Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Plant Biology

Actin dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game

Laurent Blanchoin[1], Rajaa Boujemaa-Paterski[1], Jessica L Henty[2], Parul Khurana[2] and Christopher J Staiger[2,3]

Gazing at a giant redwood tree in the Pacific Northwest, that has grown to enormous heights over centuries, does little to convince one that plants are built for speed and versatility. Even at the cellular level, a system of polymers — the cell skeleton or cytoskeleton — integrates signals and generates subcellular structures spanning scales of a few nanometers to hundreds of micrometers that coordinate cell growth. The term cytoskeleton itself connotes a stable structure. Clearly, this is not the case. Recent studies using advanced imaging modalities reveal the plant actin cytoskeleton to be a highly dynamic, ever changing assemblage of polymers. These insights along with growing evidence about the biochemical/biophysical properties of plant cytoskeletal polymers, especially those obtained by single filament imaging and reconstituted systems of purified proteins analyzed by total internal reflection fluorescence microscopy, allow the generation of a unique model for the dynamic turnover of actin filaments, termed stochastic dynamics. Here, we review several significant advances and highlight opportunities that will position plants at the vanguard of research on actin organization and turnover. A challenge for the future will be to apply the power of reverse-genetics in several model organisms to test the molecular details of this new model.

Addresses
[1] Institut de Recherches en Technologies et Sciences pour le Vivant – iRTSV, Laboratoire de Phyiologie Cellulaire et Végétale, Commissariat à l'Energie Atomique /Centre National de la Recherche Scientifique/ Institut National de la Recherche Agronomique/Université Joseph Fourier, CEA Grenoble, F38054, Grenoble, France
[2] Department of Biological Sciences, Hansen Life Sciences Research Building, Purdue University, West Lafayette, IN 47907-2064, USA
[3] The Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA

Corresponding authors: Blanchoin, Laurent (laurent.blanchoin@cea.fr) and Staiger, Christopher J (staiger@purdue.edu)

Current Opinion in Plant Biology 2010, **13**:1–10

This review comes from a themed issue on
Cell biology
Edited by Christian Luschnig and Claire Grierson

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2010.09.013

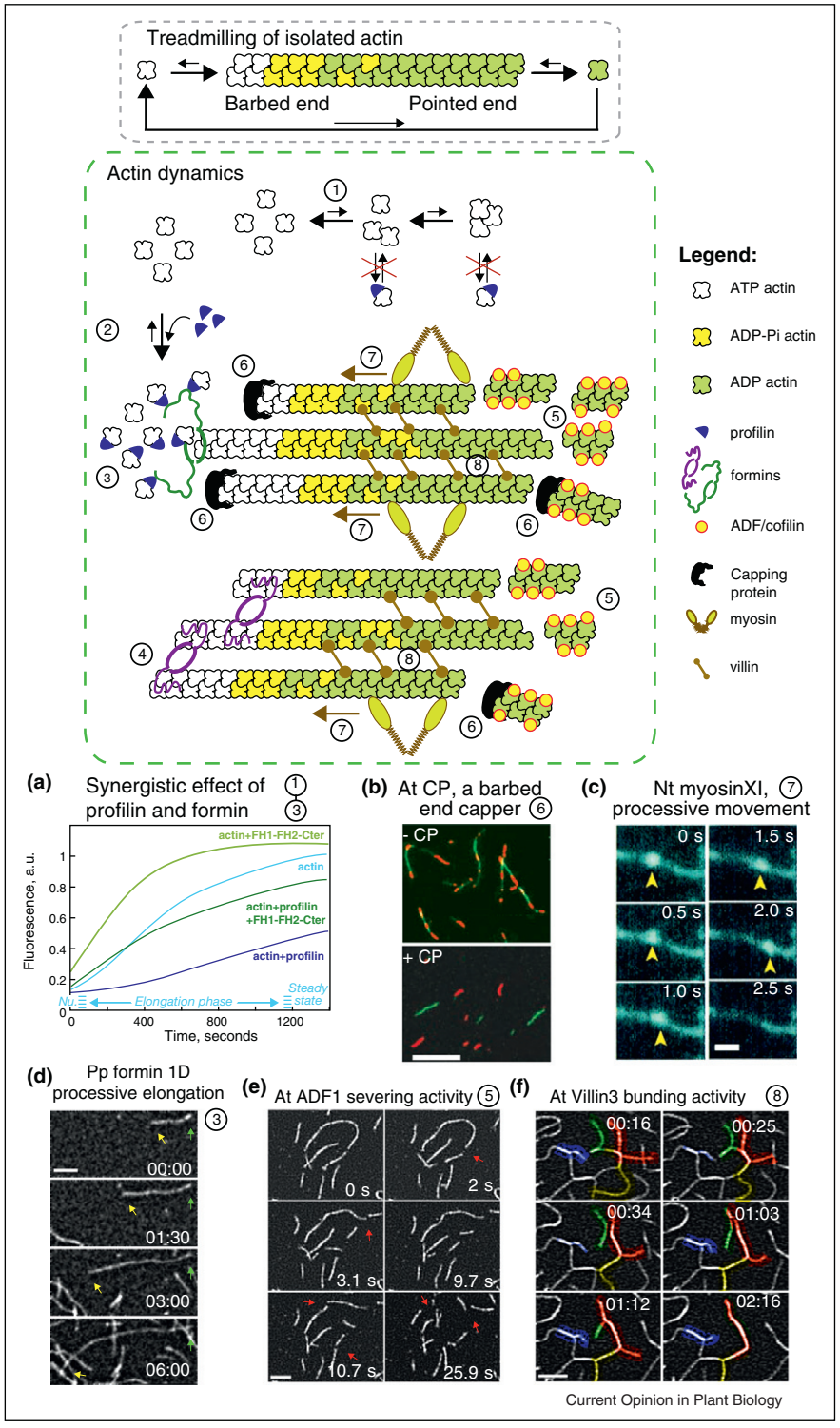
Actin dynamics are well understood from *in vitro* analyses

In eukaryotes, the actin cytoskeleton is an essential molecular machine that creates structures and generates forces that support a diverse array of cellular functions, including morphogenesis, establishment of polarity and motility [1,2]. Powering these cellular functions often depends on the ability of the cytoskeleton to auto-organize and modulate its dynamics to create novel and often transient higher-order structures. In plant cells, responses to hormones or to attack by micro-organisms, along with cell morphogenesis pathways, induce signaling cascades that correlate with the rearrangement or turnover of actin-based structures [3,4].

Actin dynamics consist of the assembly and disassembly of a helical polymer, or 'actin filament', with mechanical properties that are tuned for its specific cellular functions [5]. Assembly of actin filaments occurs through two fundamental steps, called nucleation and elongation (Figure 1, green box; [6]). Nucleation is a set of reactions involving the association of actin monomers into dimers, followed by trimer formation (Figure 1, Step 1). These trimers are thermodynamically unstable, but provide the 'seeds' or nuclei necessary for actin filament assembly [7]. Certain accessory proteins, like profilin, potently inhibit spontaneous nucleation of actin ([3,8]; Figure 1, Step 1). Following this nucleation step, the elongation of actin trimers occurs rapidly at a rate that depends on the actin monomer concentration and the particular end of the actin filament at which these monomers are added. Indeed, the two ends of actin filaments are not equivalent; for historical reasons, they are referred to as 'barbed' and 'pointed'. In the absence of regulatory proteins, growth at the barbed ends of actin filaments is up to 10 times faster than at the pointed ends [9]. However, this difference can climb up to 100 times in the presence of proteins that accelerate actin assembly, such as formins (Figure 1, Step 3; [10,11,12•]). Following elongation, aging of actin filaments is controlled by the hydrolysis and phosphate release of the nucleotide bound to actin subunits [7]. At steady state *in vitro*, the concentration of actin monomers reaches a critical concentration that varies from 0.1 μM if barbed ends are free, to 0.7 μM if only pointed ends are available [7]. Under these conditions, the turnover of actin filaments utilizes a mechanism called 'treadmilling' (Figure 1, grey box), where the rate of assembly at the barbed ends is balanced by the rate of depolymerization at the pointed ends with a rate of about

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

Figure 1



Control of actin dynamics. (Grey box) Actin polymerization in the absence of actin-binding proteins leads to a steady-state regime wherein actin in the filament pool is in equilibrium with actin in the monomer pool at the so-called 'critical concentration'. The ATP-loaded actin monomers (in white) mostly associate with the fast growing, barbed end of filaments. Aging of actin subunits within the filament occurs first through ATP hydrolysis at a time scale of seconds (ADP-Pi subunits, in yellow), secondly with the release of inorganic phosphate at a time scale of minutes (ADP subunits, in green), and finally by dissociation of subunits from the filament pointed end. Recycling of subunits occurs after exchange of the nucleotide. The overall mechanism at steady state represents a dynamic equilibrium referred to as 'treadmilling'. (Green box) Summary of actin dynamics in the presence of a limited set of accessory proteins whose interactions with actin filaments have been studied kinetically. The modulation of actin dynamics depicted in this cartoon is highlighted

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

2 μm/h ([13,14]; Figure 1). However, the situation is almost certainly different *in vivo* since the pool of free actin monomers is at least an order of magnitude larger than the critical concentration at the barbed ends [7]. This is particularly the case for plant cells where the concentration of unpolymerized actin is estimated to be dramatically larger than the concentration of actin in filamentous form [3,15]. Although the critical concentration at both ends of plant actin filaments is significantly higher than that of muscle actin (0.4 and 1.2 μM, respectively; [16]), the monomer pool is up to 500 times higher than the critical concentration at the barbed ends. Assuming that the k_{on} for plant ATP-G-actin is the same as for skeletal muscle α-actin, these values support a prediction that actin filament elongation in cells will occur at rates of ~3 μm/s whenever barbed ends are available [8]. Support for this hypothesis has recently been obtained from live-cell imaging of single actin filaments [17•]. Further questions remain about how the explosive elongation of actin filaments *in vivo* is balanced by extremely fast disassembly to maintain a large pool of actin monomers, thereby insuring the ability to rapidly and precisely turn over actin-based structures in plant cells. A thorough understanding of the dynamic behavior of actin in plants will require integrating knowledge about the biochemical and biophysical properties of actin and actin-binding proteins with a high resolution kinetic analysis of turnover in living cells.

In this context, our brief review will focus on recent findings about actin dynamics *in vitro*, on new imaging systems and fluorescent probes to study actin dynamics in living plant cells, and on the emergence of model systems other than *Arabidopsis thaliana* to study the structure/function of actin-binding proteins *in planta*. Our objective is to propose a general overview on how these findings can be integrated to understand similarities and differences between the dynamic organization of the actin cytoskeleton in plants and others organisms.

Methods to study actin dynamics *in vitro* and *in vivo*

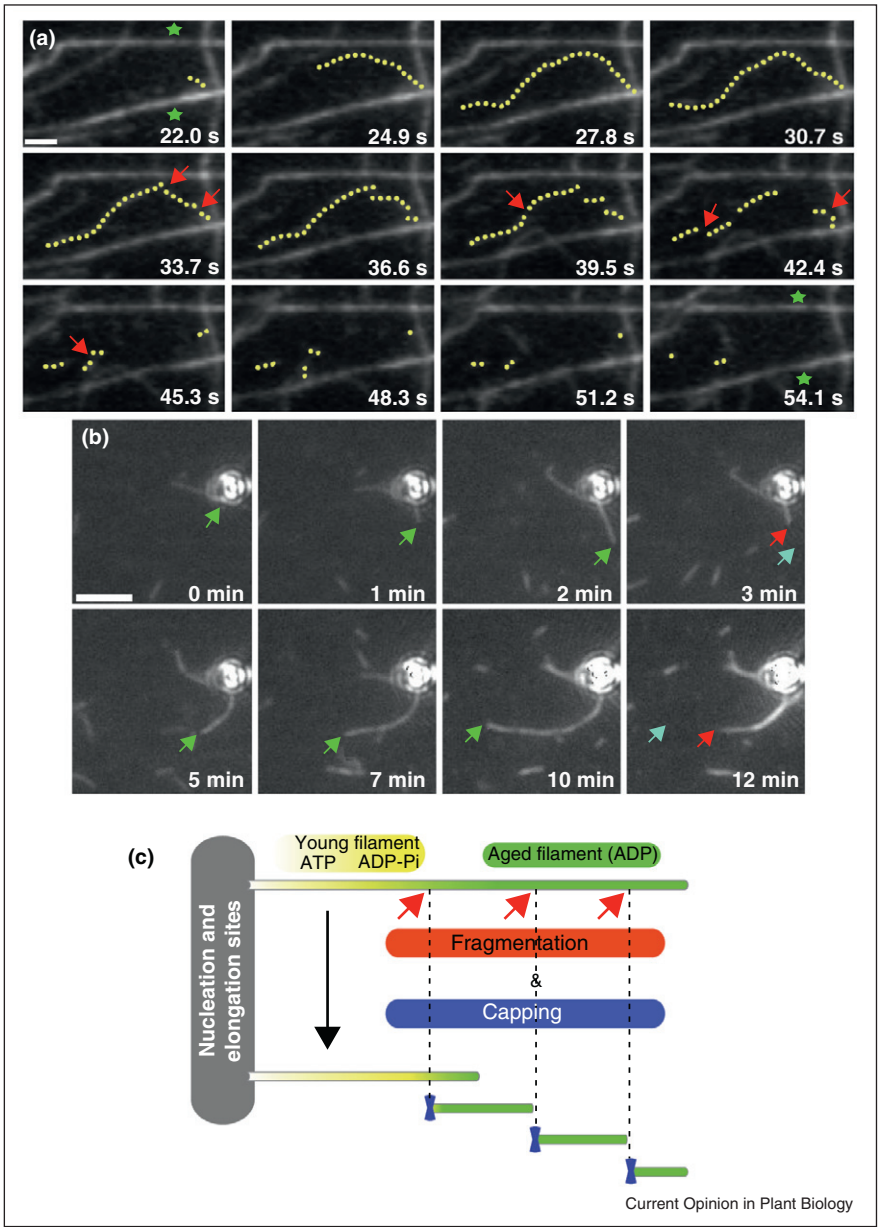
For decades actin dynamics have been studied *in vitro* with purified muscle actin and bulk assays that follow a change in fluorescence during the polymerization or disassembly of actin filaments (Figure 1a). For this purpose actin monomers are derivatized with a fluorescent probe to create an analog, pyrene-actin, that increases up to 20-fold in emission upon assembly into filaments. This allows the kinetics of actin polymerization to be followed in a fluorimeter, resulting in S-shaped assembly curves that roughly correspond to the nucleation, elongation and dynamic steady-state phases, respectively (Figure 1a). An useful alternative to this approach, that does not require monomer labeling, is 90° light scattering [18,19]. However, both techniques are complicated by several overlapping reactions that occur as soon as actin filaments are decorated or assembled into higher-order structures by actin-binding proteins. Moreover, certain properties like filament severing can only be inferred from these bulk assays, rather than demonstrated directly. Electron microscopy has been a valuable tool for determining the rate constant at the two ends of actin filaments [9]; however, EM studies provide little quantitative information about the different reactions involved during actin dynamics. More recently, individual actin filaments *in vitro* have been observed with conventional wide-field fluorescence microscopy but only after decoration with fluorescent-phalloidin, a drug which perturbs filament dynamics ([20,21]; Figure 1b). A breakthrough in studying actin dynamics, that overcomes these obstacles, was achieved with the application of total internal reflection fluorescence microscopy (TIRFM) to visualize individual filaments that are generated from a mixture of unlabeled actin monomers and actin derivatized with fluorescent dyes [22]. This approach is now the standard method for studying the dynamics of individual actin filaments in the presence of various actin-binding proteins ([23–25]; Figure 1d–f) or to visualize the interaction of molecular motors with actin tracks ([26,27]; Figure 1c). By varying the reaction conditions, all aspects of filament generation and turnover can be quantitatively measured with TIRFM including nucleation, elongation (Figure 1d), depolymerization, capping and severing (Figure 1e) reactions. The combination of this powerful imaging method with photosensitive dyes can even be applied to study the formation of higher-order structures, such as actin bundles, in real time in the presence of a plant actin-binding proteins (Figure 1f; [25]). In addition, the combination of TIRFM of single actin filament dynamics *in vitro* using a minimum set of proteins has allowed the reconstitution of

(Figure 1 Legend Continued) through eight numbered steps, each related to specific experimental data (**a**–**f**). (**a**) Profilin inhibits spontaneous nucleation by binding to actin monomers (bulk pyrene assay, compare light and dark blue curves; Step 1; [24]). This inhibition is overcome in the presence of *nucleation-promoting factors* from the formin family (green curves; Steps 2 and 3; [24]). In addition, the *Arabidopsis thaliana* formin, AFH1, promotes filament bundling (Step 4; [25]). (**b**) Heterodimeric capping protein from *Arabidopsis*, AtCP, blocks filament barbed ends as demonstrated by the inhibition of annealing between two populations of green- and red-labeled actin filaments (epifluorescence microscopy; Step 6; [20]). It also inhibits filament assembly and disassembly from filament barbed ends [20]. (**c**) Tobacco myosin XI (arrowhead) moves toward the barbed end of actin filaments at rates of up to 7 μm/s (optical trap nanometry, image reprinted by permission from Macmillan Publishers Ltd, EMBO Journal [26], copyright (2003); Step 7). (**d**) The moss formin, Pp Formin 1D, catalyzes the processive elongation at actin filament barbed ends (Step 3; [12•]). (**e**) Severing of pre-assembled actin filaments induced by *Arabidopsis thaliana* ADF1 (Step 5; see also Supplemental Movie 1; J Henty *et al.*, unpublished). (**f**) Upon addition of *Arabidopsis thaliana* villin, VLN3, some pre-polymerized rhodamine actin filaments (colorized) undergo bundling by a 'catch and zipper' mechanism (Step 8; see also Supplemental Movie 2; [70]). Bundlers also stabilize filaments against depolymerization and inhibit the activity of ADF/cofilin [70]. (**d**–**f**) Montages of time-lapse TIRFM images where green arrows indicate the pointed end, yellow arrows the barbed end, and red arrows the severing events. Scale bars are 2 μm in **c**; 4 μm in **d**; and 10 μm in **b**, **e** and **f**. (Nu., Nucleation).

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

actin-based motility systems ([28]; Figure 2b). The molecular insights obtained reveal that rapid actin filament turnover in such a system occurs through a stochastic dynamics mechanism dominated by ADF/cofilin-mediated filament severing [28,29]. Dampening of stochastic dynamics results in bundle formation that, following symmetry-breaking events, is capable of propelling polystyrene beads [28]. These biomimetic systems are an ideal way to model the motility of intracellular pathogens that hijack the host cytoskeleton as well as organelle or

Figure 2



Stochastic dynamics of single actin filaments. (**a**) Single actin filaments exhibit stochastic dynamics in the cortical array of epidermal cells from five DAG *Arabidopsis thaliana* hypocotyls expressing GFP-fABD2 [17•]. A montage of time-lapse VAEM images showing every other frame at ~3 s intervals is given. The images show a single actin filament elongating rapidly (marked with yellow dots), and then being severed (red arrows) into many small fragments, which disappear rapidly. Green stars denote two large actin filament bundles, which do not disassemble or move during the same time period. See also Supplemental Movie 3. Scale bar is 5 μm. (**b**) Single actin filaments show stochastic dynamics in the presence of ADF/cofilin in a reconstituted system, observed by time-lapse TIRFM [28]. A processive formin adsorbed on the surface of a polystyrene bead initiates actin filament nucleation and growth that oscillates between elongation (green arrow) and shrinking phases. The blue arrows indicate the last filament pointed-end position prior to fragmentation events (red arrows). Scale bar is 5 μm. (**c**) The cartoon illustrates the mechanism of stochastic dynamics mediated by severing factors like ADF/cofilin or villin/gelsolin. The severing factors act in synergy with barbed-end cappers, like AtCP or villin/gelsolin, to sever the growing filaments in their aged part and block the re-growth from the barbed-end of fragments.

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

vesicle motility, and they fill the gap between fundamental biochemical assays and cell biological analyses.

Following on from this pioneering work that uses advanced imaging to evaluate the biochemical and biophysical mechanisms of actin dynamics, the next step will be to compare the behavior of actin and actin-binding proteins *in vivo* and *in vitro*. To assess the many layers of regulation that organize actin filaments into the dynamic higher-order structures necessary for cellular function will require detailed analysis of the plant actin cytoskeleton at high spatial and temporal resolutions. However, visualization of actin dynamics in live materials has been somewhat challenging [3,8,30]. One major limitation is that directly tagging actin, either by protein derivatization or generation of fluorescent fusion proteins, leads to formation of anomalous structures or fails to decorate actin filament arrays *in vivo*. Recently, marked improvements in studying the actin cytoskeleton have come from the engineering of new probes such as the fusion of an Actin-Binding Domain from Arabidopsis Fimbrin1 (fABD2) to single or multiple GFP [31–34] or Lifeact, a 17-amino-acid peptide from yeast Abp140 [35], both of which stain filamentous actin in patterns that resemble the best fixation protocols and do not cause growth or morphological defects in plants when expressed at low levels [17•,31,34,36•,37•,38•,39•]. Another notable advance in the field is the application of new imaging methods, such as variable-angle epifluorescence microscopy (VAEM) and spinning disk confocal microscopy, which in combination with these actin reporters allow high resolution visualization of protein and vesicle dynamics in the plant cell cortex [17•,36•,37•,38•,40]. The plant community is now in an excellent position to propose a mechanistic description of actin dynamics in live plant cells and to build a bridge between this analysis and the molecules involved in controlling such dynamics.

What advantages do plant systems offer over animal and microbial cell models — a new view of actin dynamics

Most differences between a crawling animal cell and the typical walled plant cell are quite obvious. Locomotion of migrating animal cells is coordinated by the regulated polymerization of a dense actin filament array near the plasma membrane of the leading edge or lamellipodium [2,41]. Sensing of the environment and invasive growth depend on short protrusions from the leading edge, or filopodia, that are generated and stabilized by the assembly of actin filament bundles [2,42]. Even the non-motile animal cell can take on various morphologies and these shapes, in turn, depend on a dynamic cortical network of actin filaments [5]. These dense arrays of short actin filaments, which are below the limits of resolution for light microscopy, are largely recalcitrant to single filament imaging, but fluorescent speckle microscopy (FSM) has been used to infer that actin nucleation and elongation of filaments, with their barbed ends near the plasma membrane, drives membrane protrusion at rates of 1–2 μm/min [43,44]. Monomers are recycled from these arrays at sites distal to the plasma membrane by the action of actin depolymerizing factor (ADF)/cofilin, leading to a polarized flux of actin through the network in a process that superficially resembles treadmilling.

Because plant cells do not crawl and do not have lamellipodia or filopodia, one might speculate that actin dynamics will be less prominent and/or play little role in cell physiology and behavior. On the other hand, plant cells exhibit constant or inducible motions of many organelles, including Golgi [45–47], ER [48,49], mitochondria and plastids [50]. Moreover, the myosin motors isolated from plants are among the fastest mechanochemical enzymes on the planet, capable of processive movement at rates of 7.7 ± 0.5 μm/s ([26]; Figure 1c and Step 7), compared to rates of 0.6 ± 0.1 μm/s for *Saccharomyces cerevisiae* class II myosin (myo1p; [51]), 0.5 μm/s for *Schizosaccharomyces pombe* class II myosin (myo2p; [52]), 0.29 ± 0.02 μm/s for mammalian class V myosin [53], and 0.31 ± 0.02 μm/s for mammalian class VI myosin [54]. Elegant genetic studies demonstrate a role for class XI myosins in these organelle movements [45–47,49], and even hint that myosins are important for actin dynamics [48,55]. On the other hand, work in the moss *Physcomitrella patens*, suggests that actin dynamics are not altered when two myosin XI isovariants are silenced [38•]. Although plants do not have contractile actomyosin stress fibers, much of their actin is organized into prominent bundles that serve as tracks or molecular highways for these organelle movements. Similarly, although the Arp2/3 complex does not appear to generate dendritic networks responsible for plasma membrane protrusions, the distorted morphology of trichomes and the modest perturbation of epidermal pavement cell shape in mutant *Arabidopsis* plants hints at a function for actin nucleation in regulating cellular morphogenesis [56]. The recent demonstration of Arp2/3 and SCAR complex components on membrane fractions [57,58] suggests that regulated actin polymerization on endomembranes or at the plasma membrane somehow underpins this process. A goal for the future will be to identify the nature of these compartments and to understand what role actin polymerization plays in organelle function or trafficking. As pointed out earlier, the high concentration of available monomers, and the corresponding inhibition of spontaneous nucleation by the equally abundant monomer-binding protein profilin, will support explosive rates of actin elongation when new barbed ends are created, indicating that regulated actin polymerization near membranes may also be used to do work within plant cells. Finally, plant cells have several classes of novel actin-binding proteins, including CHUP1 [50,59,60] and actin-binding kinesins [61,62], whose precise activity on actin needs to be further explored. Nevertheless, these players and others may be

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

partially responsible for the formation or stabilization of actin filament arrays at the leading edge of motile chloroplasts and between the plastid and the plasma membrane [50,61], and are therefore good candidates for blue-light regulation of actin dynamics that contributes to chloroplast movements or anchoring.

Another advantage of plant systems is that they are one of the few examples where imaging of single actin filaments and quantitative analyses of dynamic parameters have been reported ([8,17•,39•]; Figure 2a; Supplemental Movie 3). Using VAEM imaging to examine the cortical cytoskeletal array in elongating hypocotyl epidermal cells from dark-grown *Arabidopsis* seedlings, our view of actin turnover as being potentially docile and unimportant has been shattered [17•]. Actin filaments in the cortical array exist in two populations: individual actin filaments arranged in random orientations and with short lifetimes (10s of seconds), and larger, stiffer and brighter actin filament cables that exist for many minutes and are mostly longitudinally oriented with respect to the cell long axis (Figure 2a; Supplemental Movie 3). Qualitatively similar views have been obtained recently by imaging the tip-growing cells from *Physcomitrella* [38•], the surface of plastids in mesophyll protoplasts [50], and other *Arabidopsis* epidermal cells as well as the liverwort, *Marchantia polymorpha* [36•]. The dynamic behavior of these two populations of actin filaments are described in more detail below and the candidate actin-binding proteins necessary to support each parameter of turnover are discussed. Suffice to say, individual actin filament turnover is one- to two-orders of magnitude faster than observed at the leading edge of crawling animal cells and the behavior does not resemble treadmilling. Instead, actin filaments undergo rapid elongation balanced by prolific severing or fragmentation, in a process termed 'stochastic dynamics' that can be mimicked by a simple reconstituted system *in vitro* ([17•,28]; Figure 2).

Molecular players responsible for plant actin specific behaviors

Despite the methods to probe actin cytoskeleton organization and dynamics in fixed or live plant cells, actin filaments organized into longitudinal bundles or cables are the most obvious organized structures within plant cells [63•,64]. Bundles are easier to visualize because they are brighter and less motile compared to the fine actin filament structures or individual actin filaments ([17•,36•,37•,65]; Figure 2a, green stars; Supplemental Movie 3). Nevertheless, the geometrical organization, the localization and the lifetime of these structures need to be tightly controlled to assure their physiological functions. In addition to supporting myosin-driven organelle movements, bundle formation and turnover are also implicated in guard cell opening/closing events [66], cytoarchitecture and transvacuolar strand dynamics [67,68], and auxin transport mechanisms [69]. Based on their relative resistance to latrunculin B (LatB) treatments [15,17•], a drug which preferentially disassembles dynamic actin filaments by binding monomers and inhibiting their polymerization, it can be inferred that bundles are more stable than single filaments. It is logical to assume that bundle stability is conferred by the association of filaments with side-binding and bundling proteins [3,8,63•,64]. At least four different families of actin-binding proteins, fimbrins, formins, LIMs and villins have been characterized for the ability to generate actin filaments bundles (Figure 1, Steps 4 and 8; [3,8,63•,64]). However, the dynamic formation of actin bundles at single filament resolution *in vitro* has been studied only for formins and villins ([25,70]; Figure 1f; Supplemental Movie 2). Cooperation of these two families of protein in bundle initiation and stabilization is an interesting hypothesis and is supported by demonstrated roles for the formins, AtFH1 and AtFH3, in the formation of actin cables in pollen tubes [71,72•].

The general principle of actin bundle formation appears to follow a 'catch and zipper' mechanism whereby the actin-bundling protein(s) first binds to the side of an actin filament, then catches a neighbor filament and zippers the two filaments together to form a bundle ([25,70]; Figure 1f; Supplemental Movie 2). Iteratively repeating this process generates actin bundles of different sizes and thickness ([25,70]; Figure 1f). This mechanism seems relevant for the formation of cortical actin bundles in *Arabidopsis* epidermal cells [39•,70] and liverwort cells [36•]. An open question is how this mechanism is related to or dictates the polarity of filaments within the bundle [3,63•,64]. Assuming that all plant myosins move in the same direction, the polarity of filaments within a bundle will determine transport directionality for various cargos, with antiparallel actin filament bundles supporting bidirectional movements and parallel bundles allowing unidirectional movements [8]. A related question is whether all actin filament bundles are equivalent for transport of cargo by myosins or whether the quality of the bundle and the presence of unique suites of bundling proteins impacts on myosin activity. In this regard, the observation that different classes of mammalian myosin move selectively and at unique velocities on different actin filament arrays is perhaps significant [27]. Similarly, the biochemical composition of the actin tracks may regulate the spatial distribution of myosin motors in fission yeast [73]. Testing this hypothesis in plants will require genetic studies as well as reconstitution of the various types of possible actin filament bundles along with *in vitro* motility assays and single-molecule imaging.

Bundling proteins on their own also stabilize the actin filaments from depolymerization by dilution or LatB *in vitro* [19,74–76] and *in vivo* [75,77], and their binding can prevent the activity of ADF [19,70,78]. A similar mechanism has been implicated in generation of bundles on

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

polystyrene beads in a biomimetic motility system where lateral contact between actin filaments modulates their stability against severing by ADF [28]. These observations may explain why bundles are not as dynamic as individual actin filaments *in vivo* [17•] but raise the question of how actin filament bundles stabilized against severing by ADF are disassembled. An interesting hypothesis is that members of the villin family of bundling proteins, such as VLN3 or VLN5 which also have severing activity that is regulated by calcium [70,77], may facilitate destabilization or fragmentation of actin bundles as a function of local or cellular calcium fluxes [70]. Alternatively, members of the gelsolin family represent another calcium-regulated protein [79] and may assist in the fragmentation of these structures. Further analysis of bundles generated in the presence of fimbrins or LIMs using time-lapse TIRFM will be required to understand the molecular mechanisms that underpin their formation, stabilization and turnover [63•]. Finally, how bundling proteins from different classes work synergistically or antagonistically, as occurs in animals, yeasts and flies, needs to be studied.

Besides creating actin bundles, actin-binding proteins maintain a second cellular population comprising extremely dynamic cortical actin filaments [17•,36•]. These individual filaments are unique in their ability to appear, grow and disappear very rapidly ([17•]; Figure 2a, yellow dots; Supplemental Movie 3). The origin or nucleation of new filament growth occurs at roughly equal frequency from three locations in plants: *de novo* in the cytoplasm; from the side of pre-existing bundles or filaments; and from short fragments or recently severed ends [17•]. Formin-mediated actin-filament nucleation, in coordination with profilin-mediated elongation, is an obvious mechanism to initiate and elongate these filaments (Figure 1, Steps 2 and 3; and [11]) and may even link the actin and microtubule cytoskeletons [80•,81]. However, to reach rates of actin filament elongation as fast as 550 subunits/s or 1.7 μm/s [8,17•], three mechanisms should be considered. As pointed out above, a huge pool of polymerization-competent actin monomers [15] could drive explosive elongation from available filament barbed-ends. Estimation of the size of this pool from the measured elongation rates of single actin filaments *in vivo* indicates that a pool of just 50 μM actin monomer would be sufficient for barbed-end growth at 580 subunits/s [8]. Decreasing the size of the monomer pool with LatB, leads to a dose-dependent reduction in barbed-end elongation rates and provides evidence in support of this mechanism. The complementary experiment, increasing the monomer pool by overexpression of actin isovariants [82,83], is now possible and would provide additional support for the direct dependence of filament growth rates on the size of the monomer pool. Second, processive elongating factors such as the formins could work in concert with the large pool of unpolymerized actin to elevate rates of growth even further than supported by the monomer concentration alone. Formins are conserved proteins capable of both nucleating filament formation and processively elongating actin filaments by binding to filament barbed ends (Figure 1, Step 3). In the formin family, a class II formin (For2A) from the moss *Physcomitrella patens* is the fastest actin-elongating machine characterized to date and this activity is critical for driving the formation of apical filamentous actin necessary for polarized growth [12•]. Finally, a 'bursting' mechanism can be envisioned whereby small filament fragments or oligomers add on or anneal to filament ends, resulting in quantum jumps in filament length. Convincing examples of this mechanism have been reported recently for yeast at cortical endocytic sites in an *aip1*Δ mutant background [84], as well as in the cortical actin array of plant cells [39•].

To balance this fast rate of elongation and maintain the large pool of actin bound to profilin [15], the cortical actin filaments must be disassembled at high rates. Disassembly of long, individual filaments does not occur appreciably through depolymerization or loss of subunits from filament ends. Instead, prolific severing destroys single actin filaments, resulting in the production of numerous small fragments (Figure 2a, red arrows; Supplemental Movie 3). Indeed, a typical 10 μm long filament will suffer on average six breaks every minute, resulting in rather short filament lifetimes [17•]. The similarities between this actin filament turnover *in vivo* and the 'stochastic dynamics' described in a reconstituted system *in vitro* are quite obvious (Figure 2b; [28]). The fact that after severing the majority of fragments or filament ends do not resume fast growth *in vivo*, suggests coordination between severing and capping during actin filament disassembly. Several proteins may play a role in these severing and capping activities, including ADF/cofilin (Figure 1, Step 5; Figure 1e; Supplemental Movie 1), heterodimeric capping protein (Figure 1, Step 6; Figure 1b), villin/gelsolin or Aip1 [8,34]. How the small fragments are depolymerized to return subunits to the monomer pool remains unclear, because most are visible for only a frame or two before disappearing from the field of view (Figure 2a; Supplemental Movie 3). One possibility is that actin-binding proteins like ADF/cofilin and cyclase-associated protein function synergistically to disassemble these fragments [8,16,85]. As proposed above for growth mechanisms, the fragments may not disassemble completely, but instead could add on to filament ends through annealing reactions ([39•,84]; Figure 1b). Addressing this will require imaging single actin filaments at even higher temporal resolutions and perhaps making use of the *Aip1* inducible-RNAi lines [34]. In addition to the actin dynamics caused by assembly/disassembly, single actin filaments or bundles can be mechanically deformed by tension generated by molecular motors [8,17•,64]. Importantly, recent data suggest that part of the turnover of actin structures involves actomyosin contractility [86].

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

This is an intriguing hypothesis that needs to be investigated further considering the effect of a putative myosin inhibitor on cortical actin dynamics [17•] and hints that myosin XI mutants alter actin organization [48,55].

Conclusions perspectives for the field

The future holds great promise to further dissect the above mechanisms for regulation of actin dynamics and the roles of individual actin-binding proteins in plants. Reverse-genetics in both *Arabidopsis* and *Physcomitrella* will allow facile and unparalled opportunities to provide unambiguous evidence for the role of each molecular player in actin dynamics *in vivo*. The moss system has indeed emerged as a powerful model organism for testing the function of actin-binding proteins and actin dynamics in plants [12•,37•,38•,87,88]. By combining knock-out and knock-down strategies in both systems with advanced imaging technologies, like VAEM and spinning disk confocal microscopy, it should be possible to quickly test each protein family for its proposed function. We look forward to many such advances in coming years, which will further propel plants into a premier position as a leading system in cytoskeletal research.

Acknowledgements

Work in the lab of LB is supported by Agence Nationale de La Recherche (ANR). The Staiger lab is funded by the Physical Biosciences Program of the Office of Basic Energy Sciences, US Department of Energy under contract number DE-FG02-04ER15526; the US National Science Foundation (IOS-1021185); and a Dept. of Energy-sponsored Center for Direct Catalytic Conversion of Biomass to Biofuels (C3Bio), an Energy Frontier Research Center (DE-SC0000997).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbi.2010.09.013.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Pollard TD, Borisy GG: **Cellular motility driven by assembly and disassembly of actin filaments**. *Cell* 2003, **112**:453-465.

2. Pollard TD, Cooper JA: **Actin, a central player in cell shape and movement**. *Science* 2009, **27**:1208-1212.

3. Staiger CJ, Blanchoin L: **Actin dynamics: old friends with new stories**. *Curr Opin Plant Biol* 2006, **9**:554-562.

4. Hussey PJ, Ketelaar T, Deeks MJ: **Control of the actin cytoskeleton in plant cell growth**. *Annu Rev Plant Biol* 2006, **57**:109-125.

5. Fletcher DA, Mullins RD: **Cell mechanics and the cytoskeleton**. *Nature* 2010, **463**:485-492.

6. Pollard TD, Cooper JA: **Actin and actin-binding proteins. A critical evaluation of mechanisms and functions**. *Annu Rev Biochem* 1986, **55**:987-1035.

7. Pollard TD, Blanchoin L, Mullins RD: **Molecular mechanisms controlling actin filament dynamics in nonmuscle cells**. *Annu Rev Biophys Biomol Struct* 2000, **29**:545-576.

8. Staiger CJ, Poulter NS, Henty JL, Franklin-Tong VE, Blanchoin L: **Regulation of actin dynamics by actin-binding proteins in pollen**. *J Exp Bot* 2010, **61**:1969-1986.

9. Pollard TD: **Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments**. *J Cell Biol* 1986, **103**:2747-2754.

10. Romero S, Le Clainche C, Didry D, Egile C, Pantaloni D, Carlier M-F: **Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis**. *Cell* 2004, **119**:419-429.

11. Blanchoin L, Staiger CJ: **Plant formins: diverse isoforms and unique molecular mechanism**. *Biochim Biophys Acta–Mol Cell Res* 2010, **1803**:201-206.

12. • Vidali L, van Gisenbergen PAC, Guérin C, Franco P, Li M, Burkart GM, Augustine RC, Blanchoin L, Bezanilla M: **Rapid formin-mediated actin-filament elongation is essential for polarized plant cell growth**. *Proc Natl Acad Sci USA* 2009, **106**:13341-13346.
Analysis of the function of all nine formin genes in the moss *Physcomitrella patens* demonstrates that the class II formins are essential for polarized cell growth. In contrast, knock-down of all other formins does not affect cell morphology. For2A is the fastest actin filament elongating machine characterized so far by a time-lapse TIRFM assay *in vitro*. Altogether, this study reveals that rapid elongation of actin filaments modulated by For2 is necessary for polarized cell growth.

13. Selve N, Wegner A: **Rate constants and equilibrium constants for binding of the gelsolin–actin complex to the barbed ends of actin filaments in the presence and absence of calcium**. *Eur J Biochem* 1986, **160**:379-387.

14. Bugyi B, Didry D, Carlier M-F: **How tropomyosin regulates lamellipodial actin-based motility: a combined biochemical and reconstituted motility approach**. *EMBO J* 2010, **29**:14-26.

15. Gibbon BC, Kovar DR, Staiger CJ: **Latrunculin B has different effects on pollen germination and tube growth**. *Plant Cell* 1999, **11**:2349-2363.

16. Chaudhry F, Guérin C, von Witsch M, Blanchoin L, Staiger CJ: **Identification of Arabidopsis Cyclase-Associate Protein 1 as the first nucleotide exchange factor for plant actin**. *Mol Biol Cell* 2007, **18**:3002-3014.

17. • Staiger CJ, Sheahan MB, Khurana P, Wang X, McCurdy DW, Blanchoin L: **Actin filament dynamics are dominated by rapid growth and severing activity in the Arabidopsis cortical array**. *J Cell Biol* 2009, **184**:269-280.
Using variable-angle epifluorescence microscopy (VAEM), the authors examine the organization and dynamics of the cortical actin cytoskeleton in growing and non-growing epidermal cells. Two populations of actin filaments are identified, highly dynamic single actin filaments and more stable actin cables or bundles. Continuous remodeling of actin structures in the cortical array follows a mechanism based on the stochastic dynamics principals established in Ref. [28].

18. Wegner A, Engel J: **Kinetics of the cooperative association of actin to actin filaments**. *Biophys Chem* 1975, **3**:215-225.

19. Huang S, Robinson RC, Gao LY, Matsumoto T, Brunet A, Blanchoin L, Staiger CJ: **Arabidopsis VILLIN1 generates actin filament cables that are resistant to depolymerization**. *Plant Cell* 2005, **17**:486-501.

20. Huang S, Blanchoin L, Kovar DR, Staiger CJ: ***Arabidopsis* capping protein (AtCP) is a heterodimer that regulates assembly at the barbed ends of actin filaments**. *J Biol Chem* 2003, **278**:44832-44842.

21. Blanchoin L, Pollard TD, Mullins RD: **Interactions of ADF/cofilin, Arp2/3 complex, capping protein and profilin in remodeling of branched actin filament networks**. *Curr Biol* 2000, **10**:1273-1282.

22. Amann KJ, Pollard TD: **Direct real-time observation of actin filament branching mediated by Arp2/3 complex using total internal reflection fluorescence microscopy**. *Proc Natl Acad Sci USA* 2001, **98**:15009-15013.

23. Kovar DR, Pollard TD: **Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces**. *Proc Natl Acad Sci USA* 2004, **101**:14725-14730.

24. Michelot A, Guérin C, Huang S, Ingouff M, Richard S, Rodiuc N, Staiger CJ, Blanchoin L: **The formin homology 1 domain modulates the actin nucleation and bundling activity of Arabidopsis FORMIN1**. *Plant Cell* 2005, **17**:2296-2313.

25. Michelot A, Derivery E, Paterski-Boujemaa R, Guérin C, Huang S, Parcy F, Staiger CJ, Blanchoin L: **A novel mechanism for the formation of actin-filament bundles by a non-processive formin**. *Curr Biol* 2006, **16**:1924-1930.

26. Tominaga M, Kojima H, Yokota E, Orii H, Nakamori R, Katayama E, Anson M, Shimmen T, Oiwa K: **Higher plant myosin XI moves processively on actin with 35 nm steps at high velocity**. *EMBO J* 2003, **22**:1263-1272.

27. Brawley CM, Rock RS: **Unconventional myosin traffic in cells reveals a selective actin cytoskeleton**. *Proc Natl Acad Sci USA* 2009, **106**:9685-9690.

28. Michelot A, Berro J, Guérin C, Boujemaa-Paterski R, Staiger CJ, Martiel J-L, Blanchoin L: **Actin-filament stochastic dynamics mediated by ADF/cofilin**. *Curr Biol* 2007, **17**:825-833.

29. Roland J, Berro J, Michelot A, Blanchoin L, Martiel J-L: **Stochastic severing of actin filaments by actin depolymerizing factor/cofilin controls the emergence of a steady dynamical regime**. *Biophys J* 2008, **94**:2082-2094.

30. Cheung AY, Duan Q-H, Costa SS, de Graaf BHJ, Di Stilio VS, Feijo J, Wu H-M: **The dynamic pollen tube cytoskeleton: live cell studies using actin-binding and microtubule-binding reporter proteins**. *Mol Plant* 2008, **1**:687-702.

31. Sheahan MB, Staiger CJ, Rose RJ, McCurdy DW: **A green fluorescent protein fusion to actin-binding domain 2 of Arabidopsis fimbrin highlights new features of a dynamic actin cytoskeleton in live plant cells**. *Plant Physiol* 2004, **136**:3968-3978.

32. Wang Y-S, Motes CM, Mohamalawari DR, Blancaflor EB: **Green fluorescent protein fusions to *Arabidopsis* fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots**. *Cell Motil Cytoskeleton* 2004, **59**:79-93.

33. Wang Y-S, Yoo C-M, Blancaflor EB: **Improved imaging of actin filaments in transgenic *Arabidopsis* plants expressing a green fluorescent protein fusion to the C- and N-termini of the fimbrin actin-binding domain 2**. *New Phytol* 2008, **177**:525-536.

34. Ketelaar T, Allwood EG, Anthony R, Voigt B, Menzel D, Hussey PJ: **The actin-interacting protein AIP1 is essential for actin organization and plant development**. *Curr Biol* 2004, **14**:145-149.

35. Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, Bista M, Bradke F, Jenne D, Holak TA, Werb Z *et al.*: **Lifeact: a versatile marker to visualize F-actin**. *Nat Meth* 2008, **5**:605-607.

36. • Era A, Tominaga M, Ebine K, Awai C, Saito C, Ishizaki K, Yamamoto KT, Kohchi T, Nakano A, Ueda T: **Application of Lifeact reveals F-actin dynamics in *Arabidopsis thaliana* and the liverwort, *Marchantia polymorpha***. *Plant Cell Physiol* 2009, **50**:1041-1048.
Along with Ref. [37•], this work shows that the actin filament probe, Lifeact, is a valuable tool for studying actin cytoskeleton remodeling in plant cells without impairing its function. The thickening and buckling of actin bundles and their myosin-depending sliding reveal a very dynamic actin network in several differentiated cell types.

37. • Vidali L, Rounds CM, Hepler PK, Bezanilla M: **Lifeact-mEGFP reveals a dynamic apical F-actin network in tip growing plant cells**. *PLoS ONE* 2009, **4**:e5744.
By using for the first time in plants the actin probe, Lifeact-mEGFP, along with spinning-disk confocal imaging, the authors demonstrate that this new versatile tool allows visualization of actin dynamics in tip growing plant cells. The apical actin network fundamental for tip growth appears very dynamic.

38. • Vidali L, Burkart GM, Augustine RC, Kerdavid E, Tüzel E, Bezanilla M: **Myosin XI is essential for tip growth in *Physcomitrella patens***. *Plant Cell* 2010, **22**:1868-1882.
The authors take advantage of the model system, *Physcomitrella patens,* to study the physiological role of class XI myosins. Indeed *P. patens* has only two myosin XI genes. Using RNAi, they silenced both genes and demonstrate that myosin XI plays an essential role in tip growth during cell morphogenesis. In addition, they report that this phenotype is not due to obvious perturbations of actin dynamics.

39. • Smertenko AP, Deeks MJ, Hussey PJ: **Strategies of actin reorganisation in plant cells**. *J Cell Sci* 2010, **123**:3019-3028.
By using the actin probe Lifeact in tobacco cell lines and in *Arabidopsis* plants along with confocal laser-scanning microscopy (CLSM) and evanescent wave microscopy (TIRFM) imaging, the authors demonstrated that actin filament network is highly dynamic in isotropically expanding cells. Interestingly, the actin network reorganization relies mainly on the stochastic severing *in vivo*.

40. Konopka CA, Bednarek SY: **Variable-angle epifluorescence microscopy: a new way to look at protein dynamics in the plant cell cortex**. *Plant J* 2008, **53**:186-196.

41. Urban E, Jacob S, Nementhova M, Resch GP, Small JV: **Electron tomograpy reveals unbranched networks of actin filaments in lamellipodia**. *Nature Cell Biol* 2010, **12**:429-435.

42. Medalia O, Beck M, Ecke M, Weber I, Neujahr R, Baumeister W, Gerisch G: **Organization of actin networks in intact filopodia**. *Curr Biol* 2007, **17**:79-84.

43. Waterman-Storer CM, Desai A, Bulinski JC, Salmon ED: **Fluorescent speckle microscopy, a method to visualize the dynamics of protein assemblies in living cells**. *Curr Biol* 1998, **8**:1227-1230.

44. Ponti A, Vallotton P, Salmon WC, Waterman-Storer CM, Danuser G: **Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy**. *Biophys J* 2003, **84**:3336-3352.

45. Prokhnevsky AI, Peremyslov VV, Dolja VV: **Overlapping functions of the four class XI myosins in *Arabidopsis* growth, root hair elongation, and organelle motility**. *Proc Natl Acad Sci USA* 2008, **105**:19744-19749.

46. Peremyslov VV, Prohknevsky AI, Avisar D, Dolja VV: **Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis**. *Plant Physiol* 2008, **146**:1109-1116.

47. Avisar D, Abu-Abied M, Belausov E, Sadot E, Hawes C, Sparkes IA: **A comparative study on the involvement of 17 Arabidopsis myosin family members on the motility of Golgi and other organelles**. *Plant Physiol* 2009, **150**:700-709.

48. Ueda H, Yokota E, Kutsuna N, Shimada T, Tamura K, Shimmen T, Hasezawa S, Dolja VV, Hara-Nishimura I: **Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells**. *Proc Natl Acad Sci USA* 2010, **107**:6894-6899.

49. Sparkes I, Runions J, Hawes C, Griffing L: **Movement and remodeling of the endoplasmic reticulum in nondividing cells of tobacco leaves**. *Plant Cell* 2009, **21**:3937-3949.

50. Kadota A, Yamada N, Suetsugu N, Hirose M, Saito C, Shoda K, Ichikawa S, Kagawa T, Nakano A, Wada M: **Short actin-based mechanism for light-directed chloroplast movement in *Arabidopsis***. *Proc Natl Acad Sci USA* 2009, **106**:13106-13111.

51. Huckaba TM, Lipkin T, Pon LA: **Roles of type II myosin and a tropomyosin isoform in retrograde actin flow in budding yeast**. *J Cell Biol* 2006, **175**:957-969.

52. Lord M, Pollard TD: **UCS protein Rng3p activates actin filament gliding by fission yeast myosin-II**. *J Cell Biol* 2004, **167**:315-325.

53. Mehta AD, Rock RS, Rief M, Spudich JA, Mooseker MS, Cheney RE: **Myosin-V is a processive actin-based motor**. *Nature* 1999, **400**:590-593.

54. Rock RS, Rice SE, Wells AL, Purcell TJ, Spudich JA, Sweeney HL: **Myosin VI is a processive motor with a large step size**. *Proc Natl Acad Sci USA* 2001, **98**:13655-13659.

55. Peremyslov VV, Prokhnevsky AI, Dolja VV: **Class XI myosins are required for development, cell expansion, and F-actin organization**. *Plant Cell* 2010, **22**:1883-1897.

56. Szymanski DB: **Breaking the WAVE complex: the point of Arabidopsis trichomes**. *Curr Opin Plant Biol* 2005, **8**:103-112.

57. Kotchoni SO, Zakharova T, Mallery EL, Le J, El-Din El-Assal S, Szymanski DB: **The association of the Arabidopsis actin-related protein (Arp) 2/3 complex with cell membranes is linked to its assembly status, but not its activation**. *Plant Physiol* 2009, **151**:2095-2109.

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013

58. Dyachok J, Shao M-R, Vaughn K, Bowling A, Facette M, Djakovic S, Clark L, Smith L: **Plasma membrane-associated SCAR complex subunits promote cortical F-actin accumulation and normal growth characteristics in *Arabidopsis* roots**. *Mol Plant* 2008, **1**:990-1006.

59. Oikawa K, Kasahara M, Kiyosue T, Kagawa T, Suetsugu N, Takahashi F, Kanegae T, Niwa Y, Kadota A, Wada M: **CHLOROPLAST UNUSUAL POSITIONING1 is essential for proper chloroplast positioning**. *Plant Cell* 2003, **15**:2805-2815.

60. Oikawa K, Yamasato A, Kong S-G, Kasahara M, Nakai M, Takahashi F, Ogura Y, Kagawa T, Wada M: **Chloroplast outer envelope protein CHUP1 is essential for chloroplast anchorage to the plasma membrane and chloroplast movement**. *Plant Physiol* 2008, **148**:829-842.

61. Suetsugu N, Yamada N, Kagawa T, Yonekura H, Uyeda TQP, Kadota A, Wada M: **Two kinesin-like proteins mediate actin-based chloroplast movement in *Arabidopsis thaliana***. *Proc Natl Acad Sci USA* 2010, **107**:8860-8865.

62. Preuss ML, Kovar DR, Lee Y-RJ, Staiger CJ, Delmer DP, Liu B: **A plant-specific kinesin binds to actin microfilaments and interacts with cortical microtubules in cotton fibers**. *Plant Physiol* 2004, **136**:3945-3955.

63. • Thomas C, Tholl S, Moes D, Dieterle M, Papuga J, Moreau F, Steinmetz A: **Actin bundling in plants**. *Cell Motil Cytoskeleton* 2009, **66**:940-957.
This very comprehensive and well-documented review details the importance of the organization of actin into bundles (or cables) in plants. In addition, all molecular players involved in the organization of actin filaments into bundles are described in great detail by comparing their similarities and differences.

64. Higaki T, Kojo KH, Hasezawa S: **Critical role of actin bundling in plant cell morphogenesis**. *Plant Signal Behav* 2010, **5**:484-488.

65. Kato T, Morita MT, Tasaka M: **Defects in dynamics and functions of actin filament in Arabidopsis caused by the dominant-negative actin fiz1-induced fragmentation of actin filament**. *Plant Cell Physiol* 2010, **51**:333-338.

66. Higaki T, Kutsuna N, Sano T, Kondo N, Hasezawa S: **Quantification and cluster analysis of actin cytoskeletal structures in plant cells: role of actin bundling in stomatal movement during diurnal cycles in Arabidopsis guard cells**. *Plant J* 2010, **61**:156-165.

67. Higaki T, Kutsuna N, Okubo E, Sano T, Hasezawa S: **Actin microfilaments regulate vacuolar structures and dynamics: dual observation of actin microfilaments and vacuolar membrane in living tobacco BY-2 cells**. *Plant Cell Physiol* 2006, **47**:839-852.

68. Szymanski DB, Cosgrove DJ: **Dynamic coordination of cytoskeletal and cell wall systems during plant cell morphogenesis**. *Curr Biol* 2009, **19**:R800-R811.

69. Nick P, Han M-J, An G: **Auxin stimulates its own transport by shaping actin filaments**. *Plant Physiol* 2009, **151**:155-167.

70. Khurana P, Henty JL, Huang S, Staiger AM, Blanchoin L, Staiger CJ: ***Arabidopsis* VILLIN1 and VILLIN3 have overlapping and distinct activities in actin bundle formation and turnover**. *Plant Cell* 2010, **22**:2727-2748.

71. Cheung AY, Wu H-M: **Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane**. *Plant Cell* 2004, **16**:257-269.

72. • Ye J, Zheng Y, Yan A, Chen N, Wang Z, Huang S, Yang Z: ***Arabidopsis* Formin3 directs the formation of actin cables and polarized growth in pollen tubes**. *Plant Cell* 2009, **19**:3868-3884.
Biochemical and genetic evidence suggests that AtFH3 specifically regulates the formation of axial actin cables involved in the growth of pollen tubes. The authors also show that AtFH3-mediated cables control the reverse fountain-pattern of cytoplasmic streaming, suggesting that these formin-dependent longitudinal cables act as tracks for myosin-dependent motion.

73. Clayton JE, Sammons MR, Stark BC, Hodges AR, Lord M: **Differential regulation of unconventional fission yeast myosins via the actin track**. *Curr Biol* 2010, **20**:1423-1431.

74. Wang H-J, Wang A-R, Jauh G-Y: **An actin-binding protein, LILIM1, mediates calcium and hydrogen regulation of actin dynamics in pollen tubes**. *Plant Physiol* 2008, **147**:1619-1636.

75. Thomas C, Hoffmann C, Dieterle M, Van Troys M, Ampe C, Steinmetz A: **Tobacco WLIM1 is a novel F-actin binding protein involved in actin cytoskeleton remodeling**. *Plant Cell* 2006, **18**:2194-2206.

76. Papuga J, Hoffmann C, Dieterle M, Moes D, Moreau F, Tholl S, Steinmetz A, Thomas C: ***Arabidopsis* LIM proteins: a family of actin bundlers with distinct expression patterns and modes of regulation**. *Plant Cell* 2010, doi:10.1105/tpc.110.075960.

77. Zhang H, Qu X, Bao C, Khurana P, Wang Q, Xie Y, Zheng Y, Chen N, Blanchoin L, Staiger CJ *et al.*: ***Arabidopsis* VILLIN5, an actin filament bundling and severing protein, is necessary for normal pollen tube growth**. *Plant Cell* 2010, **22**:2749-2767.

78. Thomas C, Moreau F, Dieterle M, Hoffmann C, Gatti S, Hofmann C, Van Troys M, Ampe C, Steinmetz A: **The LIM domains of WLIM1 define a new class of actin bundling modules**. *J Biol Chem* 2007, **282**:33599-33608.

79. Huang S, Blanchoin L, Chaudhry F, Franklin-Tong VE, Staiger CJ: **A gelsolin-like protein from *Papaver rhoeas* pollen (PrABP80) stimulates calcium-regulated severing and depolymerization of actin filaments**. *J Biol Chem* 2004, **279**:23364-23375.

80. • Deeks MJ, Fendrych M, Smertenko A, Bell KS, Oparka K, Cvrckova F, Zarsky V, Hussey PJ: **The plant formin AtFH4 interacts with both actin and microtubules, and contains a newly identified microtubule-binding domain**. *J Cell Sci* 2010, **123**:1209-1215.
In this study, a membrane-integrated formin, AtFH4, is shown to recognize specifically, via the GOE domain, microtubules in plants. Therefore, AtFH4 provides a potential candidate connecting the microtubule and actin networks to the lipid bilayer and controlling the cytoskeleton organization.

81. Li Y, Shen Y, Cai C, Zhong C, Zhu L, Yuan M, Ren H: **The type II Arabidopsis Formin14 interacts with microtubules and microfilaments to regulate cell division**. *Plant Cell* 2010, **22**:2710-2726.

82. Kandasamy MK, McKinney EC, Meagher RB: **Functional nonequivalency of actin isovariants in *Arabidopsis***. *Mol Biol Cell* 2002, **13**:251-261.

83. Kandasamy MK, McKinney EC, Meagher RB: **A single vegetative actin isovariant overexpressed under the control of multiple regulatory sequences is sufficient for normal *Arabidopsis* development**. *Plant Cell* 2009, **21**:701-718.

84. Okreglak V, Drubin DG: **Loss of Aip1 reveals a role in maintaining the actin monomer pool and an in vivo oligomer assembly pathway**. *J Cell Biol* 2010, **188**:769-777.

85. Deeks MJ, Rodrigues C, Dimmock S, Ketelaar T, Maciver SK, Malhó R, Hussey PJ: ***Arabidopsis* CAP1—a key regulator of actin organisation and development**. *J Cell Sci* 2007, **120**:2609-2618.

86. Wilson CA, Tsuchida MA, Allen GM, Barnhart EL, Applegate KT, Yam PT, Ji L, Keren K, Danuser G, Theriot JA: **Myosin II contributes to cell-scale actin network treadmilling through network disassembly**. *Nature* 2010, **465**:373-377.

87. Augustine RC, Vidali L, Kleinman KP, Bezanilla M: **Actin depolymerizing factor is essential for viability in plants, and its phosphoregulation is important for tip growth**. *Plant J* 2008, **54**:863-875.

88. Vidali L, Augustine RC, Kleinman KP, Bezanilla M: **Profilin is essential for tip growth in the moss *Physcomitrella patens***. *Plant Cell* 2007, **19**:3705-3722.

Please cite this article in press as: Blanchoin L, et al. Actin Q1dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game, Curr Opin Plant Biol (2010), doi:10.1016/j.pbi.2010.09.013