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Structural aspects of bulge formation during root hair initiation

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Abstract

Using light and electron microscopy, the early stages of root hair initiation were investigated under control conditions and in a situation where F-actin polymerization was effectively inhibited by latrunculin B. Trichoblasts in their early stage of bulge formation possessed large vacuole traversed by cytoplasmic strands and enclosed within a narrow peripheral layer of cytoplasm. The nucleus was settled at the inner periclinal cell wall, typically opposite the site of bulge formation. Within the bulging area, dense cytoplasm and numerous ER elements, and other organelles were accumulated, together with pleiomorphic membrane-bound structures, the identity and nature of which will require further studies. These unusual structures, which were associated with the outer cell wall, contained material similar to that of the cell wall. Similar cell wall-like bodies were observed also in the cytoplasm and sometimes within vacuoles. The possible role of these novel organelles of plant cells in cell wall thinning/degradation or in the turgor pressure maintenance are discussed. Latrunculin B treatment allowed bulge formation but prevented the switch from the slow and diffuse expansion of bulge into the rapid tip-growth characteristic of the emerging root hair. Moreover, the cytoplasm of the bulging domain became extensively vacuolated and lacked abundant ER elements and other organelles including the membrane-bound structures. These results indicate important roles of F-actin in the switch from diffuse to highly polarized tip growth.

Introduction

It becomes increasingly clear that root hair formation consists of two distinct steps: bulge formation followed by the transformation of the bulge into the tip-growing apex of the emerging root hair (reviewed by Dolan, 2001; Mathur and Hülskamp, 2001; Schiefelbein, 2000). The initiation of a root hair includes selection and isolation of a prospective bulge site beneath the outer wall of a trichoblast, determination of its size, local thinning of the cell wall, outgrowth of the bulge, and the switch to the tip growth characteristic of emerging root hairs (Baluška et al., 2000c; Ryan et al., 2001). Localized cell wall acidification (Bibikova et al., 1998), accumulation of expansins (Baluška et al., 2000b), and activation of xyloglucan endotransglycosylase (Vissenberg et al., 2001) in the cell walls of bulged domains accompany, and perhaps determine, the cell wall thinning. Later, cortical microtubules become depleted in the bulged domains (Baluška et al., 2000b,c). The switch from bulging to tip growth root hair is dictated via a dynamic actin cytoskeleton (Baluška et al., 2000b; Baluška and Volkmann, 2002; Emons and de Ruijter, 2000; Geitmann and Emons, 2000; Miller et al., 1999). This is manifested by progressive accumulation of proteins essential for actin filament dynamics (profilin, actin depolymerising factor), selective recruitment of dense meshwork of dynamic actin filaments, and the focussing of reorganized F-actin cables at the bulging domains of the emerging root hairs (Baluška et al., 2000a,b; Braun et al., 1999; Jiang et al., 1997; Miller et al., 1999).

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Interestingly, bulge formation is not inhibited in F-actin-depleted trichoblasts after cytochalasin D (Miller et al., 1999) or latrunculin B (Baluška et al., 2000b; Ovečka et al., 2000) treatments. However, the bulges formed under such conditions show aberrant shapes, are mechanically weak, and fail to transform into tip-growing root hairs (Baluška et al., 2000b; Miller et al., 1999). Similarly, *der1* mutants of *Arabidopsis* form aberrant bulges which fail to convert into tip-growing root hairs (Ringli et al., 2002). Map-based cloning of the *der1* locus showed that it is mutated in the actin2 gene, corresponding well with the above drug studies. Aberrantly shaped bulges and young root hairs are characteristic also for other root hair mutants of *Arabidopsis*. For instance, *rhd4* mutant shows irregularly thickened cell walls which can be mimicked with osmotic stress (Galway et al., 1998) while *rhd2* mutant has an altered cell wall structure at the bulging site prior to the switch to tip growth (Schiefelbein and Sommerville, 1990).

Our present study extends the knowledge on root hair formation with ultrastructural characteristics of trichoblasts in their pre-bulging and early bulging stages in control and F-actin-devoid situations.

Material and methods

Plant cultivation

Seeds of vetch, *Vicia sativa* L. cv. Arida (Central Control and Testing Institute of Agriculture, Bratislava, Slovakia) were sown on wet filter paper in Petri dishes, exposed to low temperature (4 °C) for 24 h to synchronize germination and left to germinate for 66 h at 22 °C in darkness. Seedlings with 3 to 4 cm long roots were grown in Fårhaeus (1957) nutrient solution modified by de Ruijter et al. (1998) with or without 10^{-5} M latrunculin B (Merck). Control plants were grown with 1% DMSO (solvent for latrunculin B), for 0.5 and 20–24 h. Cytoplasmic streaming in control and latrunculin-treated root hairs was checked using light microscopy. Plants of *Lepidium sativum* were grown and prepared as previously (Volkmann and Peters, 1995).

Light and transmission electron microscopy

Segments of the root apices including the root hair zone were fixed with 3% glutaraldehyde in 0.01 M Na-cacodylate buffer, post-fixed with 1% OsO_4 in the same buffer, dehydrated in ethanol series and propylene oxide, and embedded in Spurr's medium. Semithin sections stained with toluidine blue and ultrathin sections stained with uranyl acetate and lead citrate were observed using Olympus BX51 and TEM Tesla BS 500 microscopes, respectively.

Scanning electron microscopy

Seeds of the same vetch cultivar were surface sterilized, germinated and grown vertically on plates containing 0.5% phytagel (Sigma) solid medium with 1% sucrose, and Murashige and Skoog salts. Similar temperature and light regimes were used as for the roots embedded for transmission electron microscopy. Four-day-old roots were placed on moist nitrocellulose paper, mounted on a stub and immersed in liquid nitrogen slush. Roots were transferred to a cold stage. After removal of water by sublimation, roots were sputter coated with platinum under cryo-stage conditions of the Philips XL30 FEG scanning electron microscope at 3.0 kV and temperatures of −140 to −150 °C.

Results and discussion

Vicia sativa belongs to those species in which each rhizodermal cell is capable of root hair formation (Miller et al., 1999). This was found also in our cultivar Arida (Figure 1a). The nucleus was typically located against the inner periclinal cell wall, opposite the site of bulge formation (Figure 1b). However, nuclei move to the opposite cell wall as the bulge enlarges (Figure 1c) and enter the emerging hair (Baluška et al., 2000c; Miller et al., 1999, 2000).

In accordance with the previous report (Miller et al., 2000), the bulging domain is rich in cytoplasm showing abundant elements of ER oriented transversely to the future hair axis, Golgi bodies, vesicles, and mitochondria (Figure 2a). In addition, we observed numerous lipid bodies and prominent membrane-enclosed compartments filled with material similar to the material of the cell wall (Figure 2c,d, compare with Figure 2b). The strictly local presence and abundance of these structures within outgrowing bulges could indicate local wall material internalization and subsequent cytoplasmic digestion. The cell wall frequently showed irregular outline within this domain possibly indicating the beginning of internalization of fluidized cell wall portions (Figure 2a,b). In

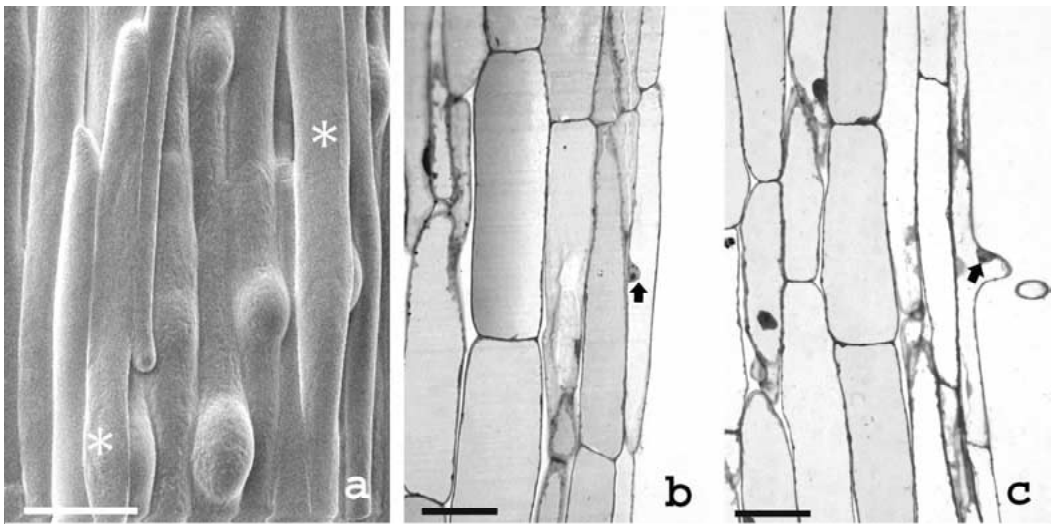

Figure 1. Trichoblasts in early stages of root hair initiation. (a) Scanning electron micrograph shows that each rhizodermal cell can form a bulge in *Vicia sativa*. Note the root cap cells (asterisk) adhering to the root surface. (b) Before bulging, the nucleus (arrow) is adjacent to the internal periclinal cell wall of the trichoblast. (c) Later when the bulge enlarges, the nucleus (arrow) moves to the opposite cell wall of the trichoblast. Bars represent 25 μm.

fact, recent studies implicate that root hair formation necessitates local, but extensive, cell wall remodelling (Baluška et al., 2000b; Baumberger et al., 2001; Bucher et al., 1997; Favery et al., 2001; Šamaj et al., 1999; Vissenberg et al., 2001; Wang et al., 2001), suggesting that local reorganization of the cell wall composition is an important aspect of root hair development. This phenomenon could be relevant for the local thinning of cell wall evident in the trichoblast of *Lepidium sativum* root (Figure 2e; see also Miller et al., 2000; Ryan et al., 2001) which culminates in F-actin independent formation of bulges (Baluška et al., 2000b; Miller et al., 1999).

Importantly, membrane-enclosed inclusions filled with the cell wall-like material described here within outgrowing bulges initiating root hairs resemble heterophagy in water-stressed maize root cells (Čiamporová and Mistrík, 1993; Nishizawa et al., 1989). Nishizawa et al. (1989) interpreted this phenomenon as a process by which cells utilize cell wall polysaccharides to acquire osmotically active compounds necessary for turgor pressure maintenance under stress. Incorporation of cell wall material into membrane invaginations was described in yeast cells during their shrinkage induced by hyperosmotic shock (Slaninová et al., 2000). Similarly, irregularly thickened cell walls of *rhd4* mutant of *Arabidopsis* can be mimicked with osmotic stress (Galway et al., 1998). Interestingly in this respect, osmotic stress-activated MAP kinase of alfalfa (Baluška et al., 2000d) accumulates within outgrowing bulges during root hair development in F-actin-dependent fashion (Šamaj et al., 2002). In trichoblasts, osmotically active compounds may be required to keep sufficient turgor pressure necessary for the outgrowth of root hair from the bulging domain. Alternatively, internalization of the cell wall material may be part of massive and fast remodelling process preparing local cell wall domains for the forthcoming tip growth.

Latrunculin B inhibited cytoplasmic streaming in vetch root hairs within 10 min of exposure. After 24 h, root extension was reduced to about 30%. This was apparently due to both dramatic alterations of cell division planes observed in longitudinal sections of the treated roots and to reduced final lengths of the root cells (data not shown). Disturbed orientation of cell division planes occurred in all root tissues. This resulted in deformation of the root apex with a thinner tip and swollen subapical part, similarly as shown by Baluška et al. (2000b) in maize. These results corroborate the findings that F-actin-devoid maize root cells divide chaotically and fail to accomplish rapid cell elongation (Baluška et al., 2001).

It has been shown before that root hair growth, but not formation of bulges, is inhibited in F-actin-devoid situation (Baluška et al., 2000b; Miller et al., 1999). In comparison to the control trichoblasts (Figures 2, 3a), the peripheral cytoplasmic layer of F-

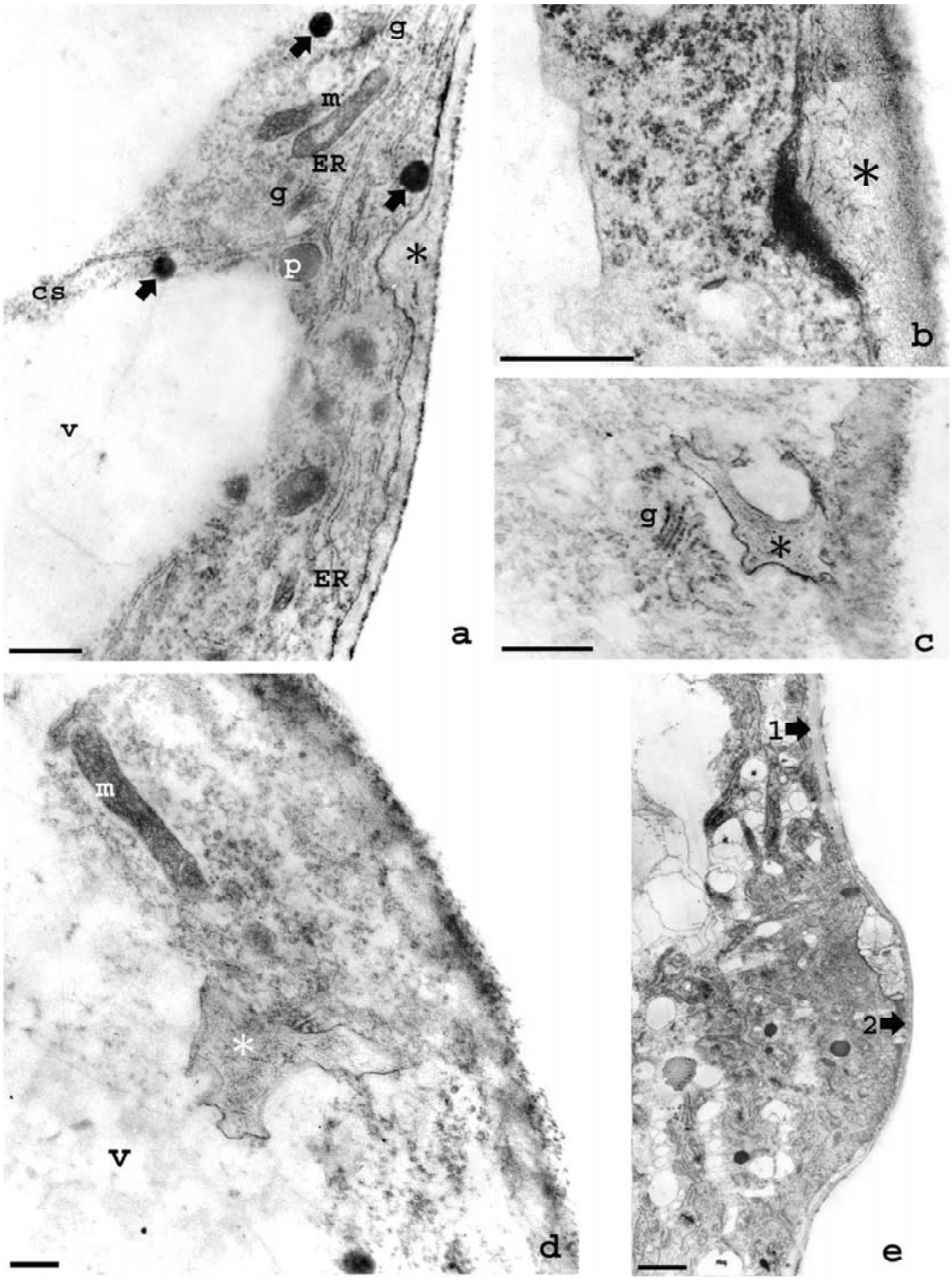

Figure 2. Cytoplasm and outer cell wall of the bulging area opposite to the nucleus. (a,b) Long and numerous elements of ER, mitochondria (m), Golgi bodies (g), plastids (p), and lipid bodies (arrows) are present within the narrow layer of the cytoplasm. Cytoplasmic strand (cs) extends towards the nucleus adjacent to the opposite cell wall. Asterisks indicate the beginning of cell wall material internalization. (c,d) Membrane-bound inclusions (asterisks) containing cell wall-like material (compare with b) associated with the outer periclinal cell wall (c) and entering the vacuole (d) within the bulging domain of the trichoblasts. (e) Longitudinal section of the trichoblast showing a markedly thicker cell wall beyond the bulge (arrow 1) compared to that surrounding the bulge (arrow 2) in *Lepidium sativum* root. Bars represent 1 (a), 0.5 (b,c,d), and 2 (e) μm.

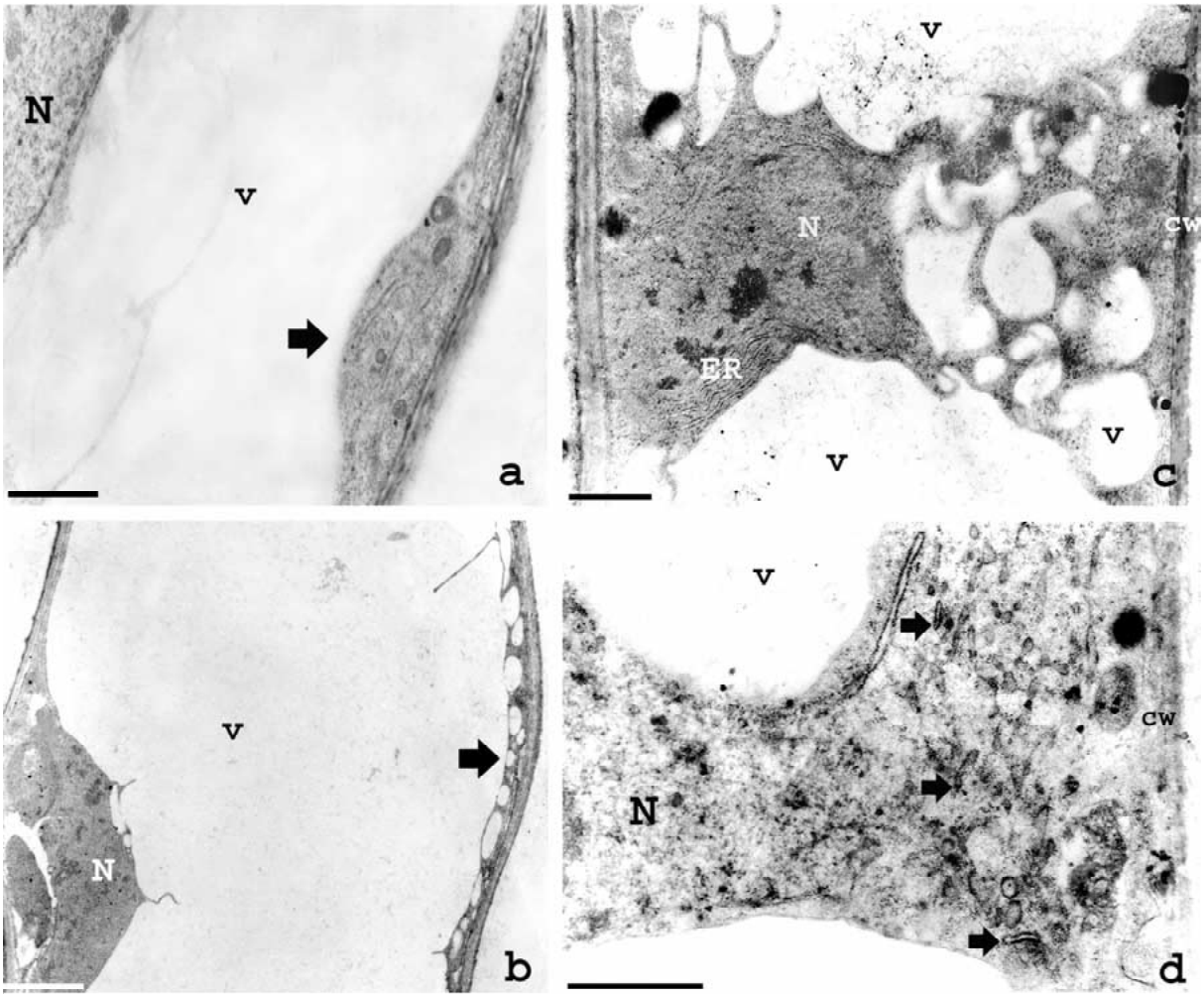

Figure 3. Control (a) and F-actin devoid (b–d) trichoblasts after exposure to latrunculin B for 24 h. (a,b) Beginning of bulge formation (arrows) opposite to nucleus (N) in a trichoblast of a control (a) and a latrunculin-treated (b) root with large vacuole (v). Note the numerous small vacuoles in the cytoplasm within the bulging domain (b). (c) Trichoblast with nucleus (N) adjacent to the inner periclinal cell wall and highly vacuolated cytoplasm within the future bulging domain. Note the unusual position of long elements of ER next to the nucleus. (d) Mostly short fragments of ER (arrows) occur within the prospective bulging domain. cw – outer cell wall. Bars represent 2 (a,c,d) and 5 (b) μm.

actin devoid trichoblasts after latrunculin B treatment contained numerous small vacuoles (Figure 3b) but, importantly, there was no accumulation of long ER elements or other organelles in the bulging domain. Instead, the dense cytoplasm was filled with small vacuoles and short fragments of ER elements (Figure 3c,d). Long elements of ER were observed only close to the nucleus (Figure 3c). This corresponds well with the findings that even a short exposure to latrunculin B results in a rapid disintegration of the cytoplasm-rich zone, known also as a 'clear zone', with the rapid vacuolation of the root hair tip (Ovečka et al., 2000). Dynamic F-actin in tip growing cells is regulated by Rop GTPase signalling and similar loss of polarity, associated with delocalization of tip-focused calcium gradient, was reported for young root hairs of *Arabidopsis* expressing constitutively active AtRop4 and AtRop6 Rop GTPases (Molendijk et al., 2001).

The actin cytoskeleton is critical for the assembly and maintenance of cytoarchitecture and plays multiple roles in higher plants (Kost et al., 1999; Kost and Chua, 2002; Staiger et al., 2000; Volkmann and Baluška, 1999). Root hair cytoarchitecture is presumably regulated by phosphorylation and dephosphorylation processes. In a line with this, Yokota et al. (2000) reported that the protein phosphatase inhib-

itor, calyculin A, induced morphological changes of the cytoplasm associated with the formation of cytoplasmic spherical bodies. Our findings showing rapid cytoplasmic vacuolation and loss of cytoplasm, as well as organelle accumulation within the bulged domains of F-actin-depleted trichoblasts, support the importance of F-actin for cytoarchitecture. Interestingly in this respect, similar changes to the root hair cytoarchitecture, polarized actin arrangements and the rapid block of the tip growth can be induced by UO 126, an inhibitor of mitogen-activated protein kinases (Šamaj et al., 2002).

In conclusion, our structural analysis of the outgrowing bulge domain extends the already known data and suggests that local cell wall degradation and internalization of cell wall components are important for local cell wall thinning before the onset of root hair tip growth. They also indicate that the F-actin dependent presence of long ER elements, abundant mitochondria, Golgi bodies, and lipid bodies might be important for the successful transformation of diffusely outgrowing bulges into highly-focussed tip-growing root hairs. The ultrastructure within the bulges was disturbed in the F-actin depleted roots, with vacuolation of the cytoplasm and depletion of ER elements and of other organelles. Absence of cell wall internalization after latrunculin B treatment indicates its F-actin dependence. Since aberrant bulge formation occurred in F-actin depleted conditions, we propose that the cell wall remodelling, culminating in the bulge formation, is driven also via local enzymatic activities. The results indicate an essential role of dynamic F-actin in structural organization of the cytoplasm within the bulge that is critical for the successful onset of the tip growth in root hairs.

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