0.01).

Motility behavior of Golgi bodies in the cell cortex depends on the actin configuration

To investigate whether the different types of velocity behavior of Golgi bodies correlate with the local actin organization, we studied Golgi body motility in the cortex of atrichoblasts of dual labeled plants expressing the 35S::Ttm-dmRFP (monomeric red fluorescent protein) Golgi marker and the 35S::GFP:FABD2 actin markers. Fig. 5 shows frames taken from a cell in the late elongation zone in which Golgi bodies move rapidly over a defined path where an actin filament bundle was present, whereas they do not show any directional motion in an area with fine F-actin or an area without detectable F-actin (Supplementary movie S1). In an atrichoblast in the early elongation phase, the whole cortex is covered with fine F-actin (Fig. 1C), and no directional motion of Golgi bodies was observed (Fig. 6A, Supplementary movie S2). In an atrichoblast in the late elongation zone, the cortical cytoplasm contained both actin filament bundles and the fine F-actin (Figs. 1B, 6B, Supplementary movie S3). We quantified the dynamic behavior of Golgi bodies and correlated it to the different actin configurations in cells in the early and late elongation zone. To discriminate between fine F-actin and actin filament bundles we measured the fluorescence intensities of both configurations. Supplementary Fig. 1 shows that the fluorescence intensity in the majority of the cortical areas with fine F-actin is maximally 60 gray levels above the background intensity. The fluorescence intensity of the majority of the cortical areas with actin filament bundles is minimally 80 gray levels above the background intensity. We used this to define fine F-actin and actin filament bundles; every fluorescence peak of >80 gray levels above the background level we considered as an actin filament bundle, whereas any peak <80 gray levels above the background level was considered to be fine F-actin. The cut-off is indicated as a green line in Supplementary Fig. 1C. We then measured the displacement of individual Golgi bodies over five subsequent 2s intervals,

Fig. 3 Example of the image processing with our algorithm on an atrichoblast in the late elongation zone. (A) The original time series in which one Golgi body does not move while another is passing. (B) The same time series after processing with the algorithm. Each pixel contains the minimum intensity value that was present at each pixel during two subsequent images. Hence, only the immobile Golgi body is visible. (C and D) Maximum projections of the four images of the time series on the left. Bar = 1 μm.
with a total duration of 10 s. Only Golgi bodies maximally 4 pixels (0.45 μm) away from actin filaments during the 10 s time interval were considered. Average velocities were calculated by measuring the displacements over 2 s intervals (Table 1). The directionality of the movement was calculated by dividing the sum of the displacements during five 2 s intervals (average summed displacement; Table 1) by the absolute displacement over a 10 s interval (average net displacement; Table 1). These measurements show that in cortical areas with fine F-actin (in both the early and late elongation zone), Golgi bodies move significantly more slowly than in cortical areas with actin filament bundles (Table 1, Fig. 7). In areas with actin filament bundles, the directionality of Golgi body motion is significantly larger than that in areas with fine F-actin. Thus, the configuration of the actin cytoskeleton correlates with the type of Golgi body motion in the cortex of Arabidopsis root epidermal cells. Since the Golgi bodies switch from one type of motion to the other (Fig. 4), this result shows that the actin configuration determines the motility type of Golgi bodies.

**Differences in motility in cortical areas with fine F-actin and actin filament bundles are not specific for Golgi bodies: mitochondria behave similarly**

Besides actin organization, other properties of the Golgi bodies that are independent of actin organization could be involved in...
their motility. We measured the motility of mitochondria in areas with fine F-actin and actin filament bundles in the cortex of atrichoblasts to test whether the behavior that we observed is specific for Golgi bodies or generic for all organelles. Like Golgi bodies, mitochondria moved by fast, directed movement along cortical areas with actin filament bundles and exhibited wiggling in cortical areas with fine F-actin configuration. Although the motility behavior of mitochondria and Golgi bodies was similar, the absolute velocity of mitochondria was lower, mainly because fewer mitochondria in the vicinity of actin filaments were moving than Golgi bodies. Even so, the same trend is clearly visible: the local actin organization, and not the organelle properties, determines the organelle motility (Table 1, Fig. 7).

Discussion
Golgi body motility correlates with actin filament organization in the cortex of root epidermal cells

Here we show that the movement of Golgi bodies and mitochondria in the cell cortex depends on the local actin configuration in the cortical cytoplasm, namely on the level of bundling of actin filaments. In the cortical cytoplasm of all atrichoblasts in the early elongation zone, the actin cytoskeleton is organized as fine F-actin. Collings et al. (2006) describe fine F-actin in the cortex of elongating plant cells. Staiger et al. (2009) show that the randomly oriented highly dynamic, short-lived, structures most probably represent single actin filaments. Golgi bodies in these small young cells exhibit slow (<2 μm s⁻¹) undirected motion, wiggling, which was previously described by Nebenführ et al. (1999) to occur in the cortical cytoplasm of tobacco BY-2 suspension cultured cells (Nebenführer et al. 1999).

In all cells in the late elongation zone, we found two types of spatially separated areas with distinct actin filament configurations and motility behavior of Golgi bodies. In cell areas with an actin cytoskeleton similar to that in the cortex of cells in the early elongation zone of the root, Golgi bodies wiggled. In cell areas with actin filament bundles Golgi bodies moved as fast as those in cytoplasmic strands. The highest velocity that we measured in the cell’s cortex was 7 μm s⁻¹. There is large variation in the velocities of Golgi bodies reported for higher plant cells, and those that have been measured previously in similar cells are considerably lower: 0.8–4.2 μm s⁻¹ (Boevink et al. 1998, Nebenführ et al. 1999, Peremyslov et al. 2008). These differences may be explained by the different time intervals that have been used for analysis. The time interval that we used, 0.4 s, was much shorter than those used in other works. Indeed, when we used longer time intervals, velocities dropped sharply (Fig. 7, Table 1). In vitro, the maximum measured velocity of the higher plant myosin XI is 7 μm s⁻¹ (Tominaga et al. 2000), which we measured in the fastest moving organelles, and has been found for organelle movement in various plant cell types.

Fig. 5 Golgi movement depends on the local actin configuration. Frames with 10 s intervals are shown in which four Golgi bodies that exhibit different types of motion are indicated with arrowheads. The Golgi bodies labeled with the orange and yellow arrowhead show a burst of rapid, directed movement when located close to an actin filament bundle. When they dissociate from the bundle, their movement changes to the wiggling type. The other two labeled Golgi bodies do not associate with actin bundles and only show wiggling motion. The complete time series is shown in Supplementary movie S1. Bar = 10 μm.
Why do myosins move their organelle cargo more slowly along fine F-actin than over actin filament bundles? There could be several reasons for this: the dynamicity of fine F-actin could negatively affect prolonged binding of Golgi bodies for directed transport. Myosin XI binding sites on fine F-actin could be occupied by other actin-binding proteins, a minimal number of actin filaments in a bundle could be required for prolonged directional transport or myosin XI attached at different surface areas of Golgi bodies could bind to different actin filaments in fine F-actin networks such that counteracting forces are produced. In vitro studies could provide insight into these matters.

If the local actin organization determines Golgi body motility, it is possible that other organelles, which are at least partially equipped with the same myosins (Prokhnevsky et al. 2008, Avisar et al. 2009), behave similarly. Zheng et al. (2009) indeed have shown that mitochondria in root hairs show very similar motility behavior to Golgi bodies. Our observation that mitochondria show the same overall motility behavior, albeit slower on average, as the Golgi bodies in root epidermal cells agrees with a hypothesis that the actin configuration is the source of the signal causing the specific conduct of the organelles.

In animal cells, the role of myosins in long-distance transport remains controversial since long actin cables with uniform polarity are lacking (Pollard and Cooper 2009, Woolner and Bement 2009) and long-distance transport is mainly governed by microtubules (reviewed in Kapitein and Hoogenraad 2011). In budding yeast cells, myosin V is involved in directional transport of vesicles over actin cables, but does not localize to actin patches that are involved in endocytosis (reviewed in Pruyne et al. 2004). This shows that the budding yeast myosin V somehow discriminates between actin cables and patches and that also in budding yeast the type of actin network determines the mode of transport, albeit by a mechanism that probably differs from that in plant cells. Besides transporting organelles, myosins themselves are capable of reorganizing the actin cytoskeleton in vitro (Soares e Silva et al. 2011) and in plant cells (Hoffmann and Nebenführ 2004, Van der Honing et al. 2010), although there are no indications that the myosins that are involved in actin reorganization are the same as those that decorate and transport organelles.

The actin cytoskeleton organization and Golgi membrane trafficking

In the Arabidopsis arichoblasts that we studied, Golgi bodies move along actin filament bundles and wiggle in areas with fine F-actin. Collings et al. (2006) discriminated between fine F-actin, present in the cell cortex and involved in anisotropic cell expansion, and cytoplasmic actin filament bundles responsible for organelle motility. Disruption of the actin filaments reduces Arabidopsis root cell elongation and induces radial swelling in a dose-dependent manner (Baskin and Bivens 1995, Collings et al. 2006), in onion (Thomas et al. 1973) and maize roots (Blancaflor 2000, Baluska et al. 2001). In accordance with these results, mutations in actin-binding proteins that disrupt actin organization affect cell elongation (Ramachandran et al. 2000, Dong et al. 2001, Barrero et al. 2002, Nishimura et al. 2003, Ketelaar et al. 2004).

With the reported exception of etiolated Arabidopsis hypocotyl cells (Refregier et al. 2004, Derbyshire et al. 2007), plant cell elongation and cell wall formation are coupled processes in...
which the cell wall keeps more or less its original width as a result of simultaneous stretching and deposition of new material (Roberts 1989). The wall matrix precursors are being made in the Golgi system and deposited against the existing cell wall by exocytosis (Vaughan and Vaughn 1987, Boevink et al. 1998, Nebenführ et al. 1999, Wasteneys and Galway 2003), whereas cellulose microfibrils are produced by CESA complexes moving in the plane of the plasma membrane, which are also thought to be inserted into the plasma membrane via the membrane of Golgi vesicles, i.e. the exocytosis process (reviewed in Emons et al. 2002). Since one SmaCC can deliver at least three CESA complexes to the plasma membrane (Collings et al. 2006), it could possibly represent the secretory vesicle cluster (SVC; Toyooka et al. 2009). Insertion of CESA complexes occurs preferentially in the proximity of cortical microtubules.

Besides our limited knowledge about the nature of SmaCCs, it has not been proven whether exocytotic vesicles carry and are being moved by motor proteins or not. Although myosins have been found on the surface of Golgi body-derived vesicles (Romagnoli et al. 2007), it has not been proven that these are exocytotic vesicles. There is no evidence for motor-based movement of SmaCCs. They end-track depolymerizing microtubule ends, which could be a motor-independent process.

The above-described research (Crowell et al. 2009, Gutierrez et al. 2009) makes clear that somehow at the end of the anterograde trafficking of CESA complexes in the Golgi membranes microtubules take over from the actin cytoskeleton. Making use of the temperature-sensitive Arabidopsis mutant *microtubule organization 1* (*mor1-1*; Whittington et al. 2001), Collings et al. (2006) have already shown an interdependency of actin filaments and cortical microtubules in the process of plant cell elongation. Moreover, actin and microtubule organization in the cell cortex depend on each other (Sampathkumar et al. 2011).

<table>
<thead>
<tr>
<th>Average velocity (µm s⁻¹)</th>
<th>Actin filament bundles</th>
<th>Fine F-actin</th>
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<tr>
<td>0.60 ± 0.51</td>
<td>0.14 ± 0.12</td>
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<tr>
<td>0.59 ± 0.53</td>
<td>0.75 ± 0.67</td>
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<tr>
<td>5.97 ± 3.96</td>
<td>1.40 ± 0.86</td>
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<td>78%</td>
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<td>122</td>
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Table 1 Measured velocities and displacements of Golgi bodies and mitochondria

The number of measured velocities within one interval (2 s).

The number of measured displacements in 10 s.

Standard deviations are shown.
Golgi bodies themselves is irrelevant (Young et al. 2008). The determination of the plasma membrane region that accepts Golgi body-derived Golgi vesicle clusters/vesicles for exocytosis is likely to be a crucial factor. What determines their docking to and tethering at the plasma membrane, to deliver the cell wall material, cell wall polymer-producing enzymes and other secreted products and transmembrane proteins, is of prime importance for plant cell growth and therefore plant development.

Materials and Methods

Plant growth

Arabidopsis transgenic plants expressing 35S::GFP:FABD2 (Ketelaar et al. 2004), 35S::STtmd:GFP or 35S::STtmd:mRFP (kindly provided by C. Hawes, Oxford Brookes University, UK) were germinated on a glass microchamber containing half-strength Murashige and Skoog (MS) medium with vitamins, pH 5.7 (Duchefa), as described previously (Ketelaar et al. 2002), plated out on Petri dishes with the same medium, complemented with 1% phyto agar (Duchefa), or grown in ‘biofoil sandwiches’ as described by Ketelaar et al. (2004). Selection of the two types of cells that were compared was on the basis of their position in the root: small young cells just above the meristem, large mature cells above the root hair growth cone.

Image acquisition

Root epidermal cells were imaged on a Zeiss LSM510 META confocal microscope, with a ×40 oil immersion objective (PlanApo, NA 1.3) using standard filter combinations. The pinhole of the confocal microscope was adjusted such that the thickness of optical sections was approximately 2 μm. This thickness allowed imaging of only the Golgi bodies in the outer cortical layer of cytoplasm and covered a large surface area. For quantitative analysis of the stop behavior, all images of atrichoblasts were acquired with the same pinhole settings and the same scan rate. Time series were generated by collecting images with 0.4 s intervals. Cell lengths were measured by counterstaining cell walls with 1 μg ml⁻¹ propidium iodide (VWR International).
Actin imaging and combined actin and organelle imaging was performed on a Roper Spinning Disk confocal microscope (Roper Scientific) using standard filter settings.

**Data analysis**

*Velocity of Golgi bodies in the cortical cytoplasm.* Measurements and tracking of Golgi bodies was performed using ImageJ (rsb.info.nih.gov/ij/). The velocity of individual Golgi bodies was calculated by dividing the measured displacement by the elapsed time.

*Fraction of wiggling Golgi bodies.* The number of Golgi bodies that showed random movement \(<2 \mu \text{m s}^{-1}\) was counted and divided by the total number of Golgi bodies present in the focal plane. This was done at an arbitrary time point in the movie, for nine atrichoblasts from the early elongation zone and nine atrichoblasts from the end elongation zone.

**Fraction of fluorescence of arresting and slow moving Golgi bodies.** A novel algorithm was developed to analyze Golgi body motility. Processing a time series by the algorithm renders a movie that only shows fluorescence from Golgi bodies that move slower than \(2 \mu \text{m s}^{-1}\). The algorithm is based on the principle of ‘overlapping’: the average diameter of the detected image of a Golgi body was \(0.8 \mu \text{m}\) for both developmental stages (data not shown). When a Golgi body moves at \(2 \mu \text{m s}^{-1}\), it moves \(0.8 \mu \text{m}\) in \(0.4\) s. Thus when a Golgi body, in a time series in which the images are taken every \(0.4\) s, is overlapping itself in a time projection, it moves with a velocity of \(<2 \mu \text{m s}^{-1}\). A resulting time series after algorithm processing only shows these ‘overlaps’ by selecting for each pixel the lowest intensity level during two subsequent images. An example is shown in **Fig. 3.** Processing of the time series by the algorithm was performed in Matlab.

Quantification of the slow movement was achieved as follows: fraction of fluorescence of arresting and slow moving Golgi bodies = total intensity after processing – background intensity/total original intensity – background intensity.

**Supplementary data**

*Supplementary data* are available at PCP online.

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**References**


