Conservation of boundary extension mechanisms between plants and animals

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Locomotion clearly sets plants and animals apart. However, recent studies in higher plants reveal cell-biological and molecular features similar to those observed at the leading edge of animal cells and suggest conservation of boundary extension mechanisms between motile animal cells and nonmotile plant cells.

Boundary extension: a necessity for living organisms

Survival requires a constant refurbishment of resources. Therefore, living organisms persistently explore their environment for extending their boundaries into favorable regions. One fundamental difference we are taught early on between motile animal and nonmotile plant cells is the way in which they carry out their boundary extension. Motile cells achieve whole-body displacement or locomotion, which allows them to leave resource-depleted domains behind while moving on to new areas. Plant cells, however, become rooted to a spot and are only able to extend outwards from it in response to different stimuli.

Two major conditions have to be met for achieving locomotion; a flexible bounding layer of the cell, which allows for rapid change in its shape, and the ability to retract one end of the cell while extending the other (Small et al., 2002a). Plant cells do not fulfill these requirements because they are encased in a relatively rigid cellulosic cell wall and, clearly, they do not have rear-end retractability. However, the cell wall of actively expanding plant cells is actually quite labile in contrast to its later, rigid nature in a mature, fully expanded cell (Mathur, 2004). Further, though lacking rear-end retractability, the forward extension of a plant cell, like that of an animal cell, also involves membrane protrusion (Vidali et al., 2001).

Recent cell-biological and molecular evidence from higher plants suggests that, despite their sessile and wall-encased nature, plant cells possess a core machinery similar to the one required for forward motility of animate cells.

## Boundary extension requires a leading edge

Although usually used in the context of crawling amoeboid locomotion, here I use the term "leading edge" for both animal and plant cells as the part of a cell that extends or "leads."

Though descriptions vary from cell to cell, the generalized leading edge of an amoeboid cell comprises a 2–5-μm-wide veil-like, organelle free, cytoplasmic extension called the lamellipodium. The leading edge has an actin-rich zone comprising of a fine F-actin mesh followed by a microtubule-rich region (Etienne-Manneville, 2004) (Fig. 1 A). A few pioneering microtubules do extend into the F-actin mesh (Small et al., 2002b; Raftopoulou and Hall, 2004).

A very similar intracellular zonation is seen in plants (Fig. 1, B–D; Mathur, 2004). For plant cells, two kinds of expansion modes are recognized; one called tip-growth, where the growth processes is limited to a small region that extends to form a tubular structure, and the second, designated diffuse growth, where the process of growth is dispersed over a large area of the cell (Mathur, 2004). Tip-growing cells (Fig. 1, B and D) are best typified by elongating root-hair and pollen tubes and, like lamellipodia, exhibit an apical region with a stretched plasma membrane, followed by a organelle-depleted clear zone (Vidali and Hepler, 2001). A fine, labile F-actin zone is defined next and leads into a dense F-actin region where the filaments become progressively bundled (Fu et al., 2001; Ketelaar et al., 2003). In active tip-growing cells, most cytoplasmic microtubules extend only to the edge of the fine F-actin mesh (Sieberer et al., 2002). A large vacuole fills the rest of the cell (Carol and Dolan, 2002). By comparison, the volume occupied by vacuoles is quite large in diffuse-growing cells so that the cytoplasm is compressed into a thin layer against the plasma membrane (Fig. 1, B and C; Mathur et al., 2003a,b). Although this thin cytoplasmic layer has not allowed intracellular zonation in these cells to be appreciated as clearly as in tip-growing cells, a fine cortical mesh, underlying cytoplasmic F-actin bundles (Fu et al., 2002; Mathur et al., 2003b), and microtubules (Saedler et al., 2004a) observed in different diffuse-growing cells suggest a very similar zonation (Fig. 1 C). The apparent similarity in zonal relationship between the plasma membrane and cytoskeletal elements in the region of active protrusive growth between animal and plant cells (Fig. 1) extends to the molecular mechanisms responsible for the creation of a leading edge.
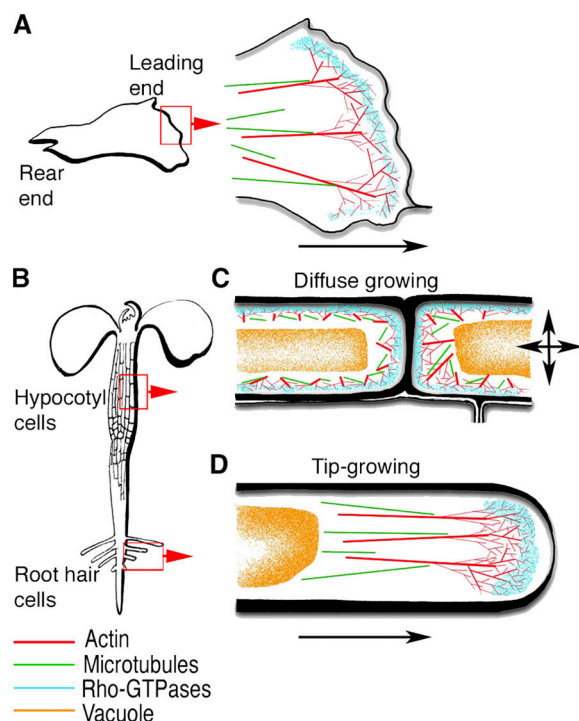
Figure 1. **Schematic depiction of similar intracellular zonation at the leading edge of animal and plant cells.** (A) A generalized motile animal cell with a rear end and leading edge. Enlarged view of the lamellipodium displays an organelle-free area near the absolute edge, followed by an actin-rich zone with dynamic, rapidly polymerizing actin. The microtubular zone usually lies behind the actin-packed zone, though pioneering microtubules make their way into the actin zone. Arrow shows the direction of membrane extension. (B) A seedling depicting two of the regions where "diffuse"-growing cells, such as hypocotyl cells and "tip"-growing cells such as root hairs are found. (C) Plant cells exhibiting diffuse growth have large expanding vacuoles that press the cytoplasm into a thin layer against the plasma membrane. A fine F-actin meshwork lying below the plasma membrane is followed by cytoplasmic microtubules on its inner side. Plant cell–specific cortical microtubule arrays have not been shown. Arrows suggest the multidirectional, diffuse nature of cell expansion. (D) Plant cells that extend by tip-focused growth are characterized by an apical accumulation of vesicles in an organelle-free zone. A fine actin meshwork with its distal region interspersed by dynamic microtubules follows. A vacuole occupies the rest of the nongrowing, mature part of the tubular cell. Arrow shows the direction of membrane extension. Note that the conserved region (blue speckled) of Rho-GTPase/actin interaction leading to a fine F-actin mesh in each cell type has been based on the localization of Rac in live animal cells (Kraynov et al., 2000) and AtROP localizations for different plant cells (Fu et al., 2001, 2002; Molendijk et al., 2001; Jones et al., 2002).

Creating the leading edge

A considerable body of information exists about the multiple protein complexes and regulatory molecules that get activated to create the leading edge in an animal cell (Kraynov et al., 2000; Pollard and Borisy, 2003; Rodriguez et al., 2003). Three key interacting components—members of the Rho/rac/Cdc42 superfamily of GTPases, and actin and microtubule cytoskeletons—stand out. Although Rho-GTPase loops interact with and regulate both actin and microtubule cytoskeletons and microtubules provide directionality to the leading edge, it is actin dynamics that play a major role in membrane protrusion (Small et al., 2002b; Pollard and Borisy, 2003). One of the proposed pathways for actin–cytoskeleton regulation

in animal cells involves Cdc42/Rac-GTPase–triggered activation of a suppressor of cAMP receptor from *Dictyostelium* (SCAR)/Wiskott-Aldrich syndrome protein family verprolin-homologous protein (WAVE) complex. This complex in turn activates a 7-subunit actin-related protein (ARP2/3) complex to enhance actin polymerization (Millard et al., 2004; Vartiainen and Machesky, 2004). The final outcome is a fine dendritic mesh of filamentous actin seen at the leading edge (Fig. 1).

Plants, too, possess a unique subfamily of Rho-family GTPases, called Rho-related GTPase of plants (ROP; Vernoud et al., 2003), whose members localize to areas of active growth (Fu et al., 2001, 2002; Fig. 1, C and D) and play numerous roles, including that of actin–cytoskeleton regulation (Gu et al., 2004). Further, different components of a putative SCAR/WAVE-like complex (Millard et al., 2004; Vartiainen and Machesky, 2004), namely SCAR-related proteins (Frank et al., 2004), NAP125/GNARLED (Brembu et al., 2004; Deeks et al., 2004; El-Din El-Assal et al., 2004; Li et al., 2004; Zimmermann et al., 2004), PIR121/KLUNKER/PIROGI (Basu et al., 2004; Brembu et al., 2004; Li et al., 2004; Saedler et al., 2004b), HSPC300/BRICK1 (Frank and Smith, 2002), and Ab1-1–like proteins (Deeks et al., 2004), have been cloned from *Arabidopsis*. At least one ROP, AtROP2, has been shown to interact with PIR121, strongly suggesting that a SCAR/WAVE-like pathway mediates the ROP–actin interaction in plants (Basu et al., 2004). Finally, homologues of the different subunit of the ARP2/3 complex have been cloned from plants and in some cases shown to be interchangeable with their animal orthologues (Le et al., 2003; Li et al., 2003; Mathur et al., 2003a,b; El-Assal et al., 2004; Saedler et al., 2004a).

In animal cells, changes in actin-mesh density are believed to affect microtubule plus-end growth by removing a stearic hindrance that allows cytoplasmic microtubules localized access to cortical domains (Rodriguez et al., 2003). In plants, studies on actin–microtubule interactions are just beginning (Mathur, 2004). However, observations of an aberrant aggregation and stabilization of cytoplasmic microtubules in actin-compromised cells suggests an actin control over microtubules very similar to that observed in animal cells (Saedler et al., 2004a). Alternatively, microtubule control over the actin cytoskeleton has also been demonstrated in *Drosophila* by providing evidence that EB1, a microtubule plus end–binding protein, mediates the delivery of DRhoGEF2, an activator of Rho1 to cortical domains for its actomyosin-related functions in cell retraction (Rogers et al., 2004). Though not yet demonstrated, similar microtubule–ROP interactions could be envisaged in plants too, as several microtubule plus end–binding proteins (Bisgrove et al., 2004) as well as a putative ROP-GEF, *SPIKE1*, have been cloned from *Arabidopsis*. Interestingly, cells of the *spike1* mutant display an aberrant microtubule cytoskeleton (Qiu et al., 2002).

Numerous other modulators (Table I) involved in fine-tuning of the core elements and their interactions appear to be conserved between animals and plants (Goode et al., 2000; Rodriguez et al., 2003; Wasteneys and Yang, 2004) (Table I).

Table I. **Some of the key molecular players at the leading edge of animal cells and their counterparts in plants**

Animal protein/complex	Plant protein/complex
Rho family GTPases	11 ROPs in *Arabidopsis* (Vernoud et al., 2003); putative ROP-GEF (Qiu et al., 2002); multiple GAP, GDI, and novel ROP effectors (Gu et al., 2004)
Microtubule +Tip proteins	Numerous—such as EB1-like, SPIRAL1 (Bisgrove et al., 2004)
SCAR/WAVE	SCAR-related in *Arabidopsis* (Frank et al., 2004)
NAP125	GNARLED (for review see Mathur, 2005)
PIR121	KLUNKER/PIROGI (for review see Mathur, 2005)
HSPC300	BRICK1 (Frank and Smith, 2002)
Abi-1	Four predicted proteins in *Arabidopsis* (Deeks et al., 2004)
ARP2/3 complex	Different subunit homologues present (for review see Mathur, 2004, 2005)
Formin(s)	Numerous formin-like (Deeks et al., 2002)
Profilin(s)	Numerous profilin genes (Vidali and Hepler, 2001)
ADF(s)	Numerous (Hussey et al., 2002)
Capping protein	Identified in *Arabidopsis* (Huang et al., 2003)
Actin-bundling proteins	Villins and fimbrins (for review see Wasteneys and Yang, 2004)

ADF, actin depolymerizing factor; EB1, microtubule plus end–binding protein; ROP, Rho-like proteins of plants; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor.

Defects at the leading edge: mutant phenotypes

Compromised function of the three key molecular components, as mentioned in the previous section, has major implications for both motility and cell shape. A brief comparison of cellular phenotypes resulting from defects in the core elements underscores the commonalities. Actin-dependent lamellipodium protrusion and cell motility get greatly attenuated in animal cells with an aberrant actin cytoskeleton (Etienne-Manneville, 2004). Likewise, cell expansion is significantly reduced in actin-compromised plant cells (Baluska et al., 2001). This is especially apparent in *Arabidopsis* mutants such as *nap125/gnarled*, *pir121/klunker/pirogi* (Brembu et al., 2004; Deeks et al., 2004; El-Assal et al., 2004; Li et al., 2004; Saedler et al., 2004b; Zimmermann et al., 2004), and *hspc300/brick1* (mutant in maize; Frank and Smith, 2002), whose respective gene products possibly feed into the ARP2/3 complex regulatory pathway. Mutations in different subunit homologues of the putative plant ARP2/3 complex such as *arp2/wurm*, *arp3/distorted1*, *arpc2/distorted2*, and *arpc5/crooked* also exhibit similar phenotypes (for review see Mathur, 2005). Each of these mutants exhibits aberrant F-actin organization and characteristic cellular deformations resulting from abnormal, random expansion. However, as mentioned earlier in this paper, actin cytoskeleton activity is heavily dependent upon the stimulation provided by Rho-GTPases. Animal cells with aberrant Rho-GTPase activity display numerous defects in pseudopod extension and chemotaxis (Chung et al., 2000; Raftopoulou and Hall, 2004). Overexpression of ROPs in plants results in increased fine F-actin meshworks and a swollen cell morphology, suggesting an overall reduction in growth polarization (Molendijk et al., 2001; Fu et al., 2002; Jones et al., 2002). Because loss of polarity is a characteristic feature of microtubule defective cells too, in both animal (Small et al., 2002b) and plant cells (Mathur, 2004) these observations again point to a conserved, intimate relationship between Rho-family GTPases, and the actin and microtubule cytoskeletons.

Although the similarities described above emphasize conserved mechanisms for membrane protrusion between animal and plant cells, they also reflect the profound impact the mechanism of boundary extension has on the survival ability of an organism. For motile cells, loss of motility leads to an inability to move to resource-rich areas and ultimately leads to death. Consequently, many animal mutants for the core elements display lethal phenotypes. For the nonmotile but photosynthetic (and therefore relatively self-sufficient) plant cells, a change in cell shape does not lead to death directly. As a result, many comparable plant mutants, though misshapen and underweight (El-Assal et al., 2004; Li et al., 2004), are able to grow and complete their life cycle, giving the erroneous impression that the core genes are not as vital for plants as for animals. However, when grown in a population of siblings, the inability of mutant plants to extend properly becomes a severe handicap that affects their survival ability.

Conclusions and perspectives

A comparison of key cell biological and molecular features in two apparently disparate processes, namely, forward motility of animal cells and extension growth of plant cells, reveals a core machinery that has been conserved by both motile and nonmotile life forms for achieving boundary extension; an essential requirement for survival. Given this realization, future studies on boundary extension mechanisms of different life forms should prove exciting, not only for uncovering the extent of similarities, but also for discovering the range of cell biological and molecular variations, and the adaptations and innovations introduced by different organisms for surviving in their specialized niches.

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