

Actin-based motility of endosomes is linked to the polar tip growth of root hairs

Boris Voigt^a, Antonius C.J. Timmers^{a,b}, Jozef Šamaj^{a,c}, Andrej Hlavacka^a, Takashi Ueda^d, Mary Preuss^e, Erik Nielsen^e, Jaideep Mathur^f, Neil Emans^g, Harald Stenmark^h, Akihiko Nakano^{d,i}, František Baluška^{a,j,*}, Diedrik Menzel^a

^a*Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany*

^b*Laboratoire Interactions Plantes-Microorganismes, INRA/CNRS, Chemin de Borderouge, F-31326 Castanet-Tolosan, France*

^c*Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademicka 2, SK-949 01 Nitra, Slovakia*

^d*Department of Biological Sciences, Graduate School of Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033 Tokyo, Japan*

^e*Donald Danforth Plant Science Center, 975 N. Warson Road, St. Louis, Missouri 63132, USA*

^f*Department of Plant Agriculture, University of Guelph, Guelph, Ont., Canada N1G 2W1*

^g*Biology VII, RWTH Aachen, Worringerweg 1, D-52074 Aachen, Germany*

^h*Department of Biochemistry, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway*

ⁱ*Molecular Membrane Biology Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

^j*Institute of Botany, Slovak Academy of Sciences, Dubravska cesta 14, SK-84223, Bratislava, Slovakia*

Received 29 October 2004; received in revised form 15 December 2004; accepted 15 December 2004

Abstract

Plant tip growth has been recognized as an actin-based cellular process requiring targeted exocytosis and compensatory endocytosis to occur at the growth cone. However, the identity of subcellular compartments involved in polarized membrane trafficking pathways remains enigmatic in plants. Here we characterize endosomal compartments in tip-growing root hair cells. We demonstrate their presence at the growing tip and differential distribution upon cessation of tip growth. We also show that both the presence of endosomes as well as their rapid movements within the tip region depends on an intact actin cytoskeleton and involves actin polymerization. In conclusion, actin-propelled endosomal motility is tightly linked to the polar tip growth of root hairs.

© 2005 Elsevier GmbH. All rights reserved.

Keywords: Actin; Endosomes; Root hair; Tip growth; *Arabidopsis thaliana*; *Medicago truncatula*

Introduction

In eukaryotic cells, endosomes are defined as pleiotropic tubulo-vesicular compartments which accumulate internalized cargo (Gruenberg, 2001; Zerial and McBride, 2001). Classically, endosomes are involved in maintenance of plasma membrane homeostasis, nutrient

*Corresponding author. Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany.
Tel.: +49 228 73 4761; fax: +49 228 73 9004.

E-mail address: baluska@uni-bonn.de (F. Baluška).

uptake, cellular defence responses, and the termination of signaling pathways through internalization and down-regulation of activated receptor-ligand complexes. More recent evidence reveals that endosomes are involved in signaling, transcytosis and synaptic cell-to-cell communication (Sorkin and von Zastrow, 2002). Thus, endocytic compartments are highly dynamic and this activity is clearly linked with many important functions within eukaryotic cells (Gruenberg, 2001; Sorkin and von Zastrow, 2002; Zerial and McBride, 2001).

In both yeast and mammals, endosomes recruit FYVE-domain proteins due to high levels of PI(3)P when the FYVE sequence acts as a PI(3)P-binding module (Gillooly et al., 2000; Jensen et al., 2001). This is mediated via specific recruitment of PI3Ks, supporting local production of PI(3)P to these membranes by regulatory GTPases, such as Ypt51p and Rab5 (Gruenberg, 2001; Zerial and McBride, 2001). While both FYVE-domain proteins as well as endosomally localized Rab GTPases are conserved in plants, it is not known, whether these two sets of proteins are localized to the same compartment as demonstrated in other systems. More recently, the importance of cytoskeletal elements in endosomal sorting and trafficking events has been highlighted. In several important instances, both sorting and trafficking of proteins has been shown to be dependent upon the ability of endosomes to associate with and move along cytoskeletal elements (Gruenberg, 2001; Zerial and McBride, 2001). The current view, established largely from animal models, suggests that long-range intracellular movements of endocytic compartments are accomplished along microtubules, whereas the actin cytoskeleton is responsible for short-distance movements (Goode et al., 2000).

Important roles for endocytosis during polarized root hair expansion, which is restricted to the tips of these cells, have been postulated based on electron microscopic observations that plasma membrane-associated clathrin-coated vesicles are preferentially enriched at the tips of root hairs (reviewed by Hepler et al., 2001). This apical zone, also known as a 'clear zone', is devoid of any larger organelles and contains only vesicles embedded in meshworks of F-actin (Baluška et al., 2000; Šamaj et al., 2004a, b). The apical domain recruits actin together with several actin-binding proteins such as ADF and profilin (Baluška et al., 2000; Baluška and Volkmann, 2002; Gilliland et al., 2002; Jiang et al., 1997; Miller et al., 1999; Nishimura et al., 2003; Ringli et al., 2002; Vantard and Blanchoin, 2002). Moreover, the tip growth of both root hairs and pollen tubes requires continuous actin polymerization (Baluška et al., 2000; Gibbon et al., 1999; Jiang et al., 1997; Miller et al., 1999; Nishimura et al., 2003; Vantard and Blanchoin, 2002; Vidali et al., 2001). Recently, the 'clear zone' in pollen

tubes was reported to accumulate large amounts of endosomal membranes (Parton et al., 2001, 2003), as revealed with the endocytic marker FM4-64 (Meckel et al., 2004).

To address the possible role of endosomes in actin-driven plant cell tip growth, we developed F-actin- and endosome-specific stably transformed transgenic GFP reporter lines of *Arabidopsis thaliana* and *Medicago truncatula* for in vivo visualization of both actin and endosomes. Here, we show that plant endosomes are labeled by a double FYVE domain-GFP/DsRed fusion construct (Gillooly et al., 2000), as well as with the plant Rab GTPases Ara6 (Ueda et al., 2001) and RabF2a fused to GFP or YFP. Importantly, small and motile endosomes were observed in the organelle-depleted and vesicle-enriched zone ('clear zone') of growing root hairs. Moreover, vigorous endosomal motility in this 'clear zone' typically does not follow any sustained directions, is independent of cytoskeletal tracks, but requires continuous actin polymerization. Our data suggest a surprising new link between the actin-driven polar tip growth of root hairs and the actin polymerization-propelled motility of endosomes in plants.

Materials and methods

Plasmid construction

The sequences of two FYVE domains from the mouse Hrs protein were connected with the linker sequence QGQGS and fused to the N-terminus of enhanced green fluorescent protein (plasmid pEGFP-C3 from Clontech) as described before (Gillooly et al., 2000). This gene construct was cloned into the binary vector pBLTI221, thereby putting its expression under the control of the CaMV 35S promoter. For DsRedT4 (Bevis and Glick, 2002) tagging the tandem FYVE domain was PCR-amplified and cloned in frame with DsRedT4 within the vector pRTL2 under the control of the CaMV 35S promoter. The coding sequence for ARA6-GFP under control of the Cauliflower mosaic virus 35S promoter and nopaline synthase terminator (Ueda et al., 2001) was excised with HindIII and EcoRI and was inserted into the corresponding sites of pBI121 (Clontech). The resulting vector was introduced into *Agrobacterium tumefaciens* strain C58C1. AtRabF2a cDNA was amplified using the primers RabF2a forward (5'-CGGGATCCATGGCTACGTCTGGAAACAAGA-3') and RabF2a reverse (5'-GCTCTAGACTAAGCACAAACACGATGAACTC-3'), and inserted into a modified pCAMBIA expression vector with eYFP at the N-terminus under the control of the 35S CaMV promoter.

Particle bombardment

Epidermal bulb scale cells of *Allium cepa* were transiently transformed by bombardment with the Bio-Rad Biolistic PDS-1000/He system (Bio-Rad Laboratories GmbH, München, Germany) according to Hamilton et al. (1992) and as described by Timmers et al. (2002).

Plant transformation

Transformed roots of *M. truncatula* cv. Jemalong were obtained using *Agrobacterium rhizogenes* ARqual according to the protocol of Boisson-Dernier et al. (2001). About 3–6 weeks later, plants with transformed roots were put individually into square 12-cm plastic dishes (Greiner Labortechnik, Kremsmünster, Austria) on Fahraeus medium containing 1% agar. *A. thaliana* plants (ecotype Columbia) were transformed using the *A. tumefaciens*-mediated floral dip method. Cultivation of *Arabidopsis* plants was performed on half MS enriched with vitamins, 1% sucrose and 0.4% phytagel.

Microscopy

M. truncatula roots growing on agar were covered with bioFolie 25 (Sartorius AG, Vivascience Support Center, Göttingen, Germany) and observed with a LEICA TCS 4D confocal microscope (Leica, Germany) using a 63× water-immersion objective. Four-day-old *A. thaliana* seedlings were mounted in liquid half MS medium containing 1% sucrose using a spacer of one layer of parafilm between slide and coverslip, and observed by confocal microscopy. Plants were adapted to liquid medium overnight to allow application of drugs.

For the observation of transgenic *A. thaliana* expressing Ara6-GFP (Ueda et al., 2001), serial images were obtained every 1 μm using a fluorescence microscope (Olympus, Japan) equipped with 40× oil-immersion objectives and a confocal unit, CSU10 (Yokogawa Electric Corporation, Japan). Images and movies were digitally processed with Image-Pro Plus 4.1 (Media Cybernetics, L.P.), Adobe Photoshop 4.0 (Adobe Corp., Mountain View, CA) and VideoMach 2.7.2.

Drug treatments and FM4-64 staining

Growing root apices were exposed to the following drugs: 2,3-butanedione monoxime (10 mM), latrunculin B (1 μM for *A. thaliana* and 10 μM for *M. truncatula*), jasplakinolide (1 μM/5 μM), brefeldin A (10 μM for *A. thaliana* and 100 μM for *M. truncatula*), wortmannin (10 μM), and oryzalin (1 μM). The drugs were diluted in culture medium and directly added to the roots of

transgenic *M. truncatula* or *A. thaliana*. Regarding FM4-64, stock solution (1 mg/ml) was prepared in DMSO and used at 17.5 μM for *M. truncatula*. Before FM4-64 treatment, plants were incubated for 25 min at 6 °C to slow down endocytosis.

Results

A tandem FYVE construct recognizes plant endosomes

In both yeast and mammals, the phosphoinositide PI(3)P accumulates preferentially in endosomal membranes (Gillooly et al., 2000), and binding of FYVE-domain proteins to PI(3)P is sufficient to target endosomal proteins to these subcellular compartments. For *A. thaliana* it is known that the classical FYVE domain binds specifically to PI(3)P in vitro (Jensen et al., 2001). Therefore, we were interested to know if the tandem FYVE domain would be sufficient for targeting to endocytic compartments in plants. To this end, the FYVE domain from the mouse Hrs protein was tandemly fused (Gillooly et al., 2000) to the C-terminus of GFP or DsRedT4, respectively. Particle bombardment was employed to transiently express the fusion proteins (GFP-FYVE; DsRed-FYVE) in onion epidermal bulb scale cells. Confocal imaging revealed fluorescently labeled motile organelles, which were positive also for the endosome-specific plant Rab GTPases Ara6 and RabF2a, when pDsRed-FYVE and Ara6-GFP or YFP-RabF2a were transiently coexpressed. In the case of Ara6 and FYVE, the merged images showed a colocalization between the two fusion proteins in small and punctate, motile organelles (Fig. 1, yellow arrowheads in c), but not in larger static organelles, which were binding the GFP-FYVE reporter exclusively (Fig. 1c, red arrowheads). Coexpression of pDsRed-FYVE and pYFP-RabF2a also showed complete colocalization within small motile organelles (Fig. 1d–f; see also Movie 1; all 24 movies are part of the supplementary material).

Previously, Ara6-labeled compartments have been shown to accumulate newly endocytosed FM4-64 (Ueda et al., 2001). To determine, whether FYVE-labeled compartments have endosomal identity, we performed double labeling by treating the stably transformed *M. truncatula* expressing GFP-FYVE with the red fluorescent styryl dye FM4-64. This endocytic tracer binds to the plasma membrane and becomes rapidly incorporated into the cell through bulk-flow endocytosis (Ueda et al., 2001). Upon the exposure of root hairs to FM4-64 for 5 min, a fluorescent signal was observed on all FYVE-labeled endosomes (Fig. 1g–i; Movie 2). The GFP-FYVE-labeled endosomes were located in the

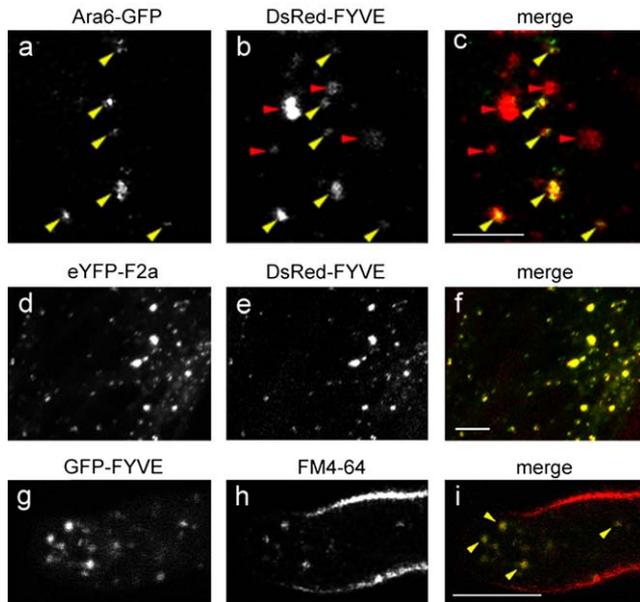


Fig. 1. Transient co-expression of endosomal markers Ara6-GFP (a) with DsRed-FYVE (b) in onion epidermal bulb scale cells. Simultaneous two-channel confocal imaging revealed that these two fusion proteins co-localize in several smaller compartments while a few larger FYVE-labeled compartments are not positive for Ara6 (c). Transient co-expression of eYFP-RabF2a (d) and DsRed-FYVE (e) in epidermal bulb scale cells showed a complete co-localization of both markers (f). To confirm the endosomal nature of the FYVE-labeled compartments, we applied endocytic tracer FM4-64 on transgenic *M. truncatula* roots expressing the double GFP-FYVE construct. After 5 min of exposure to FM4-64, FYVE-labeled endosomes (g) were enriched also with FM4-64 (h), as evidenced also by the two-channel image (i). Yellow arrowheads indicate co-localization between Ara6 and FYVE (a–c), as well as between FM4-64 and FYVE (i). Bars = 10 μ m.

vesicle-rich tip region of root hairs and co-localized with FM4-64-labeled endosomes (Fig. 1g). Taken together, the fluorescently labeled FYVE reporter co-localizes with the plant endocytic Rab GTPases Ara6 and RabF2a on endosomes which accumulate the reliable endocytic tracer FM4-64 (for plant cells see Meckel et al., 2004).

Motile endosomes are present in all root cells

After having identified the GFP-FYVE-labeled plant endosomes in *M. truncatula* root hair cells, we analyzed the pattern of their distribution in other root cells. In stably transformed *M. truncatula* roots, GFP-FYVE was detected on highly motile endosomes in all root cells. GFP-FYVE-labeled endosomes were especially abundant in root cap and meristem cells (Fig. 2a and b). In the small meristem cells, the GFP-FYVE-labeled endosomes appeared evenly distributed throughout the cytoplasm and excluded from the nuclei (Fig. 2b, stars).

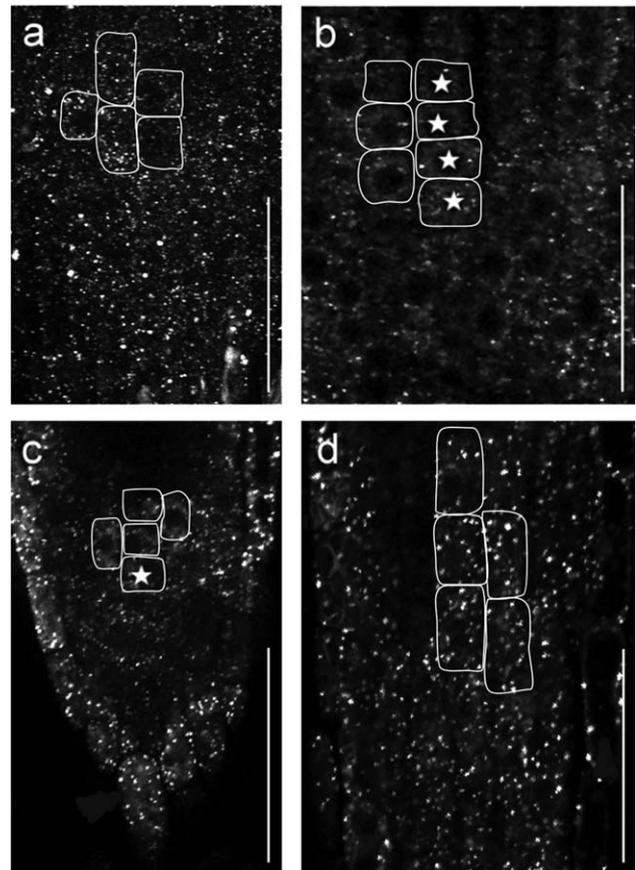


Fig. 2. In stably transformed roots of *M. truncatula*, FYVE-labeled endosomes were abundant in all cells. The most prominent early endosomes were scored in secretory cells of the root cap (a) while they were less abundant in dividing cortical cells of the root meristem localizing preferentially around centrally positioned nuclei marked with stars (b). In stably transformed roots of *A. thaliana* (c,d), FYVE-labeled endosomes were abundant in all cells, closely resembling the situation in the much larger roots of *M. truncatula*. Some cells are outlined using white lines, positions of some nuclei are indicated with stars. Bars: a–c = 50 μ m; d = 25 μ m.

GFP-FYVE was localized to endosomes that moved without any preference for a specific subcellular location. In *A. thaliana* roots, we found nearly the same distribution of FYVE-tagged endosomes (Fig. 2c and d).

Abundant and highly motile endosomes are present at sites of actin-driven polar growth

Both atrichoblasts (non-hair cells) and trichoblasts (hair cells) of *M. truncatula* exhibited a uniform distribution of GFP-FYVE-labeled endosomes throughout the length of the cell (Fig. 3a), but in the trichoblasts FYVE-labeled endosomes became enriched at the outgrowing bulges (Fig. 3a and b). At a later stage, when hairs had already emerged and were actively growing, GFP-FYVE endosomes were extraordinarily motile and

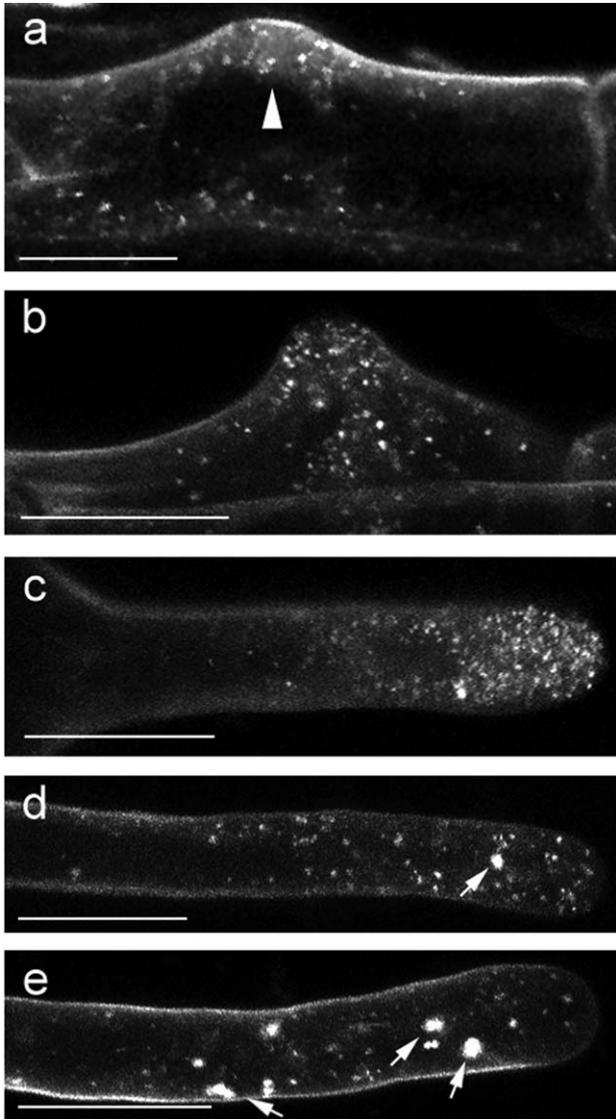


Fig. 3. During root hair formation in *M. truncatula*, FYVE-labeled early endosomes were present at the bulging site (a, b; arrowhead in (a)). In tip-growing root hairs, abundant and very motile small endosomes were present within the growing hair tip (c). Cessation of tip growth was associated with randomization of endosomes at the hair tip and with their enlargement (d, arrow). Non-growing mature root hairs showed only a few enlarged endosomes (arrows) outside the root hair tip (e). (d) and (e) are projections of serial confocal images. Bars = 20 μm .

they were present along the root hairs including the vesicle-rich and organelle-depleted tip zone of root hairs (Fig. 3c). When root hair growth ceased, GFP-FYVE accumulated on larger structures and small endosomes were never detected at the root hair tip (Fig. 3d). Occasionally, fully grown root hairs showed only a few large endosomal aggregates without any preferential localization (Fig. 3e; Movies 3 and 4).

To confirm that this pattern is not restricted to legume roots, we examined the distribution of GFP-FYVE

compartments in stably transformed *A. thaliana*. Similarly like in *Medicago*, *Arabidopsis* root hairs showed small FYVE-labeled endosomes within outgrowing bulges (not shown) and these accumulated abundantly within the first 30 μm of growing root hair apices (Fig. 4a; Movie 5). Growing hairs exhibited abundant endosomes from the tip up to 30 μm downward the root hair shank, but retracted from the tips in mature hairs, once growth had ceased (Fig. 4b). However, unlike the situation in *Medicago*, FYVE-labeled endosomes did not form enlarged structures in mature root hairs of *Arabidopsis*. Rather, endosomes became just a little bit

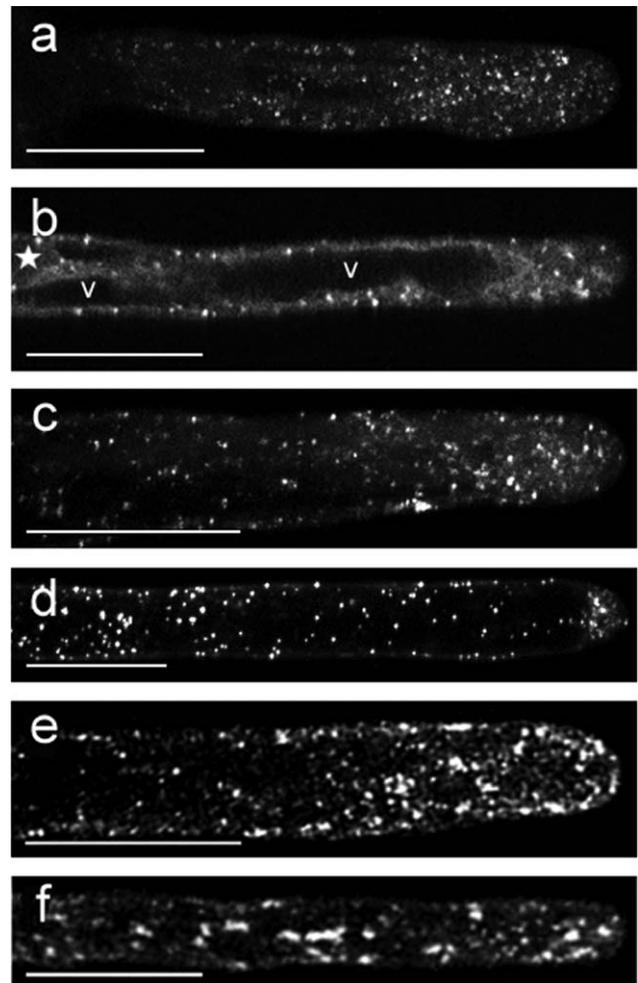


Fig. 4. In FYVE-labeled *A. thaliana* root hairs, very motile small endosomes were present at the growing hair tip (a). Root hairs with slowing growth showed endosomes at the tip, and slightly enlarged endosomes were distributed throughout the hair in which a prominent vacuole (v) protruded towards the hair tip (b). Similar distributions were scored also for Ara6-labeled endosomes in growing (c) and growth-ceasing root hairs (d). The same pattern of endosome localization in growing (e) and growth-terminated root hairs (f) was also observed with the eYFP-RabF2a marker. Star in (b) indicates the position of the nucleus. Bars = 25 μm .

larger and spread throughout the entire root hair (Fig. 4b).

Next, we wanted to determine if the endosomal Rab GTPases, Ara6 and RabF2a, are also present at the tips of growing root hair cells. In cells of stably transformed *A. thaliana* seedlings expressing Ara6-GFP or YFP-RabF2a, both these Rab GTPase reporters labeled membrane compartments that distributed along the root hairs including the growing tips. We scored distribution patterns for Ara6-labeled endosomes similar to those described above for the tandem GFP-FYVE construct, in both rapidly growing hairs (Fig. 4c) and hairs with ceased tip growth (Fig. 4d). Furthermore, the growing and growth-terminated root hairs of eYFP-RabF2a-transformed *A. thaliana* seedlings showed the same distribution pattern of endosomes as that shown for the transgenic FYVE and Ara6 seedlings (Fig. 4e and f; Movies 6 and 7).

Motile F-actin patches in root hair tips

Both the tip localization as well as the highly dynamic motility of the GFP-FYVE- and YFP-RabF2a-labeled endosomes suggested a role of the actin cytoskeleton in the subcellular distribution of these endosomal compartments within growing root hair cells. Because an intact actin cytoskeleton is also required for expansion of root hair cells (Baluška et al., 2000; Baluška and Volkmann, 2002; Gilliland et al., 2002; Jiang et al., 1997; Miller et al., 1999; Ringli et al., 2002; Šamaj et al., 2002; Vantard and Blanchoin, 2002), we wanted to understand the potential link between actin-based motility and subcellular distribution of the GFP-FYVE- and Ara6/YFP-RabF2a-labeled endosomes and cell expansion in root hair cells. Interestingly, this motility did not appear to be completely random, based on the observed presence of these compartments at the tips of root hairs. In vivo analysis of root hairs, transformed with the GFP-FABD2 construct visualizing F-actin (actin-binding domain 2 of fimbrin, Voigt et al., 2005), revealed mobile F-actin patches (Fig. 5a and c). The rate of tip growth and cell morphology did not show any obvious differences to wild-type root hairs (data not shown).

The F-actin patches resembled closely the above-described endosomes with respect to speed, locations and directions of movements. Generally, in the tips of growing root hairs of *M. truncatula* as well as of *A. thaliana*, we could detect actin patches and very dynamic short actin filaments (Fig. 5a and c). However, non-growing root hairs showed prominent actin bundles, which protruded up to the extreme tip (Fig. 5b and d), while enlarged actin patches were still present but less motile (Movies 8 and 9).

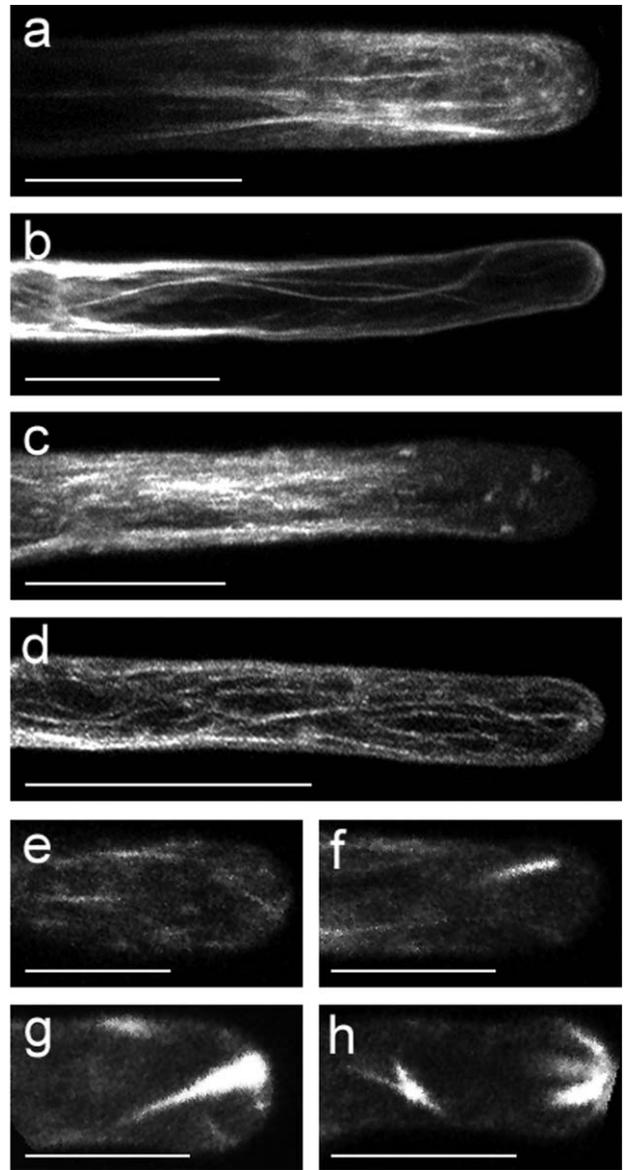


Fig. 5. Root hairs of *M. truncatula* (a, b) and *A. thaliana* (c, d) transformed with GFP-FABD2 show the characteristic actin pattern. In growing root hairs the very tip is almost free of actin bundles, but some actin-patches are visible (a, c). In growth-terminated root hairs larger actin bundles are running throughout the very tip (b, d). A normal situation for F-actin in a growing root hair tip is shown in (e). After 12 min treatment with 1 μ M jasplakinolide, the first larger paracrystalline-like F-actin bundles appeared at the tip (f). After 40 min (g), respectively 80 min (h) even larger paracrystalline-like structures of bundled F-actin were tightly associated with the very tips of jasplakinolide-treated root hairs. Bars: a–d = 20 μ m; e–h = 10 μ m.

Treatment of root hairs with 1 μ M jasplakinolide resulted in the formation of thick actin structures composed of presumably aberrantly bundled F-actin, which were formed at or near the root hair tip (Fig. 5e–h). In movies, it is obvious that these structures

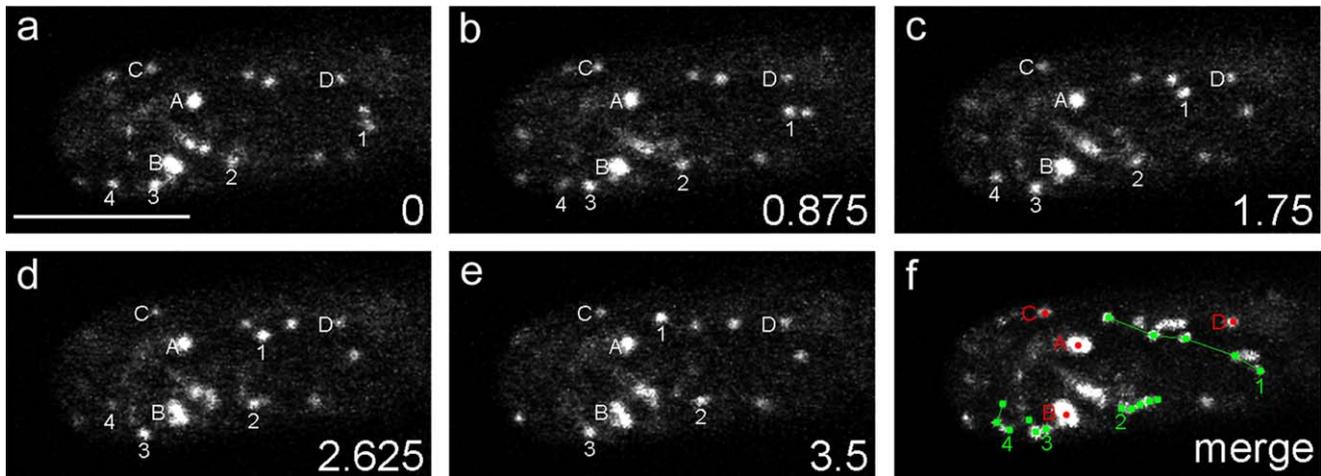


Fig. 6. Time-lapse imaging (a–f) revealed that individual endosomes in root hair tips of *M. truncatula* follow different patterns of motilities. Some were rather stationary (see endosomes marked with capital letters A, B, C, D), what we denoted ‘resting phase’. Other endosomes were moving slowly (endosomes marked with numbers 2, 3, 4) or rapidly (number 1). Bar = 10 μ m.

are polarly organized and move towards the hair tip. Finally, they stopped their movements completely and associated tightly with the very tips of jasplakinolide-treated root hairs (Movies 10 and 11).

Actin polymerization propels endosomes

The observation that F-actin patches were present at the tips of root hairs and displayed similar motility characteristics as endosomes labeled with GFP-FYVE and Ara6/RabF2a reporter constructs suggested that perhaps the dynamics of these compartments was intimately linked to the actin cytoskeleton. To identify the driving forces behind the motility of these plant endosomes, we performed time-lapse confocal imaging of root hair tips. A single focal plane in the middle of the tip was monitored for 70 s. Our observations revealed that the motile behavior of endosomes was highly variable (Fig. 6). In some rare cases endosomes were nearly stationary, displaying restricted motility of only a few micrometers forwards and backwards (Fig. 6f, red). Alternatively, stationary compartments suddenly initiated movements and dashed away at high speed. Other GFP-FYVE-labeled endosomes displayed slow and directional movements (Fig. 6f and 2), while yet others showed sustained, rapid motility (Fig. 6f and 1; Movie 3).

In order to ascertain which cytoskeletal elements and processes underlay these endosomal movements, effects of several drugs on endosomal motility were tested. In the untreated root hair tips, the majority of endosomes changed their position within 14–28 s (Fig. 7a–c). Measurements and subsequent statistical analysis of the movements revealed an average speed of 0.54 μ m/s

Table 1. Speed and rest times of the GFP-FYVE-labeled endosomes in *M. truncatula* root hair tips

	Speed range (μ m/s)	Average rest time (s)	Time spent stationary (%)
Control ($n = 100$)	0.09–17.77	1–4	26–39
BDM ($n = 54$)	0.11–18.92	0–1*	0–10*
LatB ($n = 48$)	0–1.04*	2.5–4.35	55–67*

Significant differences between treated and control root hairs are indicated by asterisks.

For the control 100 endosomes out of 11 root hairs, for the BDM treatment 54 endosomes out of three root hairs and for LatB treatment 48 endosomes out of three root hairs were measured.

(0.21 μ m/s SE) for the GFP-FYVE-labeled endosomes (Table 1). During the time of observation, they spent up to 40% of this period as stationary organelles, the duration of resting time was in the range of 1–4 s.

Upon application of 10 mM 2,3-butanedione monoxime (BDM), a general myosin ATPase inhibitor (Šamaj et al., 2000), GFP-FYVE-labeled endosomes continued to display subcellular movements within the ‘clear zone’, even if we extended the treatment for up to 1 h (Fig. 7d–f; Movie 12). Nevertheless, the characteristics of endosome motility were altered in the presence of BDM (Table 1). In particular, the resting times changed dramatically, as the BDM-treated FYVE-labeled endosomes spent less than 10% of the observed time in a stationary phase as compared to 40% in control conditions. Application of latrunculin B (LatB), an efficient F-actin-depolymerizing agent (for plant cells, see Gibbon et al., 1999; Baluška et al., 2001), resulted in

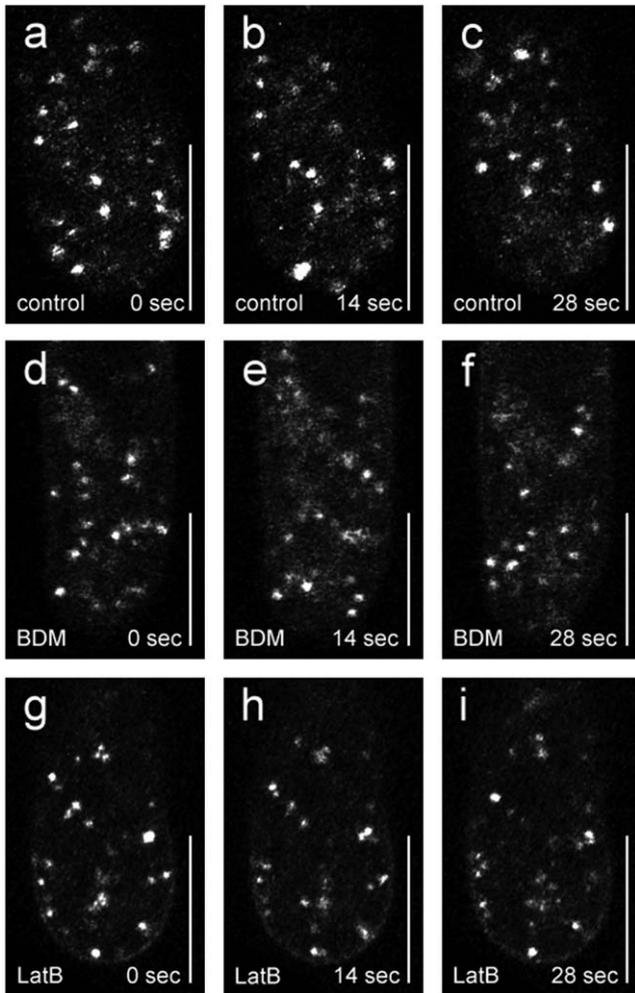


Fig. 7. Rapid motilities of tip-localized endosomes scored in the untreated root hairs of *M. truncatula* (a–c) were not affected by the inhibition of myosin motor-based activities with 2,3-butanedione monoxime (d–f) but were almost instantly inhibited by the exposure to latrunculin B which rapidly depolymerizes F-actin (g–i). See also Table 1. Bars = 10 μ m.

almost instant inhibition of all endosomal movements (Fig. 7g–i; Movies 13–16). Treatment with the microtubule-depolymerizing drug, oryzalin, had no discernible effect on endosomal motility (Movie 17).

Brefeldin A, wortmannin, and jasplakinolide affect motility and morphology of endosomes

To test if endosomal movements were influenced by inhibitors of membrane trafficking through endomembrane compartments, we applied brefeldin A (BFA) and wortmannin to root hairs of *M. truncatula*. The effect of BFA was traceable after 10 min (Fig. 8a) in the form of an enlargement of FYVE-labeled endosomes which concomitantly slowed down their motility. After 60 min of exposure to 100 μ M BFA, we scored

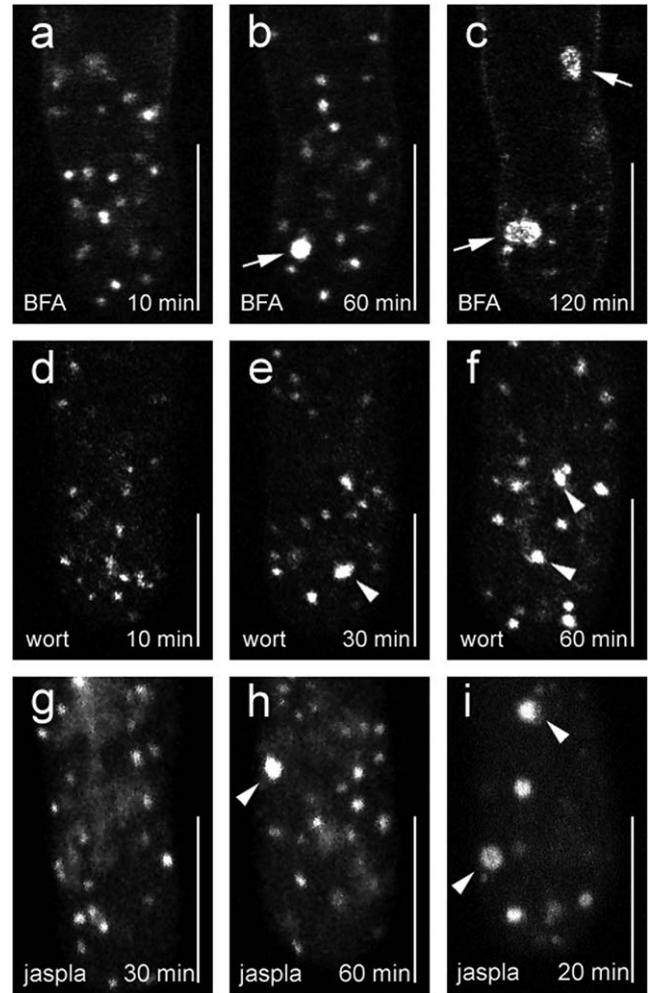


Fig. 8. Brefeldin A inhibited motilities and caused enlargement (arrows) and aggregation of FYVE-labeled early endosomes in root hairs of *M. truncatula* (a–c). Similar, but less dramatic effects (arrowheads) were induced also with wortmannin which inhibits PI(3)P production in plants (d–f). Jasplakinolide (1 μ M) inhibited the motility of the FYVE-labeled endosomes in root hairs of *Arabidopsis* after 30 min (g), whilst after 60 min of exposure first larger aggregates (arrowhead) became visible (h). After 20 min of 5 μ M jasplakinolide treatment the same effects but in a more dramatic way were scored, the motility was greatly decreased and the endosomes were enlarged (i, arrowheads). Bars = 10 μ m.

significant inhibition of endosomal motility and the size of endosomes enlarged dramatically (Fig. 8b). Two hours of BFA treatment resulted in large agglomerates of aggregated endosomes (Fig. 8c, arrows), known as the ‘BFA compartments’ in plant cells, with a greatly reduced motility. Exposure of hairs to 10 μ M wortmannin produced similar, although less prominent, effects on FYVE-labeled endosomes (Fig. 8d–f; Movies 18–21 for BFA and Movie 22 and 23 for wortmannin).

After scoring the dramatic changes of the actin cytoskeleton upon jasplakinolide treatment, we also

tested *A. thaliana* root hairs expressing GFP-FYVE with 1 and 5 μ M jasplakinolide (Fig. 8g–i). Our data revealed that FYVE-labeled endosomes considerably enlarged in hairs treated with jasplakinolide, suggesting that dynamic F-actin is essential also for their morphology. With increasing time of exposure and concentration of jasplakinolide, endosomes slowed down their movements (Movie 24).

Discussion

Using stably transformed seedlings of *A. thaliana* and *M. truncatula* as well as in vivo microscopy, we describe the specific localization of the PI(3)P reporter, GFP-FYVE, to dynamic and motile endosomal compartments. Their identity was confirmed in vivo using the reliable endocytic tracer FM4-64 (for plant cells, see Meckel et al., 2004) as well as GFP/YFP constructs of the endosome-specific plant Rab GTPases, Ara6 and RabF2a. Furthermore, we characterize actin-dependent motility of these plant endosomes in root hairs of *M. truncatula* and *A. thaliana*. Our observations highlight both conserved and unique aspects of plant endosomes with regard to mechanisms controlling membrane trafficking and subcellular dynamics. Based on the well documented and highly conserved PI(3)P-binding capacity of the FYVE domain (Gillooly et al., 2000; Jensen et al., 2001), we assume that plant endosomes identified here are enriched with PI(3)P. This is in agreement with the situation in yeast and mammalian cells. However, in contrast to animal and fungal endosomes, the motility of which is driven by dyneins and kinesins and accomplished along microtubules (Nielsen et al., 1999; Wedlich-Söldner et al., 2000, 2002), the subcellular motility of plant endosomes at tips of root hairs relies fully upon polymerization and dynamics of actin (this study).

In yeast and animals, endosomal membranes accumulate PI(3)P (Gillooly et al., 2000) due to the specific recruitment of PI-3 K by the endosome-localized Rab GTPases Vps21 and Rab5 (for review see Zerial and McBride, 2001). It has previously been shown that in transiently transformed *A. thaliana* protoplasts, overexpression of a FYVE domain-containing fragment of the mammalian EEA1 protein resulted in its targeting to subcellular membranes thought to partially overlap with a late endosome-like compartment (Kim et al., 2001; Sohn et al., 2003). Rab GTPases with significant similarity to yeast and mammalian endosomal Rab GTPases were observed on subcellular compartments that accumulate the endocytic tracer, FM4-64 (Ueda et al., 2001). However, two important questions remained unanswered from these earlier investigations. First, it was left unclear, whether the FYVE domain-containing fragment of EEA1 was sufficient for the

subcellular targeting to plant membranes because removal of the Rab5-binding region of this fragment resulted in a cytosolic localization (Kim et al., 2001; Sohn et al., 2003). Second, while the EEA1 FYVE domain localized on membranes might be consistent with a suggested endosomal-like prevacuolar compartment, it was not clear if this was a result of overexpression and whether these compartments were the same as those that could be labeled with internalized FM4-64 and the endosomal Rab GTPase Ara6 (Ueda et al., 2001). In fact, when we strongly overexpressed the FYVE construct in onion cells we always detected its artificial association with bigger structures similar to prevacuolar compartments and vacuoles (not shown). Here, we show recruitment of the new tandem FYVE reporter from mouse Hrs protein to endosomes, which accumulate endosomal marker FM 4-64 (Meckel et al., 2004). Unlike the EEA1 carboxy-terminal domain, this construct does not contain a Rab GTPase-binding domain. Therefore, the sole targeting determinant is based on the PI(3)P-binding specificity of the double FYVE domains. We further demonstrate the endosomal identity of these compartments by labeling with GFP reporters of two plant endosomal Rab GTPases, both being highly similar to mammalian Rab5 and yeast Vps21p. This raises the possibility that the recruitment of PI(3)K and subsequent accumulation of PI(3)P on endosomal membranes is a conserved feature not only in animals and yeast but also in plants.

Trichoblasts accomplish a developmentally unique signal-mediated switch in cell polarity, when they initiate a new bulging growth domain which rapidly transforms into a long tubular protrusion known as root hair (Baluška et al., 2000, 2001; Baluška and Volkmann, 2002; Gilliland et al., 2002; Jiang et al., 1997; Miller et al., 1999; Ringli et al., 2002; Šamaj et al., 2002, 2004a). We show here that motile endosomes are present at outgrowing bulges and within the apices of growing root hairs that extend via highly polar tip growth. It is known that bulging domains of hair-forming trichoblasts organize dense meshworks of the actin cytoskeleton via recruitment of actin, profilin, ADF, ROPs (Baluška et al., 2000; Jiang et al., 1997; Jones et al., 2002), and mitogen-activated protein kinases (Šamaj et al., 2002). Motile endosomes might participate in the recruitment of molecular components essential for the local assembly of the actin cytoskeleton, which drives the polarized tip growth of root hairs (Baluška et al., 2000; Gilliland et al., 2002; Jiang et al., 1997; Miller et al., 1999; Nishimura et al., 2003; Ringli et al., 2002; Šamaj et al., 2002, 2004a, b; Vantard and Blanchoin, 2002). This is the case in budding yeast, where the endosomal protein Cdc50p recruits the profilin-binding formin, Bni1p, to sites of polarized growth (Misu et al., 2003).

Myosins do not seem to be directly involved in driving motility of endosomes at growing tips because BDM has

no inhibitory effect on endosomal movements in this subcellular domain. Similarly microtubules, which are the favored long-range motility tracks in mammalian systems (Goode et al., 2000), are not essential for the motility of plant endosomes because depolymerization of microtubules did not inhibit rapid motility of FYVE/Ara6/RabF2a-based endosomes, as it also does not inhibit tip growth per se (Baluška et al., 2000; Miller et al., 1999). The only cytoskeletal drug, which almost instantly blocked the motility of endosomes, both at the tip and in the shank, was latrunculin B. Importantly, plant endosomes are particularly dynamic within the very tips of root hairs lacking prominent cytoskeletal tracks (Baluška et al., 2000; Ketelaar et al., 2004; Miller et al., 1999; Voigt et al., 2005). On the basis of these findings, we tentatively propose that endosomes move by actin comet tails (Taunton, 2001; Plastino and Sykes, 2005) in root hair tips. Comet tail movement requires profilin, ADF, and Arp2/3 in animal cells. It is therefore important to note that both ADF and profilin were localized to root hair tips (Jiang et al., 1997; Baluška et al., 2000) whereas the *Arabidopsis* genome contains genes of the Arp2/3 complex (Deeks and Hussey, 2003; McKinney et al., 2002) and mutants of the Arp2/3 complex show aberrant root hair phenotypes (Mathur et al., 2003a, b). Furthermore, Arp3-like protein is reported to be enriched at tips of early root hairs as well as in association with multivesicular endosomes (Van Gestel et al., 2003).

Spatially controlled, and signal-mediated actin polymerization is crucial for maintaining motilities and shapes of animal cells (Goode et al., 2000). Walled plant cells are non-motile and believed to expand just by the force of turgor pressure. But recent advances in the studies of pollen tubes and root hairs, two tip-growing plant cell types, surprisingly indicated that actin polymerization-driven expansion of the cell periphery might be at the heart of this highly polarized growth of walled plant cells (Baluška et al., 2000, 2001, 2002; Baluška and Volkman, 2002; Gibbon et al., 1999; Gilliland et al., 2002; Jiang et al., 1997; Ketelaar et al., 2004; Miller et al., 1999; Nishimura et al., 2003; Ringli et al., 2002; Šamaj et al., 2002, 2004a, b; Vantard and Blanchoin, 2002; Vidali et al., 2001; Voigt et al., 2005). Intriguingly, plant tip growth requires dynamic actin polymerization that is unrelated to cytoplasmic streaming (Gibbon et al., 1999; Vidali et al., 2001). Whereas most of these data were obtained using the highly potent F-actin drug latrunculin B, recent studies presented convincing genetic evidence in favor of actin polymerization-driven cell expansion in root hairs (Gilliland et al., 2002; Nishimura et al., 2003; Ringli et al., 2002). Here, we report that actin-dependent tip growth is tightly linked to the actin-driven movements of plant endosomes. A similar scenario is plausible also for tip-growing pollen tubes (Parton et al., 2001, 2003).

Invagination of the plasma membrane and endosomal motility during early endocytosis is well known to be dependent on actin polymerization (Engqvist-Goldstein et al., 2004; Kaksonen et al., 2003). In budding yeast, endocytic vesicles which have pinched off the plasma membrane use dynamic actin comet tails for rapid transport deeper into the cytoplasm, when endocytic complexes serve as nucleation sites for burst-like actin polymerization (Engqvist-Goldstein et al., 2004). These events are known to be abundant especially at sites of polar growth in yeast cells which, in some respects, resembles tip growth of plant cells (Pelham and Chang, 2001; Pruynne and Bretscher, 2000; Smith et al., 2001). Intriguingly, yeast actin patches are propelled by actin polymerization (Carlsson et al., 2002; Pelham and Chang, 2001; Smith et al., 2001). Recent live-cell imaging of budding yeast cells revealed that actin patches co-localize with endosomes both at the plasma membrane as well as along F-actin cables during their retrograde transport (Huckaba et al., 2004).

In both plants and yeast, polar expansion occurs in cells that are constrained by robust cell walls. Mechanistically, it would make sense when secretory cargo as well as endocytic traffic are recruited from and delivered into zones of high actin polymerization. As the delivery of newly synthesized cell wall components via exocytosis would also result in localized surplus of membrane material, concomitant active endocytosis would also be predicted within these zones of high actin dynamics. But how can local actin polymerization drive expansion of walled plant cells? This problem could be perhaps solved, if one considers that actin-driven endosomes participate in cell wall remodeling and turnover and hence contribute to the plasticity of the wall. In fact, root cells internalize particularly cross-linked cell wall pectins (Baluška et al., 2002) which are the major cell wall component of root hair tips. Moreover, root hairs actively maintain thin and loosened cell walls at their tips. A similar scenario is attractive also for the fungal tip growth which is tightly linked with endocytosis (Oberholzer et al., 2004), having endosomes accumulated at sites of polar growth (Walther and Wendland, 2004; Wedlich-Söldner et al., 2000). Moreover, endosomes and F-actin also accumulate at domains of cell wall remodeling during cytokinetic cell separation (Wedlich-Söldner et al., 2000, 2002). Last but not least, actin-driven endosomes might eventually push against the plasma membrane similarly like actin-driven microbial pathogens (Goldberg, 2001) and lysosome-based melanosomes (Scott et al., 2002), producing long filopodia facilitating their cell-to-cell spreading.

Besides explaining actin-driven polarized growth in walled cells, our present data could potentially also shed fresh light on the elusive actin polymerization-driven spots/foci at the actin-based protrusive surfaces of animal and human cells (Kaksonen et al., 2000; Rochlin

et al., 1999; Weiner et al., 1999) because these too, were linked with endosomes (Kaksonen et al., 2000). In conclusion, actin-propelled endosomes are inherently linked with polarized growth of root hairs, and future studies should reveal if this new link is relevant also to other eukaryotic cells that show polarized growth.

Acknowledgments

We thank Claudia Heym for excellent technical assistance. This work was supported by grants from Deutsches Zentrum für Luft- und Raumfahrt (DLR, Bonn, Germany) and by EU Research Training Network TIPNET (project HPRN-CT-2002-00265) obtained from Brussels, Belgium. F. Baluška and J. Šamaj receive partial support from the Slovak Academy of Sciences, Grant Agency VEGA (Grant No. 2031) and APVT (Grant No. APVT-51-00-23-02), Bratislava, Slovakia.

Appendix A. Supplementary materials

The online version of this article contains additional supplementary data. Please visit [doi:10.1016/j.ejcb.2004.12.029](https://doi.org/10.1016/j.ejcb.2004.12.029).

References

- Baluška, F., Volkmann, D., 2002. Pictures in cell biology. Actin-driven polar growth of plant cells. *Trends Cell Biol.* 12, 14.
- Baluška, F., Salaj, J., Mathur, J., Braun, M., Jasper, F., Šamaj, J., Chua, N.-H., Barlow, P.W., Volkmann, D., 2000. Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. *Dev. Biol.* 227, 618–632.
- Baluška, F., Jasik, J., Edelmann, H.G., Salajova, T., Volkmann, D., 2001. Latrunculin B-induced plant dwarfism: plant cell elongation is F-actin-dependent. *Dev. Biol.* 231, 113–124.
- Baluška, F., Hlavacka, A., Šamaj, J., Palme, K., Robinson, D.G., Matoh, T., McCurdy, D.W., Menzel, D., Volkmann, D., 2002. F-actin-dependent endocytosis of cell wall pectins in meristematic root cells. Insights from brefeldin A-induced compartments. *Plant Physiol.* 130, 422–431.
- Bevis, B.J., Glick, B.S., 2002. Rapidly maturing variants of the *Discosoma* red fluorescent protein (DsRed). *Nat. Biotechnol.* 20, 83–87.
- Boisson-Dernier, A., Chabaud, M., Garcia, F., Becard, G., Rosenberg, C., Barker, D.G., 2001. *Agrobacterium rhizogenes*-transformed roots of *Medicago truncatula* for the study of nitrogen-fixing and endomycorrhizal symbiotic associations. *Mol. Plant Microb. Interact.* 14, 695–700.
- Carlsson, A.E., Shah, A.D., Elking, D., Karpova, T.S., Cooper, J.A., 2002. Quantitative analysis of actin patch movement in yeast. *Biophys. J.* 82, 2333–2343.
- Deeks, M.J., Hussey, P.J., 2003. Arp2/3 and ‘The shape of things to come’. *Curr. Opin. Plant Biol.* 6, 1–7.
- Engqvist-Goldstein, A.E., Zhang, C.X., Carreno, S., Barroso, C., Heuser, J.E., Drubin, D.G., 2004. RNAi-mediated Hip1R silencing results in stable association between the endocytic machinery and the actin assembly machinery. *Mol. Biol. Cell* 15, 1666–1679.
- Gibbon, B.C., Kovar, D.R., Staiger, C.J., 1999. Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell* 11, 2349–2363.
- Gilliland, L.U., Kandasamy, M.K., Pawloski, L.C., Meagher, R.B., 2002. Both vegetative and reproductive actin isoforms complement the stunted root hair phenotype of the *Arabidopsis* act2-1 mutation. *Plant Physiol.* 130, 2199–2209.
- Gillooly, D.J., Morrow, I.C., Lindsay, M., Gould, R., Bryant, N.J., Gaullier, J.M., Parton, R.G., Stenmark, H., 2000. Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J.* 19, 4577–4588.
- Goldberg, M.B., 2001. Actin-based motility of intracellular microbial pathogens. *Microbiol. Mol. Biol. Rev.* 65, 595–626.
- Goode, B.L., Drubin, D.G., Barnes, G., 2000. Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.* 12, 63–71.
- Gruenberg, J., 2001. The endocytic pathway: a mosaic of domains. *Nat. Rev. Mol. Cell Biol.* 2, 721–730.
- Hamilton, D.A., Roy, M., Rueda, J., Sindhu, R.K., Sanford, J., Mascarenhas, J.P., 1992. Dissection of a pollen-specific promoter from maize by transient transformation assays. *Plant Mol. Biol.* 18, 211–218.
- Hepler, P.K., Vidali, L., Cheung, A.Y., 2001. Polarized cell growth in higher plants. *Annu. Rev. Cell Dev. Biol.* 17, 159–187.
- Huckaba, T.M., Gay, A.C., Pantalena, L.F., Yang, H.-C., Pon, L.A., 2004. Live cell imaging of the assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 167, 519–530.
- Jensen, R.B., La Cour, T., Albrethsen, J., Nielsen, M., Skriver, K., 2001. FYVE zinc-finger proteins in the plant model *Arabidopsis thaliana*: identification of PtdIns3P-binding residues by comparison of classic and variant FYVE domains. *Biochem. J.* 359, 165–173.
- Jiang, C.J., Weeds, A.G., Hussey, P.J., 1997. The maize actin-depolymerizing factor, ZmADF3, redistributes to the growing tip of elongating root hairs and can be induced to translocate into the nucleus with actin. *Plant J.* 12, 1035–1043.
- Jones, M.A., Shen, J.-J., Fu, Y., Li, H., Yang, Z., Grierson, C.S., 2002. The *Arabidopsis* Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *Plant Cell* 14, 763–776.
- Kaksonen, M., Peng, H.B., Rauvala, H., 2000. Association of cortactin with dynamic actin in lamellipodia and on endosomal vesicles. *J. Cell Sci.* 113, 4421–4426.

- Kaksonen, M., Sun, Y., Drubin, D.G., 2003. A pathway for association of receptors, adaptors, and actin during endocytic internalization. *Cell* 115, 475–487.
- Ketelaar, T., Allwood, E.G., Anthony, R., Voigt, B., Menzel, D., Hussey, P.J., 2004. The actin-interacting protein AIP1 is essential for actin organization and plant development. *Curr. Biol.* 14, 145–149.
- Kim, D.H., Eu, Y.J., Yoo, C.M., Kim, Y.W., Pih, K.T., Jin, J.B., Kim, S.J., Stenmark, H., Hwang, I.I., 2001. Trafficking of phosphatidylinositol 3-phosphate from the trans-Golgi network to the lumen of the central vacuole in plant cells. *Plant Cell* 13, 287–301.
- Mathur, J., Mathur, N., Kernebeck, B., Hülskamp, M., 2003a. Mutations in actin-related proteins 2 and 3 affect cell shape development in *Arabidopsis*. *Plant Cell* 15, 1632–1645.
- Mathur, J., Mathur, N., Kirik, V., Kernebeck, B., Srinivas, B.P., Hülskamp, M., 2003b. *Arabidopsis* CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* 130, 3137–3146.
- McKinney, E.C., Kandasamy, M.K., Meagher, R.B., 2002. *Arabidopsis* contains ancient classes of differentially expressed actin-related protein genes. *Plant Physiol.* 128, 997–1007.
- Meckel, T., Hurst, A.C., Thiel, G., Homann, U., 2004. Endocytosis against high turgor: intact guard cells of *Vicia faba* constitutively endocytose fluorescently labelled plasma membrane and GFP-tagged K⁺-channel KAT1. *Plant J.* 39, 182–193.
- Miller, D.D., De Ruijter, N.C.A., Bisseling, T., Emons, A.C., 1999. The role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. *Plant J* 17, 141–154.
- Misu, K., Fujimura-Kamada, K., Ueda, T., Nakano, A., Katoh, H., Tanaka, K., 2003. Cdc50p, a conserved endosomal membrane protein, controls polarized growth in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 14, 730–747.
- Nielsen, E., Severin, F., Backer, J.M., Hyman, A.A., Zerial, M., 1999. Rab5 regulates motility of early endosomes on microtubules. *Nat. Cell Biol.* 1, 376–382.
- Nishimura, T., Yokota, E., Wada, T., Shimmen, T., Okada, K., 2003. An *Arabidopsis* ACT2 dominant-negative mutation, which disturbs F-actin polymerization, reveals its distinctive function in root development. *Plant Cell Physiol.* 44, 1131–1140.
- Oberholzer, U., Iouk, T.L., Thomas, D.Y., Whiteway, M., 2004. Functional characterization of myosin I tail regions in *Candida albicans*. *Eukaryot. Cell* 3, 1272–1286.
- Parton, R.M., Fischer-Parton, S., Watahiki, M.K., Trewavas, A.J., 2001. Dynamics of the apical vesicle accumulation and the rate of growth are related in individual pollen tubes. *J. Cell Sci.* 114, 2685–2695.
- Parton, R.M., Fischer-Parton, S., Trewavas, A.J., Watahiki, M.K., 2003. Pollen tubes exhibit regular periodic membrane trafficking in the absence of apical extension. *J. Cell Sci.* 116, 2707–2719.
- Pelham Jr., R.J., Chang, F., 2001. Role of actin polymerization and actin cables in actin-patch movement in *Schizosaccharomyces pombe*. *Nat. Cell Biol.* 3, 235–244.
- Plastino, J., Sykes, C., 2005. The actin slingshot. *Curr. Opin. Cell Biol.* 17, 62–66.
- Pruyne, D., Bretscher, A., 2000. Polarization of cell growth in yeast. *J. Cell Sci.* 113, 571–585.
- Ringli, C., Baumberger, N., Diet, A., Frey, B., Keller, B., 2002. ACTIN2 is essential for bulge site selection and tip growth during root hair development of *Arabidopsis*. *Plant Physiol.* 129, 1464–1472.
- Rochlin, M.W., Dailey, M.E., Bridgman, P.C., 1999. Polymerizing microtubules activate site-directed F-actin assembly in nerve growth cones. *Mol. Biol. Cell* 10, 2309–2327.
- Šamaj, J., Peters, M., Volkmann, D., Baluška, F., 2000. Effects of myosin ATPase inhibitor 2,3-butanedione 2-monoxime on distributions of myosins, F-actin, microtubules, and cortical endoplasmic reticulum in maize root apices. *Plant Cell Physiol.* 41, 571–582.
- Šamaj, J., Ovecka, M., Hlavacka, A., Lecourieux, F., Meskiene, I., Lichtscheidl, I., Lenart, P., Salaj, J., Volkmann, D., Bogre, L., Baluška, F., Hirt, H., 2002. Involvement of the mitogen-activated protein kinase SIMK in regulation of root hair tip growth. *EMBO J.* 21, 3296–3306.
- Šamaj, J., Baluška, F., Menzel, D., 2004a. New signalling molecules regulating root hair tip growth. *Trends Plant Sci.* 9, 217–220.
- Šamaj, J., Baluška, F., Voigt, B., Schlicht, M., Volkmann, D., Menzel, D., 2004b. Endocytosis, actin cytoskeleton and signalling. *Plant Physiol.* 135, 1150–1161.
- Scott, G., Leopardi, S., Printup, S., Madden, B.C., 2002. Filopodia are conduits for melanosome transfer to keratinocytes. *J. Cell Sci.* 115, 1441–1451.
- Smith, M.G., Swamy, S.R., Pon, L.A., 2001. The life cycle of actin patches in mating yeast. *J. Cell Sci.* 114, 1505–1513.
- Sohn, E.J., Kim, E.S., Zhao, M., Kim, S.J., Kim, H., Kim, Y.W., Lee, Y.J., Hillmer, S., Sohn, U., Jiang, L., Hwang, I., 2003. Rha1, an *Arabidopsis* Rab5 homolog, plays a critical role in the vacuolar trafficking of soluble cargo proteins. *Plant Cell* 15, 1057–1070.
- Sorkin, A., von Zastrow, M., 2002. Signal transduction and endocytosis: close encounters of many kinds. *Nat. Rev. Mol. Cell Biol.* 3, 600–614.
- Taunton, J., 2001. Actin filament nucleation by endosomes, lysosomes and secretory vesicles. *Curr. Opin. Cell Biol.* 13, 85–91.
- Timmers, A.C.J., Niebel, A., Balague, C., Dagkesamanskaya, A., 2002. Differential localisation of GFP fusions to cytoskeleton-binding proteins in animal, plant, and yeast cells. *Protoplasma* 220, 69–78.
- Ueda, T., Yamaguchi, M., Uchimiya, H., Nakano, A., 2001. Ara6, a plant-unique novel type Rab GTPase, functions in the endocytic pathway of *Arabidopsis thaliana*. *EMBO J.* 20, 4730–4741.
- Van Gestel, K., Slegers, H., Von Witsch, M., Šamaj, J., Baluška, F., Verbelen, J.P., 2003. Immunological evidence for the presence of plant homologues of the actin-related protein Arp3 in tobacco and maize: subcellular localization to actin-enriched pit fields and emerging root hairs. *Protoplasma* 222, 45–52.
- Vantard, M., Blanchoin, L., 2002. Actin polymerization processes in plant cells. *Curr. Opin. Plant Biol.* 5, 502–506.

- Vidali, L., McKenna, S.T., Hepler, P.K., 2001. Actin polymerization is essential for pollen tube growth. *Mol. Biol. Cell* 12, 2534–2545.
- Voigt, B., Timmers, T., Šamaj, J., Müller, J., Baluška, F., Menzel, D., 2005. GFP-FABD2 fusion construct allows in vivo visualization of the dynamic actin cytoskeleton in all cells of *Arabidopsis* seedlings, *Eur. J. Cell Biol.* 84, ■–■.
- Walther, A., Wendland, J., 2004. Apical localization of actin patches and vacuolar dynamics in *Ashbya gossypii* depend on the WASP homolog Wallp. *J. Cell Sci.* 117, 4947–4958.
- Wedlich-Söldner, R., Bolker, M., Kahmann, R., Steinberg, G., 2000. A putative endosomal t-SNARE links exo- and endocytosis in the phytopathogenic fungus *Ustilago maydis*. *EMBO J.* 19, 1974–1986.
- Wedlich-Söldner, R., Straube, A., Friedrich, M.W., Steinberg, G., 2002. A balance of KIF1A-like kinesin and dynein organizes early endosomes in the fungus *Ustilago maydis*. *EMBO J.* 21, 2946–2957.
- Weiner, O.D., Servant, G., Welch, M.D., Mitchison, T.J., Sedat, J.W., Bourne, H.R., 1999. Spatial control of actin polymerization during neutrophil chemotaxis. *Nat. Cell Biol.* 1, 75–81.
- Zerial, M., McBride, H., 2001. Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2, 107–117.