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Actin dynamics in the cortical array of plant cells

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The actin cytoskeleton changes in organization and dynamics as cellular functions are reprogrammed following responses to diverse stimuli, hormones, and developmental cues. How this is choreographed and what molecular players are involved in actin remodeling continues to be an area of intense scrutiny. Advances in imaging modalities and fluorescent fusion protein reporters have illuminated the strikingly dynamic behavior of single actin filaments at high spatial and temporal resolutions. This led to a model for the stochastic dynamic turnover of actin filaments and predicted the actions and responsibilities of several key actin-binding proteins. Recently, aspects of this model have been tested using powerful genetic strategies in both *Arabidopsis* and *Physcomitrella.* Collectively, the latest data emphasize the importance of filament severing activities and regulation of barbed-end availability as key facets of plant actin filament turnover.

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Introduction

The actin cytoskeleton comprises an ever-changing network of individual filaments and higher-order structures called actin cables or bundles. In plants, these arrays serve as 'tracks' for the movement and positioning of diverse organelles, such as chloroplasts, mitochondria and Golgi. Actin filaments are thought to be necessary for the delivery of secretory vesicles containing polysaccharides and proteins to the plasma membrane, and they play a central role in endocytosis; however, there is only modest evidence supporting these functions for actin in plants. They are also important for responding to biotic and abiotic stimuli, such as during the innate immune response against pathogens. How exactly these tracks form, how they are organized in the cytoplasm, and how they turn over has been the subject of intense scrutiny.

Nearly two decades of protein biochemistry, along with genome sequencing, has uncovered several dozen plant actin-binding proteins (ABPs) that are likely to regulate actin organization and turnover (reviewed by [1–4]). However, understanding how these operate in cells requires model systems where individual actin filaments can be visualized at high spatial and temporal resolutions. This critical knowledge gap has been satisfied recently with advanced imaging of the cortical cytoskeletal array in *Arabidopsis* and moss cells [5••,6,7••,8••]. These studies illuminate some rather incredible rearrangements of the actin cytoskeleton. In the cortical cytoplasm of certain *Arabidopsis* epidermal cells, for example, filament arrays are both complex and constantly assembling and disassembling. Remarkably, the rates of turnover are among the fastest recorded in eukaryotic cells, with barbed-end assembly occurring at rates of ~2 μm s^{-1} or 720 subunits per second, and disassembly is achieved through prolific severing activity. The central features of this behavior have been termed 'stochastic dynamics' [5••,9,10], and this mechanism represents a new paradigm for actin turnover in eukaryotic cells.

Here, we highlight the identification of several key parameters for actin filament turnover, derived primarily from live-cell imaging studies of *Arabidopsis* epidermal cells. Based on their biochemical properties, it can be predicted which ABPs are involved in different aspects of actin dynamics. Finally, we review several recent studies that combine pharmacological and reverse-genetic approaches with quantitative image analyses to implicate key ABPs in specific steps of actin turnover *in vivo*. One key take-home message is that the cortical cytoskeletal array in plant cells may be an ideal system for understanding single filament turnover. The deep insight gained from such studies reveals that the mechanisms cells use to control actin dynamics may be more universal than previously suspected.

Parameters for individual actin filament turnover in live cells have been measured

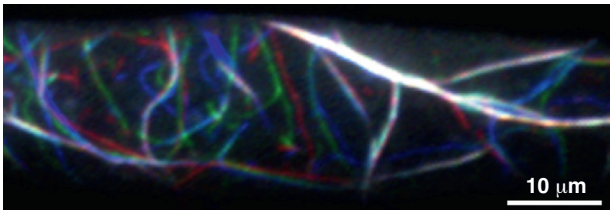
To examine cytoskeletal dynamics in living plant cells, a variety of fluorescent fusion protein reporters of the actin cytoskeleton have been utilized [11]. The original GFP-mouse Talin or GFP-humanTalin constructs have fallen from favor due to their reported stabilizing effects on actin turnover [12–14]. These have been replaced predominantly with fusions containing the second actin-binding domain from *Arabidopsis* FIMBRIN1 (fABD2; [15–17]) or the yeast-based Lifeact reporter [6,7••,18–20]. When combined with these probes, the use of fast, high-resolution imaging such as variable-angle epifluorescence microscopy (VAEM [21]) or spinning disk confocal (SDC) microscopy has dramatically advanced our view of cytoskeletal dynamics (reviewed in [22]). Moreover, several model cell types facilitate high spatial-resolution and temporal-resolution imaging of actin arrays and allow correlations to be made between cytoskeleton dynamics and cell expansion by tip-growth or diffuse growth. These include epidermal cells from the dark-grown hypocotyl and cotyledons of *Arabidopsis thaliana* seedlings [5••,19,23] and protonemal cells of the moss, *Physcomitrella patens* [6,8••]. Hypocotyl epidermal cells also provide the advantage of a deep knowledge about the mechanisms of microtubule dynamics in the cortical array and coupling to the behavior of cellulose synthase complexes that are critical for oriented cell wall deposition (reviewed by [24,25]). Moreover, the actin filaments in the cortical array can be sparsely populated facilitating the tracking of individual filament ends and actin dynamic properties over tens of seconds [5••].

The cortical cytoplasm of *Arabidopsis* epidermal cells contains at least two types of actin filament structure: putative individual filaments and actin filament bundles ([5••,11]; Figure 1). The former type of array was originally referred to as 'fine microfilaments' in epidermal pavement cells [26] and as 'short actin bundles' in pollen tubes [27], but we prefer to refer to them as single or individual actin filaments [5••]. Although the diffraction-mediated limits of fluorescence light microscopy mean that there can be no proof that these dynamic and ephemeral structures represent single filaments, their fluorescence intensity profiles, dynamic properties and sensitivity to latrunculin treatment are all consistent with the conclusion that these are single polymers [5••]. Alternatively, they may represent small bundles of filaments with dynamic ends, like those observed in budding yeast cells [28,29]. Regardless, visualizing the dynamic behavior of these structures by time-lapse fluorescence microscopy has led to testable models for actin turnover in plant cells (reviewed by [2,30,31]).

Several parameters of individual actin filament turnover have been described and measured in the cortical array of epidermal cells [5••,23,32•,33•,34•]; montages with representative filament behaviors from dark-grown hypocotyl epidermal cells are defined and illustrated in Figure 2 [5••,32•,33•]. For this review, we will focus attention on the dynamics of single filaments, rather than actin filament bundles. Whereas the filament bundles are mostly aligned longitudinal to the cell long axis, the network of individual filaments is randomly dispersed in the cortical cytoplasm (Figure 1). Single filaments have lower fluorescence intensity, are more dynamic, and have shorter lifetimes than the bundles [2]. In the rapidly expanding cells of the hypocotyl, the average filament lifetime is on the order of 20 s and they reach average maximum lengths of 12–15 μm before disappearing [2,32•]. New filaments originate or are initiated from three locations (Figure 2a): *de novo* in the cytoplasm ❶; the side of another filament or bundle ❷; or, the end of a pre-existing filament or fragment ❸ [2]. These types of filament initiation occur in varying proportions, with nucleation on the side of a nascent filament usually the most prevalent [33•]. Once an available end exists, filament elongation is rapid ❹. Growth rates in the epidermal cells of the hypocotyl are ~1.7 μm s^{-1} or, in other words, 629 actin monomers add each second onto the presumed barbed-end or growing-end of a filament [2]. This allows filaments to grow from one sidewall of the cell to the other in about 10 s, or from one endwall to another in less than a minute.

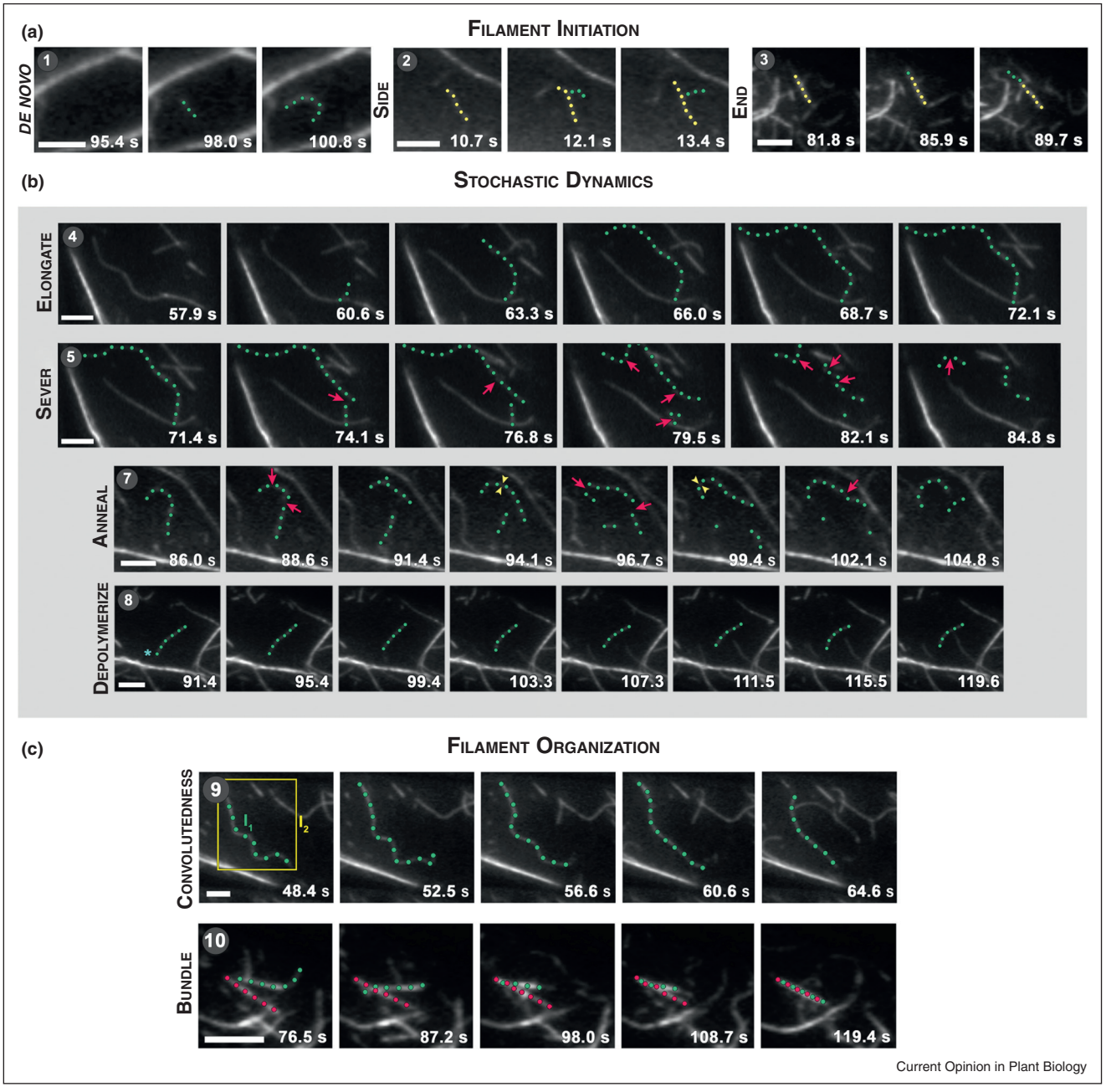
Normally, only one end of filaments in the cortical array is observed to grow at any given time, although occasionally both ends can be seen extending. Unlike the dynamic instability of cortical microtubules [35], growing ends do not typically transition to a depolymerizing phase [5••]. Moreover, filament depolymerization from the non-grow-

Figure 1



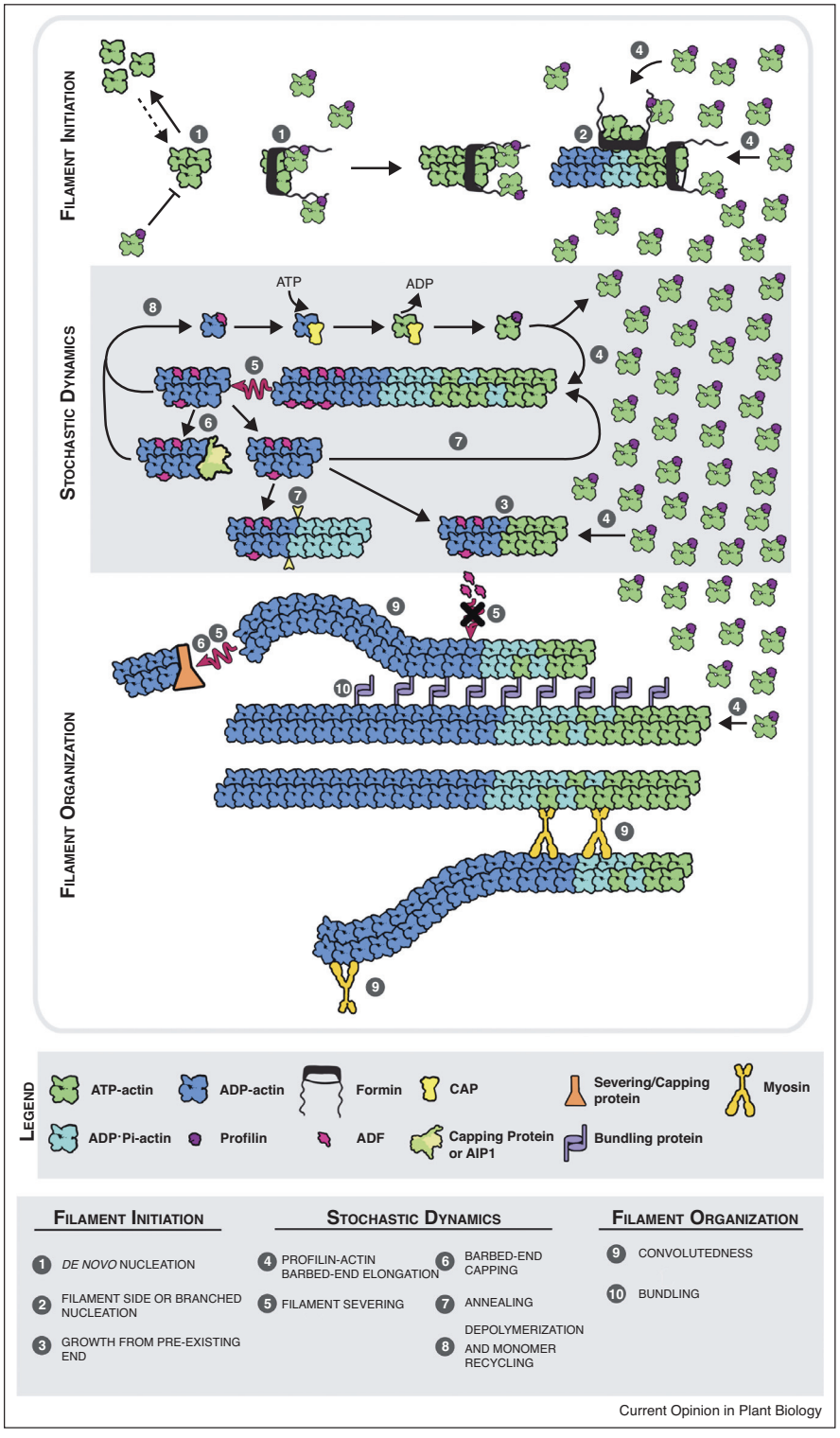
The cortical actin array in epidermal cells from *Arabidopsis* contains two populations of filaments. A timeseries projection is shown for an epidermal cell from the dark-grown hypocotyl of *Arabidopsis* expressing the actin reporter, GFP-fABD2. Time-lapse images were collected by variable angle epifluorescence microscopy (VAEM) at 1 s intervals. Three images separated by 10 s were colored red, green and blue, respectively and the three images superimposed. A white color indicates stable actin structures, like actin filament bundles, that remain relatively stationary during this time period. In contrast, fainter and more ephemeral structures appear, disappear and translocate over this time interval and appear colored in this projection. The latter structures are assumed to be individual actin filaments based on their fluorescence intensity and dynamic properties.

Figure 2



Live-cell imaging of actin filament organization and turnover reveals key features of single filament dynamics. High-resolution, time-lapse VAEM images of the cortical actin array were collected from epidermal cells of *Arabidopsis* hypocotyls expressing GFP-fABD2, as described previously [5••]. Examples of individual filament behaviors are highlighted in the following montages. Bar = 5 μm. **(a)** Actin filament initiation occurs from three distinct origins: 1 *de novo* in the cytoplasm; 2 the side of pre-existing actin filaments or bundles; and 3 pre-existing filament ends. Each representative growing filament is highlighted in green, whereas the pre-existing filament for branched nucleation is shown in yellow 2, as is the pre-existing fragment in 3. **(b)** Key features of single actin filament assembly and turnover by stochastic dynamics are shown in these time-lapse montages: 4 a representative actin filament (green dots) elongates at 2.3 μm s^{-1} and reaches a maximum filament length of 29.6 μm; 5 the same filament is disassembled via numerous severing events (red arrows); 6 recently severed ends rarely re-grow (not portrayed); 7 and sometimes, recently severed filaments re-anneal (yellow arrowheads). 8 Depolymerization, when it can be observed, occurs at a modest rate of 0.2 μm s^{-1} from the pointed-end (blue asterisk). **(c)** Actin filament organization is influenced by buckling or waving, as well as by filament bundling. 9 A representative example of filament buckling and straightening. The highlighted filament becomes more straight (or displays less waviness) over time. A unitless measure of waviness, termed convolutedness, is calculated as the length of the filament (l_1) divided by the longest axis (l_2) of a bounding rectangle (yellow box). 10 An example of actin bundle formation. The highlighted actin filament (green dots) makes contact with a second filament (red dots) then bundles through a zippering mechanism (alternating dots).

Figure 3



A model for actin stochastic dynamics. Based on biochemical properties for several plant actin-binding proteins, estimates of the total concentration of plant actin in cells and *in vivo* observations of actin filaments, this cartoon displays the major features and key molecules that regulate the assembly, disassembly and organization of actin filament arrays in plant cells. The gray box displays the key steps of filament assembly and turnover by stochastic dynamics. The numbering of steps is intended to mirror the live-cell observations of single filament turnover (Figure 2) and quantitative analyses of parameters [5••,32•,33•]. Red squiggly arrows denote filament severing events, which are a major feature of stochastic dynamic turnover. See text for details.

Table 1

Actin dynamic parameters

	Arabidopsis epidermal cell				Tobacco		Arabidopsis pollen[b,f,m]	Moss protonema[b,d,n]
	Hypocotyl[a,d,g]	Cotyledon[b,e,h]	Root[b,e,i]	Guard cell[a,e,j]	Guard cell[c,e,k]	BY-2[b,d,l]		
Elongation rate ($\mu m\ s^{-1}$)	1.7	1.7	0.9	0.3	0.05	0.6	0.3	2.3
Depolymerization rate ($\mu m\ s^{-1}$)	0.2	–	–	–	–	–	0.2	–
Shortening rate ($\mu m\ s^{-1}$)	–	1.2	0.7	0.4	0.02	0.2	–	2.8
Severing frequency								
(breaks/$\mu m\ s^{-1}$)	1.1×10^{-2}	6.4×10^{-5}	5.1×10^{-5}	4.9×10^{-3}	2.0×10^{-3}	–	3.4×10^{-2}	–
(events/$\mu m^2\ s^{-1}$)	–	–	–	–	–	–	–	3.3×10^{-3}
Max. filament length (μm)	14.8	–	–	–	–	–	2.5	–
Max. filament lifetime (s)	15.3	–	–	–	–	–	20.2	–
Regrowth of severed ends (%)	2.9	–	–	–	–	–	–	–
Annealing of ends (%)	2.1	–	–	–	–	–	–	–
Dynamic ends (ends/$\mu m^2\ s^{-1}$)	–	–	–	–	–	–	–	4.2×10^{-3}
Filament origin; *de novo*/ends/side (% per cell)	28/22/50	–	–	–	–	–	–	–
Convolutedness	1.6	1.1	1.1	–	–	–	–	–

Transgenic plants, expressing GFP-fABD2[a], GFP-Lifeact[b] or GFP-mouse Talin[c] actin cytoskeleton reporters, were imaged with variable-angle epifluorescence microscopy (VAEM)[d], confocal laser scanning microscopy (CLSM)[e], or spinning disk confocal (SDC)[f] microscopy. For *Arabidopsis thaliana* seedlings, epidermal cells on five-day-old dark-grown hypocotyls [5••,32•,33•][g]; ten-day-old light-grown cotyledons [7••][h]; four-week light-grown root elongation zone [7••][i]; or rosette leaf guard cells [38][j] were imaged. In tobacco, guard cells from the abaxial surface of 3-week leaves [36][k] or protoplasts prepared from BY-2 suspension cells [7••][l] were used for experiments. For tip-growing cells, *Arabidopsis* pollen tubes [37•][m] germinated *in vitro* were imaged, as were protonemal cells from seven-day-old light-grown moss plants [8••][n], *Physcomitrella patens*.
Metrics for measuring single actin filament turnover were described in [5••,7••,8••,32•,33•]. Elongation rates were determined from the presumed barbed-end of actin filaments, wereas depolymerization rates were measured at the opposite, putative pointed-end of actin filaments. Shortening rates include shrinkage due to subunit loss as well as disassembly due to severing or filament breakage. For most parameters, individual elongating filaments were selected at random and measured [5••,7••,32•,33•]. In moss, severing frequency and the frequency of dynamic ends were quantified as the number of events per area [8••].

ing end is infrequent and occurs at rates of just 0.2–0.3 $\mu m\ s^{-1}$ ❽ [5••,32•]. This argues against a conventional 'treadmilling' model for filament turnover, in which growth at barbed-ends is matched by loss of subunits from the pointed-ends of filaments. Instead, disassembly of the rapidly growing filaments occurs by prolific severing activity ❺ (*red arrows*) [5••]. On average, a typical 10 μm-long filament suffers nine breaks every minute. Newly-severed ends rarely re-grow, with only ~3% elongating after a break [5••,33•]. This suggests that the barbed-end is capped immediately or soon after the severing event ❻ (not shown for technical reasons). Consistent with prominent capping activity, the frequency of filament-filament annealing is also rather low, on the order of 2% of filament ends ❼ (*yellow arrowheads*) [23,33•]. Because they disappear from the field of view quite rapidly, the fate of small fragments generated by severing is not entirely clear. Theoretically, they could return subunits to the monomer pool by slow depolymerization ❽, they might anneal onto filament ends allowing bursts of growth ❼, or they might get uncapped and resume growth by monomer addition at the barbed end ❸. Collectively, this dynamic behavior of individual actin filaments — namely rapid elongation at barbed-ends balanced by filament disassembly through stochastic severing events — has been termed 'stochastic dynamics' (gray box, Figure 2b) [5••,9,10].

Values for the parameters of actin turnover do vary depending on the type and developmental status of the plant cell(s) examined [7••,8••,36,37•,38], but the overall behavior is mostly consistent between different studies (Table 1). Some notable exceptions, however, include the rather low filament elongation rates measured in pollen tubes and guard cells of *Arabidopsis* [37•,38], as well as guard cells of tobacco [36]. In quite a few studies, filament disassembly rates are nearly equal to elongation rates [7••,8••,36,37•,38], perhaps suggesting a treadmilling-like turnover mechanism. However, most of these authors' metrics include filament breakage events, rather than just putative depolymerization at pointed-ends, into a shrinkage or shortening rate [7••,8••]. Besides differences in methodology, values may vary between cell types and organisms due to variation in the amount of monomeric actin available, the ratio of F-actin to G-actin, or the particular constellation of actin-binding proteins and isoforms present in the cytoplasm. Determining whether these different rates of turnover or dynamicity correlate with a specific biological process, or whether particular parameters are predictive of cell expansion for example, requires deeper investigation.

In addition to initiation, elongation and disassembly, growing filaments show additional dynamic behaviors in the cortical array (Figure 2c). Like a snake or a

rubber-band, individual filaments can undergo dramatic contortions or waving ❾; they can also rapidly straighten [5••,7••]. The extent of filament waviness has been captured in a metric termed 'convolutedness' ❾, which is defined as the length of a filament (l_1) divided by the longest axis of a bounding rectangle (l_2) [5••]. Filaments also appear to slide over other filaments or bundles, and can move along the plasma membrane in the absence of other filament–filament contacts [7••]. Finally, higher-order structures can be generated by the lateral association of individual filaments, creating new bundles by a 'catch and zipper' mechanism ❿ [39]. These filament bundles are generally brighter, longer-lived and less dynamic than the individual actin filaments. They also suffer appreciably fewer severing events than do the single filaments. Although powerful tools for quantifying the architecture of actin cytoskeletal arrays from static images have been described [14,40], the development of additional metrics to measure actin bundle formation, stability and turnover from timelapse images is an area requiring future attention [20]. For actin arrays that are too dense to track single actin filaments, a powerful analysis based on the redistribution or change in fluorescent pixel intensities over all temporal pairs from a timelapse series has been developed and allows a unique metric for cytoskeletal dynamics [41]. This correlation coefficient analysis has been applied with great success to describe altered dynamics in myosin XI, ADF, and AIP1 mutants of the moss, *P. patens* [8••,41].

A model for the biochemical regulation of actin stochastic dynamics

Actin purified from plants can assemble rapidly *in vitro*; however, in cells, the dynamic behavior and turnover of filaments is modulated by actin-binding proteins or ABPs. Dozens of plant ABPs have been identified and their *in vitro* properties characterized extensively [1–4,30]. Based on the cellular abundance of actin pools and ABPs, the biochemical activities of ABPs *in vitro*, as well as evidence from reconstituted biomimetic systems that recapitulate mechanisms of filament turnover, a model for actin filament stochastic dynamics can be postulated. Because this topic has been reviewed previously [2,4,31], herein we will only briefly describe the relevant ABP properties and their putative roles in actin stochastic dynamics. The current model is depicted in Figure 3, with specific steps corresponding to the time-lapse montages numbered ❶–❿.

Total actin protein in plant cells is present at 50–200 μM cytosolic concentration; however, most of this actin exists in the monomer pool (G-actin), with only about 5–10% estimated to be in filamentous form (F-actin) (reviewed in [2,30]). This monomer pool is buffered by equally abundant profilin, which forms a 1:1 complex with G-actin. Profilin suppresses filament nucleation and blocks the addition of subunits to pointed ends of filaments; this has the effect of restricting growth of filaments to the barbed-end. Formins and the Arp2/3 complex can use the pool of profilin–actin to nucleate new filaments in the cytosol or on the side of a mother filament or bundle. If barbed ends are free, as in the case of Arp2/3-nucleated filaments, profilin–actin can assemble as well as actin alone. On the other hand, a processive formin that remains associated with the barbed end could accelerate polymerization rates from profilin–actin compared to actin alone. As long as a formin is associated or the barbed-end is uncapped, filament elongation continues at high rates, supported by the large pool of profilin–actin. Disassembly of growing filaments occurs mainly in their aged regions, comprising ADP-actin subunits, and is due to prolific severing activity. Both villins and ADF/cofilins are capable of severing filaments *in vitro* (*red squiggly arrow*), and the activity of ADF/cofilin is further enhanced by the accessory protein AIP1. Whereas ADF/cofilin creates free barbed-ends following severing, villin remains attached and caps the newly-severed barbed end. Additional capping factors include the heterodimeric capping protein (CP) and AIP1. CP is quite abundant in cells and has a high affinity for the barbed-end of filaments, suggesting that most available ends will be quickly capped; it also dissociates from filament ends extremely slowly *in vitro*. Cells likely use multiple capping factors with overlapping activities to regulate dynamics of different regions of networks; for example, AIP1 appears to be involved with the disassembly of aged ADP-F-actin fragments in yeast [42]. The fragments generated by severing could recycle through the monomer pool by the synergistic action of ADF/cofilin, CAP and profilin. Alternatively, some of these fragments might contribute bursts of growth by filament–filament annealing at uncapped ends. Finally, uncapping of filament ends allows new growth to resume from the profilin–actin pool.

Filament waving or sliding is perhaps mediated by the activity of myosin molecular motors of which two classes, myosin XI and VIII, exist in plants [43]. Convolutedness could be generated by endomembrane cargo trafficking along filaments, sliding of filaments along the plasma membrane or adjacent filament bundles [44]. Single filaments are collected into higher-order structures by the action of bundling proteins such as formin, fimbrin, villin, LIM, SCAB1, and EF-1α [4]. Some of these bundles are stable against the severing activity of ADF/cofilin (*red arrow with black X*) and therefore have longer lengths and lifetimes than single filaments.

Pharmacological and genetic analyses implicate ABPs in specific aspects of actin dynamics regulation

Historically, the function of actin cytoskeletal arrays in plants has been tested with small molecule inhibitors like cytochalasins, latrunculin and jasplakinolide, which are known to bind to G-actin or F-actin [45]. New classes of

pharmacological agents that target specific ABPs, or that have undefined targets but impact actin organization or dynamics, have also become available and are powerful tools for dissecting the role of the cytoskeleton in diverse cellular processes. Several of these pharmacological agents have been used to probe actin stochastic dynamics parameters [5••,34•].

Hypocotyl epidermal cells that are treated for short periods with low doses of latrunculin B (LatB) show rapid changes in the dynamics of single actin filaments [5••]. LatB binds to G-actin and inhibits monomer addition onto filament ends; thus, this compound preferentially perturbs dynamic actin filaments. The elongation rate at filament barbed-ends in epidermal cells treated with 100 nM LatB for 10 min is reduced threefold, indicating a substantial reduction in the size of the assembly-competent monomer pool. This has the consequence of reducing the maximum filament length to 4.4 μm in treated cells, but does not significantly alter the severing frequency. Another compound, 2,3-butanedione monoxime (BDM) is a myosin II inhibitor in mammalian cells but also reportedly affects several myosin-dependent activities in plants and dramatically slows actin rearrangements [5••]. The greatest effect of BDM is on filament waviness and translocations; it markedly reduces the rate of change of convolutedness *in vivo*, but also affects severing frequency [5••]. This supports the assertion that filament buckling and bending is a myosin-dependent process; however, a better test would be to use more specific myosin inhibitors or knock-out mutants. Finally, prieurianin (Pri) is a limonoid compound that was originally identified as an inhibitor of endomembrane trafficking in pollen tubes and named endosidin 1 [46]. Although its molecular target is unknown, a recent study demonstrates that Pri stabilizes actin filaments in plant and mammalian cells [34•]. Like BDM, Pri appears to have a rapid and dramatic effect on filament flexibility or convolutedness. However, other stochastic dynamics parameters are also significantly affected by 60 min treatment of hypocotyl epidermal cells with 10 μM Pri, including a 2.2-fold reduction in severing frequency; a 50% reduction in depolymerization rate; and a 60% increase in filament lifetimes.

In addition to the advantages for imaging and quantifying cytoskeletal dynamics, plants also provide powerful genetic tools to reduce or eliminate specific ABPs [47,48]. Both *Arabidopsis* and *Physcomitrella* have been used to establish the role of particular ABPs in actin stochastic dynamics [8••,32•,33•,37•]. For example, the role of ADF/cofilin was demonstrated by examining the phenotypes associated with a knock-out mutation for *ADF4* [49], a ubiquitously-expressed but minor isovariant in *Arabidopsis* [50]. Loss of ADF4 resulted in a 2.5-fold reduction in severing frequency, but had little impact on the depolymerization rate for single filaments in the cortical array of hypocotyl epidermal cells [32•]. Consequently, the maximum filament lengths and lifetimes of growing filaments were significantly increased; amazingly, some individual filaments could be observed to grow to 40–70 μm in length before suffering a few breaks. Although it is widely assumed that ADF/cofilin functions in eukaryotic cells primarily via the severing of actin filaments, these studies on plant cells provide the first genetic evidence directly supporting this hypothesis [8••,32•]. Similarly, villins which are actin filament severing and bundling proteins in *Arabidopsis* [39,51,52], have been demonstrated to regulate filament breakage in pollen tubes [37•].

Biochemically, the actin-interacting protein (AIP1) is known to enhance the activity of ADF/cofilin *in vitro* [53]. This was demonstrated in living plant cells by an elegant genetic approach targeting the single *AIP1* gene in *P. patens* [8••]. The *Δaip1* knock-out plants created by homologous recombination accumulated actin bundles and had markedly altered filament dynamics. Specifically, the frequency of severing events per unit area of cortical cytoplasm, as well as the number of dynamic filament ends, was reduced by 3.5 fold. The *Δaip1* phenotypes could be partially rescued by overexpression of ADF. By contrast, knocking down expression of the single *ADF* gene in *Physcomitrella* [54] almost completely obliterates actin filament dynamics [8••]. This work further demonstrates the critical nature of filament severing in the dynamic remodeling of actin cortical arrays.

Regulation of filament barbed-end availability is predicted to be a key factor in funneling growth to particular actin filaments and keeping the abundance of F-actin low in cells [55,56]. The heterodimeric CP has these properties *in vitro* [55,57], and recently knock-down mutants with insertions in both *Arabidopsis CP* genes (*cpa* and *cpb*, respectively) were used to characterize the role of filament capping *in vivo* [33•]. As expected, reduction in CP levels led to an increase in actin filament abundance in hypocotyl and root epidermal cells, consistent with an overall enhancement of polymerization from profilin–actin due to the greater availability of barbed ends. Stochastic dynamics parameters also reveal a significant increase in filament ends, including more filaments that originate from pre-existing ends and up to sixfold increase in filament-filament annealing. Similar to loss of ADF4, this results in longer maximum filament lengths and increased lifetimes for growing filaments. Collectively, these data show that CP functions *in vivo* to keep actin filaments short and short-lived.

Finally, the preliminary characterization of *Arabidopsis* FORMIN1 mutants (*fh1*) demonstrates a role for filament nucleation in organizing the cortical array [58]. In root epidermal cells of *fh1* mutants, the density of actin filament arrays is reduced and bundling increases. Moreover,

these cytoskeletal phenotypes are mimicked by treating roots with a small molecule formin inhibitor (SMIFH2 [59]). Understanding which population of filament origins is mediated by FORMIN1 or whether different classes of growing ends are due to processive elongation versus addition of free actin monomers awaits measurement of stochastic dynamics parameters in the *fh1* mutants. Because of potential redundancy in the large gene family of *Arabidopsis* formins, this may be better evaluated in *Physcomitrella*, where there are only two class II formins [60•]. These are essential for polarized growth and were elegantly demonstrated with a functional For2A-GFP fusion protein to nucleate new filaments and then processively elongate actin filaments or bundles at rates of 1.8 $\mu m\ s^{-1}$ [60•]. However, whether class II formins are the main molecular player for generating and elongating new filaments was not tested directly. Finally, whether filaments can be nucleated by the Arp2/3 complex in plant cells *in vivo* remains undetermined. Surprisingly, analysis of stochastic dynamics parameters in guard cells of *Arabidopsis arp2* and *arp3* mutants revealed an overall increase in actin dynamics, with elevated assembly and disassembly rates and a nearly twofold increase in severing frequency [38]. More work is obviously required to determine if this represents some sort of compensation by the mutant plants, or if these changes result from loss of Arp2/3 nucleation activity.

Future directions

The ability to resolve individual actin filaments using state-of-the-art imaging modalities has allowed the generation of a testable model (Figure 3) for regulation of actin cytoskeleton turnover in plant cells. Although aspects of this model vary quantitatively from cell type to cell type, or under different developmental conditions (Table 1), three consistent features are noteworthy: barbed ends of filaments elongate at high rates; the stochastic severing of aged filaments underpins filament disassembly; and strict regulation of barbed-end availability controls the number of growing filaments in the cortical array. The role of key ABPs has been elucidated with molecular precision using the power of reverse-genetic analyses in both *Arabidopsis* and *Physcomitrella*. In the future, it should be possible to predict the biochemical properties of novel ABPs or dissect the signaling intermediates that impinge on actin turnover by evaluating single actin filament parameters in appropriate mutant lines. A looming unanswered question is: Why do plant cells expend such a large proportion of their cellular energy (ATP) to incessantly remodel the cortical array? One hypothesis is that this is a surveillance mechanism to sense cellular stress at the level of the plasma membrane–cell wall continuum [5••]. Small changes in ABP activities or altering single parameters of turnover, for example inhibiting severing activity, could result in the generation of new actin arrays within seconds. How individual filament dynamics change in response to attack by pathogenic microbes, how this is choreographed with the vesicle trafficking necessary for cell wall assembly, and how cytoskeletal remodeling occurs following abiotic stress will provide fruitful lines of investigation in the future.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

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