Chapter 26

ACTIN AND MYOSIN VIII IN DEVELOPING ROOT APEX CELLS

Development- and tissue-specific distributions with possible relevance for diverse root cell functions

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Abstract: Root apices represent an ideal model object for studies on plant cell growth and development. We have exploited this opportunity for detailed analysis of the actin-based cytoskeleton in cells of various tissues throughout their cellular development. During mitosis, all root cells accomplish re-distribution of actin filaments (AFs) and myosin VIII molecules from the cytoplasm to the cell periphery where they accumulate at putative AF-organizing centres (AFOCs) facing the spindle poles. Postmitotic root cap cells differentiate first into gravity-sensing statocytes which are unique among postmitotic root cells due to the lack of any distinct cables of AFs. Later, statocytes, as well as peripheral cap cells, transform into secretory cells equipped with dense AF networks distributed throughout their cytoplasm. They retain abundant AFs after being shed from the root. Intriguingly, however, all root cap cells lack myosin VIII at their periphery. By contrast, all postmitotic cells of the root body, as they traverse the transition zone, show myosin VIII localized at their periphery. Myosin VIII localizes especially at the plasmodesmata in the non-growing cross walls. In cells of the transition zone, unique AF bundles develop which are proposed to participate in the onset of rapid cell elongation. These AF bundles are initiated at the nuclear peripheries and are organized via myosin VIII-enriched cross-walls, these two sites obviously act as the major AFOCs of postmitotic root-body cells. Treatment of roots with latrunculin B reveals that dynamic AFs are essential for the vacuome-driven cell elongation and for the root hair formation. In the transition zone and elongation region, cells of the inner cortex localize plant myosin VIII molecules abundantly at pit-fields. These distinct subcellular sites, similarly like cross-walls and root hair apices, represent powerfull AFOCs of root cells organizing abundant AFs.

1. INTRODUCTION

An actin cytoskeleton plays a central role in diverse intracellular processes, the list of which is far from complete (for recent reviews, see Volkmann & Baluška, 1999; Staiger, 2000; other chapters in this volume). One of the most typical role of the actin-based cytoskeleton is that its actin filaments (AFs) serve as tracks along which myosins (for plant cell myosin, see Yokota & Shimmen; and Reichelt & Kendrick-Jones, this volume) transport Golgi-based vesicles (Mollenhauer & Morré, 1976) and other diverse organelles, including small vacuoles (Verbelen & Tao, 1998), whole Golgi complexes (Boevink et al., 1998; Nebenführ et al., 1999), mitochondria (Olyslaegers & Verbelen, 1998) and endoplasmic reticulum (ER) elements (e.g., Kachar & Reese, 1988; Lichtscheidl et al., 1990; Knebel et al., 1990; Liebe & Menzel, 1995). In short, myosins are membrane-associated and actin-activated molecular machines which hydrolyse ATP and use its energy for driving intracellular movements along bundles of AFs (Titus, 1993; for plant cells, see Asada & Collings, 1997). Nevertheless, it is postulated that this picture reverses in situations where plant myosins are firmly anchored at the plasma membrane and their motor-activities are for dragging AFs along and beneath the plasma membrane. Actually, such a situation can occur experimentally *in vitro* when F-actin elements are induced to move on non-biological surfaces coated with myosins and myosin-containing extracts (Higashi-Fujime, 1991; Kohno et al., 1991; Shimmen & Yokota, 1994). This alternative scenario seems to be valid for the plant unconventional myosin VIII (Knight & Kendrick-Jones, 1993; Reichelt et al., 1997) which localises preferentially along cellular peripheries in the form of distinct dots (Reichelt et al., 1999; Volkmann & Baluška, 1999; Reichelt & Kendrick-Jones, this volume).

Movements of AFs beneath and along the plasma membrane, which is equipped with presumably anchored myosin VIII molecules (for the analogical situation with myosin I, see Zot et al., 1992), can be expected to exert local tension on the plasma membrane (e.g., Wolfe & Steponkus, 1993). Similarly, cytoplasmic strands are known to exert pulling forces on the plasma membrane causing its local distortion (Hahne & Hoffman, 1984). Such stretching of a stress/strain-loaded plasma membrane might indirectly stimulate the activity of stretch-activated ion channels (Lee et al., 1999; for plant cells, see Ding & Pickard, 1993; Garrill et al., 1996; Ramahaleo et al., 1996). Moreover, physical linkages between ion channels and elements of plasma-membrane-linked AF meshworks might also more directly control the activities of these channels (Watson et al., 1992; Cantiello & Prat, 1996; Prat et al., 1996; Hwang et al., 1997; Glogauer et al., 1998; Liu & Berg, 1999). As vacuome-driven plant cell elongation requires activities of both ion and water channels, one could propose a testable hypothesis: that actomyosin-based forces are essential for the execution of vacuome-driven

cell elongation through both direct and indirect impacts of actomyosin forces on ion channel activities.

Root apices represent a unique assemblage of different plant cell types encompassing diverse developmental pathways and contrasting cellular fates. The anatomical simplicity of root apices makes them one of the most useful experimental plant systems (e.g., Barlow, 1989, 1994; Barlow & Baluška, 2000; Silk, 1992). Moreover, the suitability of root apices for the Steedman's wax embedding technique (e.g., Baluška et al., 1992; Vitha et al., 2000; this volume) allows tissue- and development-specific visualization of actin and myosin VIII, as well as other antigens, using specific antibodies applied on sections taken from the whole root apex (Baluška et al., 1997a; Vitha et al., 1997, 2000; Reichelt et al., 1999). Most data shown or discussed in this chapter were obtained from maize root apices. However, similar observations were made also in cells of cress, alfalfa, rice, and Arabidopsis root apices (Vitha et al., this volume).

2. MITOTIC CELLS RE-DISTRIBUTE ACTIN AND MYOSIN VIII

Throughout interphase, the whole cytoplasmic interior is homogeneously pervaded by AFs. Dense AF networks are arranged as perinuclear baskets from which individual AFs extend towards the cell periphery and associate with the plasma membrane (Figs. 1, 2). In contrast, myosin VIII is present within the cytoplasm in low amounts only, whereas it is abundant at the cell periphery (Fig. 3), presumably in association with the plasma membrane (Reichelt et al., 1999; Volkmann & Baluška, 1999). This feature is less prominent in quiescent centre cells and their immediate descendents, as well as in all epidermis cells. The myosin VIII-positive dots at the cell periphery have been identified as plasmodesmata and pit-fields (Reichelt et al., 1999).

The above situation changes dramatically during mitosis when fine AFs extend from those peripheral sites, putative AF-organizing centres (AFOCs), which face towards the cytoplasmic domains that are adjacent to spindle poles (Figs. 1C-E). Myosin VIII is similarly restricted to the actin-enriched cell periphery where they form a prominent continuous labelling (Figs. 3C, D). This latter feature suggests that, besides plasmodesmata, other plasma membrane portions, and perhaps also plasmodesmata-associated cortical ER elements, recruit myosin VIII molecules released from the cytoplasm during mitosis. Mitotic re-organisation of AFs and myosin VIII, and especially the enrichment of actin and myosin VIII at cell-periphery domains facing spindle poles, appear to be involved in orienting, or anchoring, the mitotic spindles (Baluška et al., 2000). This enrichment of AFs at peripheral sites facing the spindle pole, and also the depletion of AFs at pre-prophase band-

marked domains, persist during cytokinesis, whereas myosin VIII re-distributes further and accumulates at the callosic cell plates during the final stages of cytokinesis (Reichelt et al., 1999; Volkmann & Baluška, 1999; Reichelt & Kendrick-Jones, this volume).

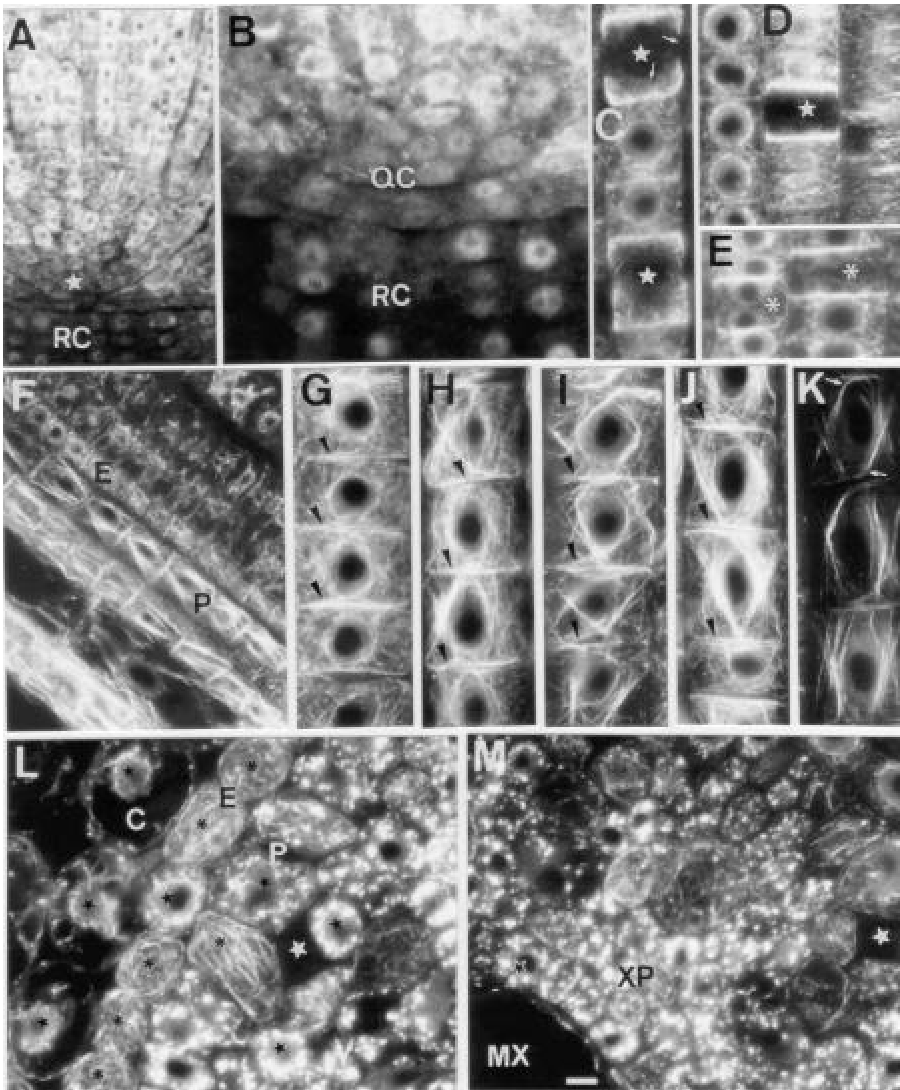

Figure 1. Actin cytoskeleton in diverse cell types in meristem and transition zone of maize root apices. A, B – Depletion of actin in cells of quiescent centre (QC) and root cap (RC). C-E – In the meristem, interphase cells show AFs organized as dense networks radiating from nuclear surfaces towards cell peripheries. This pattern changes dramatically in mitotic cells; actin becomes depleted from spindle regions (stars in C, D) and accumulates at cell-periphery domains facing spindle poles (lower arrow in C). In contrast, those cell periphery domains

which are pre-disposed for cell plate fusion are actin-depleted (upper arrow in C). This unique distribution of the actin cytoskeleton persists also during cytokinesis (asterisks in E). F – In the transition zone, actin is organized in the form of prominent bundles organized via putative AFOCs assembled at nuclear surfaces and cross-walls. This is especially prominent in cells of pericycle (P). Note the prominent actin in cells of the outer stele (lower part of F), whereas cells of inner cortex show less prominent actin cytoskeleton (upper part of F). G-K – Developmental sequence of actin bundle formation in cells of pericycle around centrally positioned nuclei and near cross-walls (black arrowheads in G-J). Note that, after leaving the nuclear region, bundles align along cross-walls (white arrows in K – this image is under-exposed to allow visualization of AF bundles alignment along the plasma membrane). L, M – Cross-sections through the transition zone of the root apex reveal abundant longitudinal (with respect of root axis) bundles around nuclei (black stars). Some cells are sectioned at their peripheries and so reveal cortical AFs near their plasma membranes which are arranged transversely to longitudinal AF bundles (black snowflakes). Note that vacuolated cells of the cortex have fewer AFs, while phloem (white stars in L and M) and metaxylem (MX in M) are almost devoid of AFs. RC – root cap, QC – quiescent centre, E – endodermis, P – pericycle, C – cortex, MX – metaxylem, XP – xylem parenchyma. Bar = 17 µm (A) and 7 µm (B-M).

3. ROOT CAP CELLS – GRAVIPERCEPTION VERSUS CELL SEPARATION

Among all root tissues, the root cap is the most intriguing one due to the progression of most of its cells through three distinct developmental stages which culminate in a dramatic disintegration event. After few mitotic cycles, the postmitotic root-cap columella cells rapidly develop into approximately isodiametric gravity-perceiving cells, also called statocytes. These cytoplasmically rich cells contain only few small vacuoles. Towards the exterior of the cap, the cells transform into elongated and more vacuolate secretory cells. These cells at the root cap periphery produce large amounts of extracellular mucilage which covers the whole root apex and performs multiple functions critical for root growth (Vermeer & McCully, 1982). Subsequently, development of root-cap cells terminates when they are shed from the cap periphery (e.g., Hawes et al., 1998). These specific cellular fates clearly distinguish root cap cells from all root body cells and are closely associated with unique re-organizations of the actin cytoskeleton.

The actin cytoskeleton is dramatically depleted from the cytoplasm of meristematic and early postmitotic root cap cells, as well as of quiescent centre cells (Figs. 1A, B). Interesting in this respect is that it is precisely these cells which show the highest endogenous levels of auxin (Sabatini et al., 1999). Statocytes are devoid of any AF bundles which makes them unique among all postmitotic root cells (Baluška et al., 1997b). Together with the absence of internal ER elements, this feature is critical in allowing free sedimentation of amyloplasts, or statoliths (Baluška & Hasenstein, 1997), which is the basis of plastid-mediated gravisensing (Sack, 1997).

Sedimented amyloplasts are enmeshed in a fine network of short AFs interlinking individual statoliths to each other as well as to the cortical ER elements and the plasma membrane. This highly specialised population of AFs is supposed to play a critical role in both the perception and transduction of gravity (Sievers et al., 1991; Volkmann et al., 1999; Volkmann & Baluška, 1999; Volkmann & Baluška, this volume).

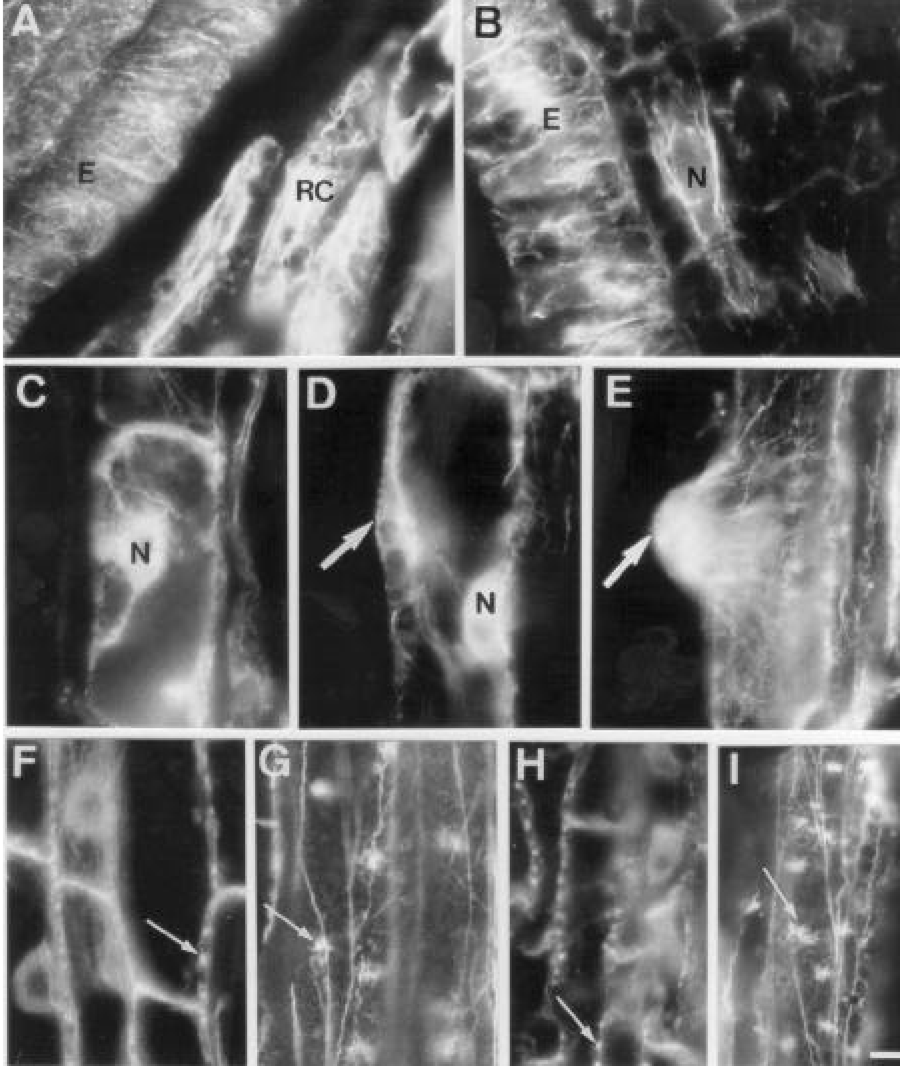

Figure 2. Distributions of AFs in elongating cells of maize root apices. A – Root cap cells (RC), when they are shed from the root periphery, are equipped with prominent AF bundles. In the root body, epidermis cells (E) show numerous cortical AFs arranged transversely with respect to the root axis. B – Transverse arrangement of of AF bundles is typical of epidermis

cells in the distal part of the transition zone. Adjacent outer cortex cells are already at the stage of early elongation and AF bundles 'embrace' the nuclei and anchor at cross-walls. C-E – Elongating trichoblasts re-arrange their actin cytoskeleton when the bulging domains (arrows in D and E) become the major AFOCs and attract most AFs. F-I – Pit-fields of the inner cortex (arrows) represent another powerful AFOCs of elongating cells, as revealed by jasplakinolide (10^{-5}, 4 h; F, G) and mild salt-stress (NaCl 200 mM for 1 h; H, I) treatments. Bar = 8 µm

In contrast, secretory cells are equipped with prominent networks of AFs and numerous ER elements, both of which support the cells' specialization for synthesis and secretion of root cap mucilage. Both statocytes and secretory root cap cells lack myosin VIII at their peripheries (not shown but see Volkmann & Baluška, this volume), and this feature seems to be relevant for the progression of the cells towards tissue disintegration. Intriguingly, cells shed from the root cap periphery retain abundant AFs, indicating their viability and active role in modulating the root-surface micro-environment (e.g., Vermeer & McCully, 1982; Hawes et al., 1998).

4. TRANSITION ZONE AS ACTOMYOSIN-BASED GROWTH REGION WHERE CELLS PREPARE FOR F-ACTIN DEPENDENT AND VACUOME-DRIVEN CELL ELONGATION

After leaving the apical meristem, root cells enter the transition growth zone which serves as some kind of preparatory region for their further growth via the vacuome-driven rapid cell elongation (Baluška et al., 1996a). Cells traversing the transition zone are, in many features, more similar to meristematic cells (although they do not divide any more) than to elongating cells (Baluška et al., 1994). Among these features, the most evident is that nuclei are still organizing active 'cell bodies' (Baluška et al., 1998). This is obvious from the approximately central positions of the nuclei which are supported by numerous cytoskeleton-based cytoplasmic strands, radiating from the nuclei towards the cellular peripheries. In contrast, the nuclei of cells performing vacuome-driven cell elongation are settled against the longitudinal (extending) cell walls. These nuclei are depleted of perinuclear AFs and microtubules, do not organize cytoplasmic strands, and represent inactive plant 'cell bodies' (Baluška et al., 1998). They can, however, be re-activated via either external signals (e.g., wounding – Mews et al., 1996) or internal signals (e.g., root hair initiation – Baluška et al., 1998, 2000a,b; and lateral root promordia).

Root cells located within the transition zone are unique with respect to their calcium and auxin responsiveness (Ishikawa & Evans, 1992, 1992;

Baluška et al., 1994). Moreover, transition-zone cells initiate diverse root tropisms (for root gravitropism see, e.g., Baluška et al., 1996b) and are the target for thigmostimulation and aluminium toxicity, both of which impinge on calcium- and auxin-related processes (Ishikawa & Evans, 1992, 1993; Sivaguru & Horst, 1998; Sivaguru et al., 1999; Kollmeier et al., 2000). The transition-zone cells are proposed to act as sensors of both environmental and developmental factors (Baluška et al., 1994, 1996a). They transmit information towards those endogenous processes which bring about vacuome-driven rapid cell elongation in an F-actin-dependent fashion (Baluška et al., 1997a; Volkmann & Baluška, 1999; Šamaj et al., 2000). In many situations, these responses can show different timings at the opposite sides of root apices. This induces differential cell growth in the apical part of the cell elongation region and results in a range of root tropisms (e.g., Baluška et al., 1994, 1996b). Actually, the above-mentioned root cap statocytes also pass through a transition phase of cellular development, as evidenced by their isodiametric shapes and the absence of a central vacuole. Thus, one could generalize that the transition phase of plant cell development is well suited for the sensing of diverse exogenous and endogenous factors, in both the root cap and the root body.

Postmitotic root body cells traversing the transition growth zone perform a prominent re-organization of the actin cytoskeleton with the perinuclear arrays of AFs transforming into axially arranged, thick bundles (Baluška et al., 1997a). The final outcome of this re-organization, which is characteristic in a more-or-less prominent form of all elongating plant cells (see below), is a population of longitudinally arranged AF bundles, which are anchored at non-growing cross walls (for elongating leaf cells, see Masuda et al., 1991; Ryu et al., 1995, 1997). The very first sign of this rather dramatic reconstruction of the actin cytoskeleton, which is prominent especially in the densely cytoplasmic cells of the outer stele, pericycle and endodermis, is a sudden proliferation of nuclear-envelope-associated AFs which then bundle together (Baluška et al., 1997a). These nucleus-based AF bundles apparently have an inherent polarity (see also Yokota & Shimmen, 1999) as they, after growing out from perinuclear sites and approaching the non-growing cross walls, exhibit contrasting architectures (for schematic overview see Fig. 4). One AF bundle end succeeds in interacting with the plasma membrane and then grows further along the plasma membrane. This anchors the whole AF bundle at a specific position. The opposite end of the AF bundle fails to interact with the plasma membrane and becomes clearly splayed in appearance. However, it might physically interact with endomembranes. This suggests that AF-bundling factors (Bartles, 2000; for plant cells see Vidali et al., 1999; Klahre et al., 2000) are either absent or not working properly at this splayed bundle end. Similarly splayed are F-actin tails of

propelling *Listeria monocytogenes* bacteria when the actin-binding protein, α-actinin, was depleted in an in-vitro movement assay (Loisel et al., 1999; Machesky & Cooper, 1999). Importantly, lateral surfaces of centrally positioned nuclei act as some kind of reference point for these AF bundles, which keep their nuclear contact areas intact, even after their ends approach the non-growing cross walls. Typically, four AF bundles are visible in 10-µm-thick median sections (for schematic overview of two such bundles see Fig. 4A). Altogether, our data identify nuclear surfaces and non-growing myosin VIII-enriched cross-walls as the major AFOCs of postmitotic cells of the transition zone. Further unconventional myosins were also proposed to anchor AFs at at the plasma membrane (for myosin I see Heintzelman & Mooseker, 1992; for myosin VI see Self et al., 1999).

One of the most characteristic features of these unique AF bundles is their straight appearance, suggesting that they are under tension. This feature is lost immediately after the onset of rapid cell elongation. The longitudinal AF bundles then acquire a crinkled appearances (Baluška et al., 1997a). This indicates that these AF bundles, and their interactions with endomembranes and the plasma membrane, are directly related to the developmental transition from the slow cytoplasmic growth to the rapid, vacuome-driven cell elongation. One possibility is that both ends of these nucleus-associated AF bundles are growing, and, after their approach to the cross walls, they exert a pushing forces on the plasma membrane (reviewed by Borisy & Svitkina, 2000). In addition, one end of these bundles laterally associates, perhaps via membrane-associated myosin VIII molecules, with the plasma membrane. The combined actin polymerization and actomyosin-based forces might thus contribute to a mechanical stretching of the plasma membrane. As root cells traverse the transition zone, the tensional stress imposed on the plasma membrane is expected to increase gradually. Our working hypothesis is that after reaching a critical threshold value, putative stretch-activated channels are switched-on, allowing an influx of osmotically-active ions and water molecules which initiate and drive rapid cell elongation. Due to this feature, the vacuome-driven rapid cell elongation starts suddenly (Kubica et al., 1991; Ivanov & Maximov, 1999). The pushing forces of the actin nano-machinery (see Borisy & Svitkina, 2000) may also help overcome the yield threshold of cell growth (i.e., the variable, Y, in the Lockhart growth equation; see Barlow, 1989).

Latrunculins represent a new generation of powerful actin drugs which bind to G-actin monomers, hence precluding their polymerization into F-actin (for plant cells, see Gibbon et al., 1999). Due to the natural dynamicity of AFs, latrunculins rapidly ‘precipitate’ the whole cellular complement of G-actin, leading to total disintegration of AFs. This property makes these drugs for ideal tools to reveal which processes are F-actin dependent and, by

contrast, which proceed unhindered in the absence of F-actin arrays. Our experiments have shown that exposing growing maize root apices to latrunculin B for 2 h increases the number of cells in the transition zone, indicating an F-actin dependence for the onset of vacuome-driven cell elongation. After a longer (12 h) absence of F-actin, root cortex cells fail to execute rapid cell elongation, although cell divisions continue. As a consequence, the whole of the former elongation region becomes filled with short cells. Similar, but milder, effects were induced with the general inhibitor of myosin motor activity, 3,4 butanedione monoxime (see Baluška et al., 1997a; Volkmann & Baluška, 1999; Šamaj et al., 2000), indicating that, besides actin polymerization, actomyosin activities are also involved in the initiation and execution of vacuome-driven cell elongation.

5. ASSOCIATION OF MYOSIN VIII AND ACTIN WITH PIT-FIELDS IN ELONGATING INNER CORTEX CELLS

In contrast to cells of the meristem and transition zone, which have myosin VIII associated preferentially with their peripheries, elongating cells have most of their myosin VIII molecules localized in the cytoplasm. The only exceptions to this are the pit-fields of the inner cortex cells which show abundant myosin VIII at their side walls in the transition zone and apical part of the elongation region. As phloem unloading is accomplished in these root zones, one interpretation of this might be that plasmodesmata-associated myosin VIII plays some role in symplasmic transport (see Overall et al, this volume). For instance, those plasmodesmata which are expected to be more active in symplasmic transport of sucrose into the meristem also show more myosin VIII associated with them. The most dramatic examples of this phenomenon are the myosin VIII-enriched sieve plates of phloem (Fig. 3O). Moreover, all root cortex cells accumulate abundant myosin VIII at their plasmodesmata/pit-fields in root apices exposed to compacted soil (Baluška, Bengough, Volkmann, in preparation). These root apices exhibit increased lateral forces, generated via excessive vacuome formation based on an additional unloading of osmotically active sucrose from phloem elements which helps to form cracks within compact soil.

Although myosin VIII-rich pit-fields of the inner cortex cells do not show abundant AFs using the ICN antibody (clone C4), AFs can be visualized with this antibody at these pit-fields after exposing the roots to mild osmotic stress (200 mM NaCl for 1 h), or to mastoparan (data not shown) and jasplakinolide treatments. However, a polyclonal antibody raised against maize pollen actin (gift of Chris Staiger) recognizes AFs at pit-fields also in

the inner cortex of control root apices (Baluška, preliminary data). All these findings identify the plasmodesmata and pit-fields as potential AFOCs.

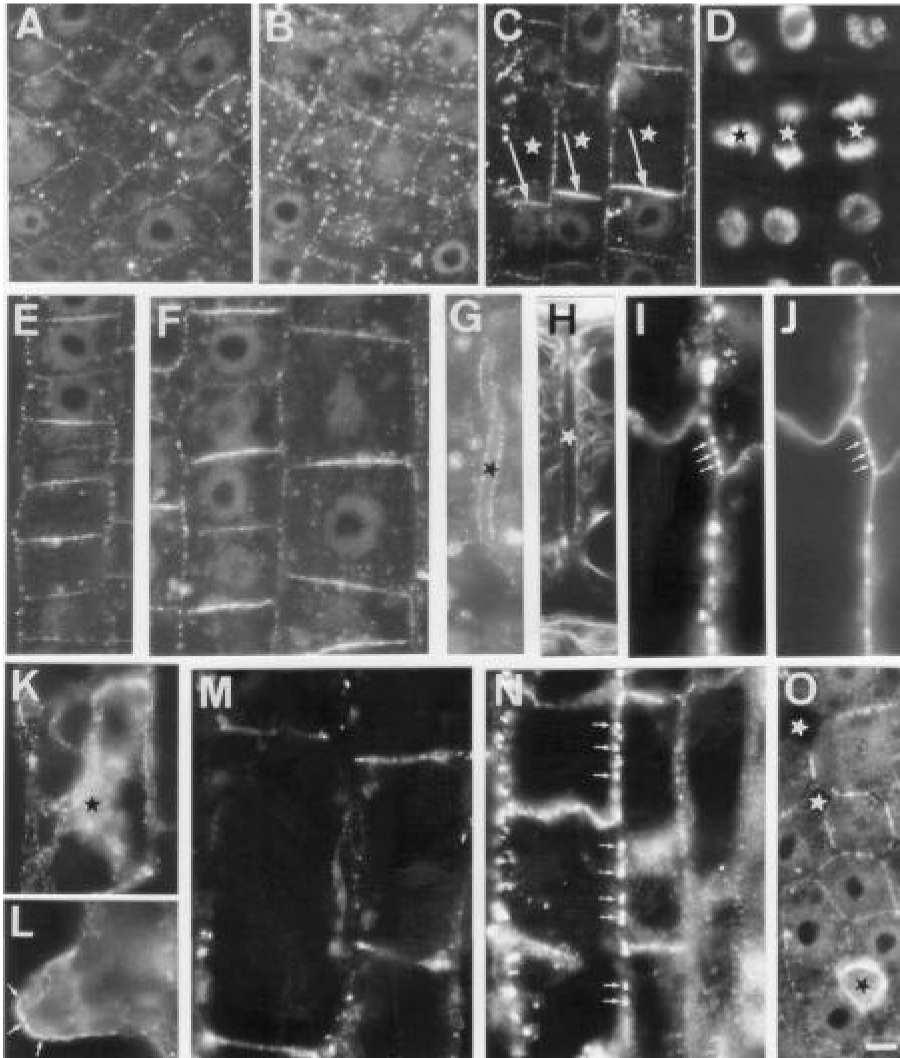

Figure 3. Localization of myosin VIII in cells of maize root apices. A-D – Meristematic cells just behind the quiescent centre (A) show numerous fluorescent dots at cellular peripheries and less fluorescence in the cytoplasm. Further back from the root tip (B), the size of these peripheral dots increases while mitotic cells (stars in C and D) accumulate abundant myosin VIII at cell walls facing the spindle poles (arrows in C and D). In the transition zone, cross-walls of inner cortex cells (E and F) show continuous labelling while side-walls are decorated with myosin VIII-positive dots. G, H – Myosin VIII-positive dots (G) and actin (H) at the cross-wall (star) of a metaxylem cell. I, J – In elongating inner cortex cells, myosin VIII-positive pit-fields (arrows in I) are enriched with callose (arrows in J), as identified by aniline blue staining. K – Abundant distributions of myosin VIII in postmitotic epidermal cells (star

indicates position of nucleus). L – Myosin VIII accumulates at the plasma membrane of emerging root hair apices (arrows). M – In the elongation region, outer and middle cortex cells show only faint labelling with myosin VIII antibody. N – In the inner cortex, elongating cells accumulate abundant myosin VIII at pit-fields (arrows). O – Cross-section showing that myosin VIII localizes at cell-cell contacts while it is depleted from cell peripheries facing extracellular spaces (white stars). Abundant myosin VIII is associated with prospective phloem elements (black star). Bar = 8 µm

6. TRICHOBLASTS: ROOT HAIR FORMATION IS F-ACTIN DEPENDENT

Of all the elongating cells of the root, those of the epidermis have the most abundant AFs (Baluška et al., 1997a, 2000b). This feature corresponds well with the dramatic increase of cytoplasmic streaming in prospective trichoblasts, which is one of the first signs of root hair initiation (Baluška & Lichtscheidl, unpublished). During the early bulge outgrowth, longitudinal AF bundles re-organize and, simultaneously, an actin-rich domain is assembled in the vicinity of the prospective bulge (Fig. 2E; Baluška et al., 2000). At this early stage of root hair formation, trichoblast AF cables become oriented transversely with respect to the root axis (Miller et al., 1999; Braun et al., 1999; Baluška et al., 2000) and are typically associated with the nuclei moving into the bulge (Baluška et al., 1998, 2000). This subcellular domain ultimately transforms into the tip-growing root-hair apex and it represents a powerful AFOC. It not only attracts to itself most of the newly assembled AF cables (Fig. 2E; Baluška et al., 2000) but it also obviously polarize the whole exocytosis apparatus. From the ‘cell body’ perspective, tip-growing root hairs can be considered as active plant ‘cell bodies’ (Baluška et al., 1998) polarized by the actin-rich cell cortex domains of the root hair tips (Braun et al., 1999; Baluška et al., 2000) which acts as a potent AFOCs.

In addition to vacuome-driven rapid cell elongation, root hair formation has been identified as another F-actin-dependent growth process (Baluška et al., 2000). In the absence of AFs, root hair formation is stopped after the bulge formation, indicating that this latter event results perphaps from localized cell wall loosening (discussed in Baluška et al., 2000). Analysis of living, latrunculin-B treated trichoblasts during the initiation of hairs has revealed an instantaneous vacuolation of the cytoplasmic bulges (Ovecka et al., 2000) which then failed to initiate tip growth. Moreover, an Arabidopsis root-hair mutant which is unable to transform the outgrowing bulges into tip-growing root hairs has been cloned and identified as defective for a potassium ion channel (Liam Dolan, personal communication). Both these findings indicate a very close relationships between the actin cytoskeleton

and vacuome formation, supporting the notion that both rapid cell elongation and tip growth depend on intact F-actin for extensive vacuome formation. Intriguingly, most of morphogenetic processes proceed normally during the long-term absence of F-actin: for example, seed imbibition and germination in the presence of latrunculin B ultimately results in dwarf seedlings which resemble various genetic dwarfs of *Arabidopsis* (Baluška, Jásik, Edelmann, Salajová, Volkmann, submited).

7. ACTIN FILAMENT ORGANIZING CENTRES

Our above data strongly implicate the existence of putative AFOCs in plant cells. Distinct domains at the nuclear envelope and plasma membrane obviously support AF assembly. Among these, we can mention plasma membrane domains facing spindle poles in mitotic cells, cross-walls in the postmitotic cells of the transition zone (Fig. 4C) and of the elongation region (Fig. 4B, C), as well as bulging domains and apices of root hairs. In addition, also myosin VIII enriched pit-fields of longitudinal walls of the inner cortex cells behave as AFOCs (Fig. 4B).

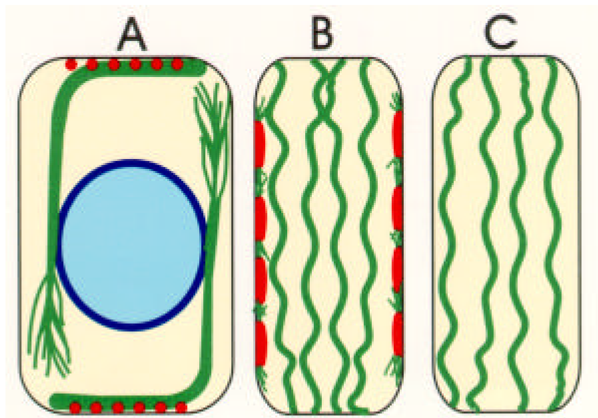

Figure 4. Schematic overview of distributions of AFs (green) and myosin VIII (red) in postmitotic maize root cells. A – Cells of the transition zone develop straight AF bundles which attach laterally to nuclear surfaces and are symmetrically organized from both cross-walls thus matching the growth axes of the cell and of the root. These AF bundles show an inherent polarity: while one end of a bundle is anchored at myosin VIII-enriched cross-walls, the other end, with a loosely-splayed configuration, resides in the cytoplasm. For simplicity, only two such AF bundles are depicted (compare with Fig. 1K), but up to eight bundles may develop in any one cell. B, C – More proximal, elongating cells are equipped with undulating, axially arranged AF cables. These are typical of elongated plant cells. In the case of inner cortex cells (B), myosin VIII-enriched pit-fields (red structures) act as further AF-organizing sites. Other elongating cells (C) lack such myosin-enriched sites.

As cross-walls are equipped with abundant primary plasmodesmata, one could speculate that plasmodesmata, irrespective if primary or secondary ones, are relevant for the organization of AFs. In accordance with this notion, both plasmodesmata and pit-fields are known to be depleted in cortical microtubules (reviewed by Baluška et al., 2000) and are enriched with myosin VIII (Reichelt et al., 1999; Reichelt & Kendrick-Jones, this volume). Studies on yeast and animal cells identified several candidates for putative members of AFOCs (e.g. Shariff & Luna, 1992; Miki et al., 1996; Frazier & Field, 1997; Moreau & Way, 1998; Machesky & Gould, 1999; Yuan & Chia, 1999; Suetsugu et al., 1999; Defacque et al., 2000; Raucher et al., 2000). Our present data on root cells indicate that the unconventional myosin VIII might be involved in rendering AFOC-like properties to discrete portions of the plasma membrane in root cells. This is in accordance with data from animal cells identifying unconventional myosins as molecules regulating AF assembly and organization (reviewed by Soldati et al., 1999; Wu et al., 2000). Moreover, preliminary data imply putative plant homologues of Arp2/3 proteins as further AFOC-relevant components (see Machesky & Gould, 1999) of myosin VIII-enriched plasmodesmata and pit-fields (Baluška, von Witsch, Šamaj, Volkmann, in preparation).

8. CONCLUSIONS

The actin cytoskeleton has been analyzed in cells of all root apex tissues throughout their diverse pathways of cellular development. For this, we applied indirect immunofluorescence, using ICN actin antibody (clone C4), to Steedman's wax sections taken from whole root apices. In combination with conventional epifluorescence microscopy, this powerful technique allows simultaneous viewing of all root apex cells arranged within the context of the three-dimensionally organized tissues. This is critical for analysis of tissue- and development-specific organisations of AFs and to assess their relationships to subcellular distributions of myosin VIII.

Our analysis allows us to reach seven tentative conclusions, listed below, which pave solid avenues for further illumination of the involvement of AFs and actomyosin-based forces in plant cell growth and development.

1. Unique organizations of the actin cytoskeleton are typical for cells of diverse tissues and for various phases of cellular development.
2. During mitosis, AFs become depleted from spindle regions and from pre-prophase band-marked cortical domains, and are recruited to cell cortex domains facing the spindle poles. The same redistribution pattern is true also for the unconventional myosin VIII.

3. Dramatic re-organization of AFs is accomplished in postmitotic root-body cells of the transition zone in preparation for their rapid cell elongation. Here, perinuclear AF networks transform into unique AF bundles.
4. In meristematic and transition zone cells, myosin VIII molecules are localized preferentially at plasmodesmata and pit-fields. In the elongation region, myosin VIII abundantly localizes to pit-fields only in the inner cortex cells. In contrast, all root cap cells lack myosin VIII at their peripheries.
5. Latrunculin B efficiently depolymerizes all AFs in root apices. Root cells devoid of F-actin continue to divide, but then fail to accomplish the rapid cell elongation.
6. Roots devoid of F-actin do not form any root hairs, although the first stage of root hair formation, the bulge outgrowth, proceeds normally.
7. Distinct subcellular domains of the nuclear envelope and the plasma membrane act as AFOCs in root cells. Plant molecules responsible for this domain-specific organization of AFs await identification but our data suggest that the plant unconventional myosin VIII is a strong candidate for this role.

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