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REVIEW PAPER

Regulation of actin dynamics by actin-binding proteins in pollen

Christopher J. Staiger[1,*], Natalie S. Poulter[2], Jessica L. Henty[1], Vernonica E. Franklin-Tong[2] and Laurent Blanchoin[3]

[1] Department of Biological Sciences and Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907-2064, USA
[2] School of Biological Sciences, University of Birmingham, Edgbaston B15 2TT, UK
[3] institut de Recherches en Technologies et Sciences pour le Vivant, iRTSV, Laboratoire de Physiologie Cellulaire Végétale, CNRS/CEA/INRA/UJF, F-38054 Grenoble, France

* To whom correspondence should be addressed: staiger@purdue.edu

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Abstract

A dynamic network of polymers, the actin cytoskeleton, co-ordinates numerous fundamental cellular processes. In pollen tubes, organelle movements and cytoplasmic streaming, organization of the tip zone, vesicle trafficking, and tip growth have all been linked to actin-based function. Further, during the self-incompatibility response of *Papaver rhoeas*, destruction of the cytoskeleton is a primary target implicated in the rapid cessation of pollen tube growth and alterations in actin dynamics are associated with the initiation of programmed cell death. Surprisingly, these diverse cellular processes are accomplished with only a small amount of filamentous actin and a huge pool of polymerizable monomers. These observations hint at incredibly fast and complex actin dynamics in pollen. To understand the molecular mechanisms regulating actin dynamics in plant cells, the abundant actin monomer-binding proteins, a major filament nucleator, a family of bundling and severing proteins, and a modulator of growth at the barbed-end of actin filaments have been characterized biochemically. The activities of these proteins are generally consistent with textbook models for actin turnover. For example, the three monomer-binding proteins, profilin, ADF, and CAP, are thought to function synergistically to enhance turnover and the exchange of subunits between monomer and polymer pools. How individual actin filaments behave in living cells, however, remains largely unexplored. Actin dynamics were examined using variable angle epifluorescence microscopy (VAEM) in expanding hypocotyl epidermal cells. Our observations of single filament behaviour are not consistent with filament turnover by treadmilling, but rather represent a novel property called stochastic dynamics. A new model for the dynamic control of actin filament turnover in plant cells is presented.

Key words: ADF/cofilin, actin, actin-binding proteins, cytoskeleton, pollen, profilin, signalling.

The actin cytoskeleton: general features and functions

The cytoskeleton comprises two highly-conserved and ubiquitous polymers, called microtubules and actin filaments, respectively. The actin cytoskeleton is a network of individual, cross-linked and/or highly bundled filamentous structures. In plant cells, these continuously remodelled arrays power intracellular locomotion and serve as cellular motorways for the transport of various organellar cargos, including mitochondria (Van Gestel *et al.*, 2002; Sparkes *et al.*, 2008), ER (Quader *et al.*, 1987; Lichtscheidl and Hepler, 1996; Boevink *et al.*, 1998; Yokota *et al.*, 2009), Golgi stacks (Boevink *et al.*, 1998; Nebenführ *et al.*, 1999), peroxisomes (Collings *et al.*, 2002; Jedd and Chua, 2002; Mathur *et al.*, 2002), and chloroplasts (reviewed by Wada *et al.*, 2003; Gabrys, 2004; Takagi *et al.*, 2009). Actin filaments are also implicated in vesicle trafficking between endomembrane compartments, especially to and from the plasma membrane (Geldner *et al.*, 2001; Grebe *et al.*, 2003; Kim *et al.*, 2005; Dhonukshe *et al.*, 2008; Wightman and

Turner, 2008; Gutierrez *et al.*, 2009). It is widely accepted that many of these movements are due to the mechano-chemical enzyme, myosin, and its ability to couple the hydrolysis of ATP with changes in conformation and binding state on the actin filament (Shimmen and Yokota, 2004; Shimmen, 2007). This allows myosin bound to various membrane-bound compartments and vesicles to carry these cargos along actin filament cables. Compelling genetic and cell biological support for this model comes from detailed analyses of single- and double-mutant knock-outs, as well as dominant-negative mutants, of class XI myosins in *Arabidopsis* (Avisar *et al.*, 2008, 2009; Peremyslov *et al.*, 2008; Prokhnevsky *et al.*, 2008; Sparkes *et al.*, 2008; Yokota *et al.*, 2009). In addition to propelling organelles and vesicles, actin filaments can also build a framework around structures or allow polar transport and positioning of organelles at particular subcellular locales. In this regard, the identification of an actin-binding protein (ABP) associated with the outer envelope of chloroplasts, CHUP1, provides intriguing evidence for a role of actin polymerization in positioning organelles (Oikawa *et al.*, 2003, 2008; Kadota *et al.*, 2009). Actin filaments and the structures they form are also responsible for maintaining cellular architecture by contributing to vacuolar and transvacuolar strand dynamics (Staiger *et al.*, 1994; Verbelen and Tao, 1998; Higaki *et al.*, 2006; Sheahan *et al.*, 2007). Finally, actin has been implicated in the regulation of cell growth for both diffuse expansion of somatic cells and localized or tip growth of pollen tubes and root hairs (reviewed by Smith and Oppenheimer, 2005; Hussey *et al.*, 2006; Szymanski and Cosgrove, 2009). Only in tip-growing cells can a solid case be made for actin contributing to cell expansion by guiding secretory vesicles and regulating docking and fusion at the plasma membrane (see below).

Individual actin filaments (F-actin) are assembled from monomeric (G-) actin subunits in a process that occurs spontaneously in the test tube, but is regulated in the cytoplasm by a host of actin-binding proteins (see section below on ABPs; Hussey *et al.*, 2006; Staiger and Blanchoin, 2006)). Monomeric actin is an asymmetric, 42 kDa polypeptide with four subdomains organized around a deep cleft that contains nucleotide- and divalent cation-binding sites (Kabsch *et al.*, 1990). The rate-limiting step for polymerization is the formation of a seed or nucleus of two or three actin subunits. After nucleation, further elongation occurs rapidly. Filaments are 7–9 nm diameter, double-stranded chains of subunits with a right-handed helical twist and 13 subunits per twist (Oda *et al.*, 2009). Each filament has a characteristic polarity conferred by the end-to-end assembly of asymmetric monomers and by differences in the nucleotide-loaded state of the subunits along the length of the filament. All filament growth and shrinkage occurs at filament ends, rather than along their length. ATP–G-actin adds preferentially to filament plus- or barbed-ends and nucleotide hydrolysis followed by P_i release lags behind assembly, resulting in a predicted ATP-ADP·P_i–cap at the filament growing ends. Older portions of the filament are comprised mainly of ADP–actin subunits and the loss of ADP subunits occurs from minus- or pointed-ends. At equilibrium, a dynamic steady-state is established whereby subunit addition at barbed-ends is exactly balanced by subunit loss from pointed ends—a process referred to as 'treadmilling'. Most models for actin dynamics within cells, for example, within the dendritic array of filaments at the leading edge of crawling animal cells, invoke some variation on the actin treadmilling mechanism for the organization, maintenance, and turnover of filament arrays.

Actin in pollen and pollen tubes: the normal situation

Perhaps the closest plant equivalent to a crawling animal cell is the pollen tube of angiosperms (see Supplementary Video S1 at *JXB* online). This tip growing protuberance of the male gametophyte, or pollen grain, has to extend through sporophytic tissue for a distance of up to 30 cm, often in a period of less than a day, to effect double-fertilization of the embryo sac. In the case of the maize pollen grain, the elongation rate *in planta* is reportedly as high as 1 cm h^{-1} (Kiesselbach, 1949), but is several orders of magnitude slower *in vitro* (8 μm min^{-1}; Gibbon *et al.*, 1999). This remarkable growth presumably requires a huge investment in new cell wall and plasma membrane materials, as well as energy to supply the vectorial delivery of the secretory vesicles to the growing apex. Live-cell imaging of growing pollen tubes reveals several other amazing features of the pollen tube (see Supplementary Video S1 at *JXB* online); the organelles and vesicles show marked cytoplasmic zonation, with large organelles excluded from the apical clear zone; prominent cytoplasmic streaming of the large organelles exhibits a 'reverse-fountain' pattern; and periods of rapid growth alternate with periods of no or slow growth, giving an oscillatory pattern to the elongation rate.

Several decades of cytological and pharmacological studies by numerous research groups have implicated the actin cytoskeleton and filament turnover as the central players in co-ordinating many of these pollen tube processes (for reviews see Taylor and Hepler, 1997; Cole and Fowler, 2006; Cheung and Wu, 2008; Yang, 2008). Indeed, careful examination of pollen tubes from a variety of species, both on fixed cells as well as living cells expressing fluorescent fusion protein reporters, gives a consensus view of actin filaments being arrayed into at least three distinct structures (Kost *et al.*, 1998; Fu *et al.*, 2001; Lovy-Wheeler *et al.*, 2005; Cheung *et al.*, 2008; Vidali *et al.*, 2009*a*). Examples of this are illustrated in Fig. 1, with pollen tubes from corn (*Zea mays*; Fig. 1a) and the field poppy (*Papaver rhoeas*; Fig. 1b) that have been stained with rhodamine-phalloidin to decorate F-actin (see also Gibbon *et al.*, 1999; Geitmann *et al.*, 2000; Snowman *et al.*, 2002; Thomas *et al.*, 2006*b*). Prominent throughout the cytoplasm along the length of the pollen tube are numerous, longitudinal actin cables or bundles. Based on polarity determination in root hairs, which also support reverse-fountain streaming, bundles in the cortical cytoplasm are likely to have the filament

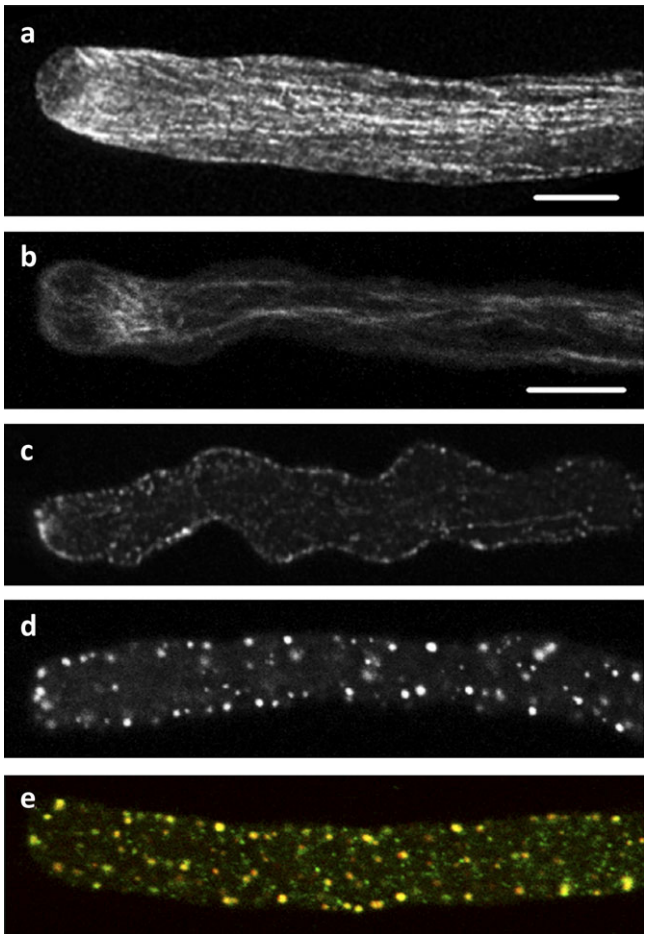

Fig. 1. Actin filament arrays in maize and poppy pollen tubes, and alterations induced by self-incompatibility (SI). (a) F-actin arrays in a *Zea mays* (maize) pollen tube. The typical organization of F-actin is indicated: the bracket shows the dense sub-apical F-actin 'collar' region; A, apical region (with a fine actin meshwork); SA, sub-apical region; SH, 'shank' region with long F-actin longitudinal bundles. (b) F-actin arrays in a *Papaver rhoeas* (poppy) pollen tube. The typical organization of F-actin is indicated, as in (a) for comparison. (c) Typical F-actin arrays in a poppy pollen tube 10 min after SI induction. F-actin organization is greatly altered, with loss of large bundles and with a marked cortical array evident. (d) F-actin arrays in a poppy pollen tube 3 h after SI induction. F-actin organization is further altered; formation of large punctate F-actin aggregates is typical. (e) Adenylate cyclase-associated protein (CAP1) is co-localized with the large F-actin foci in a typical poppy pollen tube at 1 h after SI induction. CAP1, green; F-actin, red; co-localization, yellow. Images in (a) to (d) are F-actin visualized with rhodamine phalloidin. The image in (e) is a merged image of F-actin detected using rhodamine phalloidin and CAP1 using immunolocalization with CAP antisera. All images, except (a), are single optical sections obtained with confocal imaging. Scale bars in (a) and (b) are 10 μm.

plus-ends facing the apex, whereas bundles in the centre of the tube likely have the plus-ends facing the pollen grain (Tominaga *et al.*, 2000). Both populations of bundles terminate well short of the pollen tube tip. In the subapical region, beginning roughly 1–5 μm from the extreme apex, is a dense collar of cortical actin filaments that extends basally for 5–10 μm. In different species, this subapical array assumes slightly different morphologies and has also been referred to as a cortical fringe (Lovy-Wheeler *et al.*, 2005). The exact nature of the organization and polarity of actin filaments within this subapical array have not yet been elucidated. The apical region is not devoid of actin filaments, but probably contains a population of dense, randomly-oriented individual filaments at or near the diffraction-mediated limit of resolution for light microscopy.

If one were to ascribe functions to these pollen tube actin filament arrays, it is most acceptable to suggest that the axial cables provide the tracks for organelle movements and cytoplasmic streaming. The apical and subabpical tip arrays are somewhat more problematic, but likely play a role in organizing the cortical cytoplasm and regulating vesicle trafficking to and from the tip zone. Both the actin cables and the subapical, cortical fringe also appear to be involved in movements of ER and mitochondria (Lovy-Wheeler *et al.*, 2007). Compelling evidence that the apical and subapical actin arrays are critical for pollen tube growth comes from treatments with the actin-monomer binding drug, latrunculin B (LatB), which inhibits tip growth with IC_{50} values of 2–7 nM for lily, maize, and *Tradescantia* pollen (Gibbon *et al.*, 1999; Vidali *et al.*, 2001). These low doses of LatB perturb the tip actin organization without markedly altering cytoplasmic streaming or the axial, actin cables, providing indirect evidence for rapid turnover of apical and subapical actin filaments. This is because LatB does not cause actin depolymerization by stimulating loss of actin subunits from filaments, but rather binds to monomeric actin and prevents its addition to the plus-end of filaments. Thus, actin filaments that turn over rapidly are prevented from growing and instead shrink from their ends, whereas stable filaments that are capped at one or both ends are relatively resistant to LatB.

The dynamic tip actin filament arrays play a role in co-ordinating secretory vesicle docking and fusion at the apex, as demonstrated recently by the elegant time-lapse and photobleaching studies of Zhenbiao Yang and coworkers (Hwang *et al.*, 2008; Lee *et al.*, 2008). The authors report that actin polymerization is necessary for secretory vesicles to accumulate in the apical inverted cone and actin depolymerization is necessary for vesicle docking or fusion at the plasma membrane (Lee *et al.*, 2008). Some alternative models suggest that actin polymerization contributes directly to pollen tube extension by pushing on the plasma membrane (Mathur, 2005) or permits the pollen protoplast to adhere and 'crawl' along the cell wall analogous to animal cells moving over an extracellular matrix (Lord *et al.*, 1996). These models seem rather implausible given that the growth of pollen tubes and plant cells is constrained by a semi-rigid cell wall. In all likelihood, turgor pressure is the likely driving force for growth, with actin contributing via the delivery of new plasma membrane and polysaccharides that expand the cell wall by intercalation of new polymers amongst old (Szymanski and Cosgrove, 2009). Other functions for actin filaments are certainly

possible; nonetheless, it is not too outrageous to claim that actin-based function in pollen tubes requires precise control over local actin turnover.

Indeed, time-lapse imaging of fluorescent fusion protein reporters for actin filaments suggest that actin polymerization or localization oscillates in the tip zone (Fu *et al.*, 2001; Hwang *et al.*, 2005; Cheung and Wu, 2008). Actin accumulation in the extreme apex occurs in advance of growth events, and cortical actin bundles alternate between the cortex of the apical dome and regions immediately adjacent to the apex (Fu *et al.*, 2001; Hwang *et al.*, 2005). Key players in the organization, formation, and oscillatory turnover of actin arrays are likely to include, ABPs (see later) and a battery of signalling molecules and second messengers (Cheung and Wu, 2008; Cole and Fowler, 2006; Yang, 2008). Several molecules with demonstrated oscillatory localization or activity—for example, cytosolic Ca^{2+}, pH, and Rop GTPases—are the most viable candidates for regulating actin dynamics. As described below, the tip-high, oscillatory cytosolic Ca^{2+} gradient (Holdaway-Clarke *et al.*, 1997; Messerli and Robinson, 1997) could mediate actin depolymerization through the action of ABPs like profilin and gelsolin. The subapical alkaline band (Feijó *et al.*, 1999; Lovy-Wheeler *et al.*, 2006) could regulate the activity of actin depolymerizing factor (ADF). Tip-localized, dynamic Rop GTPase signalling probably operates through two key effector proteins, RIC3 and RIC4, that have antagonistic functions (Gu *et al.*, 2005; Hwang *et al.*, 2005). RIC4 stimulates actin polymerization by an unknown mechanism, whereas RIC3 is responsible for Ca^{2+}-mediated actin disassembly (Gu *et al.*, 2005; Yang, 2008). The co-ordinated action of these pathways that impinge on actin dynamics and provide an integrated feedback system has been referred to as 'localization-enhancing network, self-sustaining' or LENS (Cole and Fowler, 2006).

Surprisingly, all of the actin-based functions in pollen are accomplished with just a modest amount of F-actin. In the first study of its kind, Gibbon *et al.* (1999) reported on levels of F-actin in pollen and pollen tubes of maize using a quantitative assay developed for measuring polymer levels in yeast and mammlian cells. Total actin available for polymerization in pollen tubes is abundant and on the order of 100–125 μM cellular concentration (Gibbon *et al.*, 1999); this value is quite consistent with estimates from animals and microbes (Pollard *et al.*, 2000), but somewhat higher than data from lily pollen where total actin is estimated to be 25 μM (Vidali and Hepler, 1997). However, polymeric actin levels in maize pollen represent just a small proportion of the total, with estimated levels of 12–15 μM actin in filamentous form (Gibbon *et al.*, 1999). Thus, only ~10% of actin in pollen is polymerized at any given time. These results were confirmed and extended by quantitative analyses of poppy pollen and pollen tubes; the estimate for total actin in poppy is 250–300 μM, whereas actin in filamentous form is just 11–15 μM (Snowman *et al.*, 2002). By comparison, a 'typical' mammalian cell like unactivated human platelets, or crawling *Acanthamoeba* and *Dictyostelium* cells, contain about half of their actin in filamentous form and half in the monomer pool (Pollard *et al.*, 2000). At the other end of the spectrum, the budding yeast *Saccharomyces cerevisiae* has a very modest total actin pool, estimated at about 2 μM, but virtually all of it is in polymeric form (Karpova *et al.*, 1995; Kim *et al.*, 2004). By extrapolation from the differences between the F-actin and total actin population, pollen must maintain a huge pool of monomeric actin available for polymerization (Gibbon *et al.*, 1999; Snowman *et al.*, 2002; Staiger and Blanchoin, 2006). Because the rate of actin polymerization in the test tube is proportional to the rate constant for addition at the plus-ends and the G-actin concentration (Pollard *et al.*, 2000; Staiger and Blanchoin, 2006), it is possible to estimate how quickly filaments would grow in pollen tubes under these circumstances. Using the association rate constant for ATP-loaded rabbit muscle α-actin (k_+=11.6 μM^{-1} s^{-1}) and the assumption that 370 subunits comprise 1 μm of actin filament length (Pollard, 1986; Pollard *et al.*, 2000), 100 μM actin would assemble at rates of ~3 μm s^{-1}. This is truly explosive growth and reveals the potential for a huge pool of subunits to assemble into new arrays at a precise location within seconds. Moreover, it is at least an order of magnitude faster than the extension of the pollen tube tips under *in vitro* growth conditions, indicating that actin polymerization rates are more than sufficient to keep up with tube growth.

Actin depolymerization and rearrangements during the SI response of poppy

It has been argued that the eukaryotic actin cytoskeleton is an excellent sensor and transducer of environmental signals into changes in cell behaviour, morphology or intracellular functions (reviewed by Machesky and Insall, 1999; Staiger, 2000). Angiosperm tip-growing cells are no exception to this assertion. Indeed, some of the most rapid and dramatic responses to biotic and abiotic stimuli are reported in pollen grains and root hairs, where controlled actin polymerization seems to be the rule. One noteworthy exception, in which destruction of the actin cytoskeleton is a key feature of the biological response to signalling events, is the self-incompatibility (SI) response of poppy pollen.

SI is a genetically-determined surveillance mechanism during which pollen is inhibited from germinating and/or growing within female tissues of the same genotype, thereby preventing self-fertilization. In *P. rhoeas*, this system is gametophytically controlled and involves specific interactions between the pistil and pollen *S*-determinants. The pistil *S*-determinants (PrsS; stigma S) are novel, small proteins secreted by the pistil (Foote *et al.*, 1994); the pollen *S*-determinants (PrpS; pollen S) are novel, small transmembrane proteins that bind to the PrsS (Wheeler *et al.*, 2009). Their interaction appears to be a receptor–ligand type of interaction, as it triggers rapid increases in intracellular free Ca^{2+} levels specifically in incompatible pollen tubes (Franklin-Tong *et al.*, 1993), resulting in the cessation of incompatible pollen tube growth within minutes.

Recognizing that increased cytosolic Ca^{2+} leads to inhibition of cytoplasmic streaming and tip growth in pollen

by altering cytoskeletal organization (Kohno and Shimmen, 1987; Kohno and Shimmen, 1988), it was necessary to examine whether actin was a target for SI-induced signalling pathways. Localization studies on fixed, incompatible pollen tubes revealed dramatic alterations in the actin cytoskeleton that correlate with cessation of growth (Fig. 1; Geitmann *et al.*, 2000; Snowman *et al.*, 2002). Within 5–10 min after incompatible recombinant PrsS treatment, many of the axial F-actin cables are lost or diminished in intensity, and the subapical actin collar disappears in pollen tubes (Fig. 1c). The remaining actin is predominantly in the cortical cytoplasm and sometimes accumulates at the apex. At later stages, actin foci appear and increase in size over time (Fig. 1d). Importantly, none of these changes are observed in pollen tubes treated with compatible PrsS. To evaluate whether the dramatic changes in organization are caused by actin filament disassembly rather than re-distribution, the quantitative assay of Gibbon *et al.* (1999) was applied to populations of pollen and pollen tubes treated with compatible or incompatible PrsS (Snowman *et al.*, 2002). Significant reductions in F-actin levels could be detected within 1 min of SI and total polymer dropped by 69% after 10 min. These low levels of actin polymer are maintained for at least an hour, and similar changes could be induced with Ca^{2+} ionophore and a G-protein agonist. Interestingly, not only is actin a target for SI, but the microtubule cytoskeleton is also rapidly and dramatically altered in incompatible pollen tubes (Poulter *et al.*, 2008). Microtubules appear to be downstream of actin, however, as treatments with LatB cause apparent microtubule depolymerization and jasplakinolide (JASP) treatments alleviate or delay microtubule disassembly during SI (Poulter *et al.*, 2008). Collectively, these results demonstrate that SI specifically induces actin depolymerization in incompatible poppy pollen tubes and that cessation of growth is mediated, at least partially, by destruction of the cytoskeleton.

Early events during SI result in the inhibition of germination or tube growth, but are followed by irreversible steps that ensure the destruction of incompatible pollen. Specifically, the SI signalling pathway triggers a caspase-mediated programmed cell death (PCD) cascade in incompatible pollen tubes (Thomas and Franklin-Tong, 2004; Bosch and Franklin-Tong, 2007). In eukaryotic cells as diverse as budding yeast and mammalian cultured cells, changes in actin dynamics are capable of eliciting PCD (Franklin-Tong and Gourlay, 2008), making it worthwhile to examine whether the same is true for poppy pollen. Short-term LatB treatments, which cause dose-dependent reductions in actin filament levels (Snowman *et al.*, 2002) and presumably dampen filament dynamics, trigger activation of a caspase-3-like activity in poppy pollen and result in DNA fragmentation (Thomas *et al.*, 2006*b*). This demonstrates that actin destruction is sufficient to induce PCD in pollen tubes. To evaluate whether this destruction is essential for PCD induction during the SI response, pollen tubes were pretreated with the actin-stabilizing drug, JASP, and then subjected to incompatible PrsS treatment. JASP on its own stabilizes actin filaments in poppy pollen tubes, causing extensively bundled actin arrays to fill the cytoplasm and inhibit tube growth. Importantly, SI-stimulated PCD in incompatible pollen tubes is alleviated by pretreatments with 0.5 μM JASP, providing the first evidence that changes in actin dynamics or polymer levels are necessary and sufficient for the induction of PCD in pollen tubes. Whether this causal relationship exists in other plant cells is an open question; however, it is noteworthy that a dominant-negative *ACTIN2* allele reportedly leads to cell death in *Arabidopsis* root trichoblasts (Nishimura *et al.*, 2003).

Although actin filament levels do not return to normal as PCD ensues, actin is organized in a rather unusual configuration that begs further study. Phalloidin-staining reveals that actin filaments accumulate into actin punctae or foci in incompatible poppy pollen tubes (Fig. 1c, d); these first appear at ~10 min after SI induction and increase in size and intensity, but decrease in number, over the next several hours (Geitmann *et al.*, 2000; Snowman *et al.*, 2002; Poulter *et al.*, 2010). These foci appear to be rather stable or non-dynamic structures because they are not disrupted by treatments with 1 μM LatB for 30 min (Poulter *et al.*, 2010). Conversely, the large actin foci do not represent non-specific aggregates of F-actin as they are not induced with JASP treatments (Thomas *et al.*, 2006*b*). These unusual structures are superficially similar to 'F-actin bodies' in quiescent budding yeast cells (Sagot *et al.*, 2006) and to Hirano bodies found in animal cells and *Dictyostelium* undergoing neurodegenerative diseases or cellular stresses (Bamburg and Wiggan, 2002). Because these structures contain unique suites of ABPs—F-actin bodies are enriched for fimbrin and heterodimeric capping protein, whereas Hirano bodies are associated with ADF/cofilin—it was necessary to analyse which ABPs might localize to the SI-induced actin foci. A quantitative analysis of actin–ABP co-localization revealed the association of ADF and adenylate cyclase-associated protein (CAP1) with actin foci (Fig. 1e; Poulter *et al.*, 2010). This was somewhat surprising, because ADF and CAP are two key mediators of actin filament depolymerization or turnover (see below). Moreover, an actin filament bundling and stabilizing protein, fimbrin, is absent from actin foci, as is the monomer-binding protein, profilin. These findings hint that pollen actin foci are more similar in structure and function to Hirano bodies than they are to yeast F-actin bodies. How these intriguing actin foci form and whether they are a cause or consequence of PCD remains to be determined.

Major pollen actin-binding proteins (ABPs) and their functions

In order to understand how pollen actin turnover is regulated *in vivo*, it is first necessary to have detailed knowledge about the biochemical properties, cellular abundance, and localization of the key actin-binding proteins (ABPs). In eukaryotic cells, more than 70 classes of ABP have been identified (Kreis and Vale, 1999; Pollard *et al.*, 2000) and an ever expanding subset of these is present in angiosperm pollen (see also Ren and Xiang, 2007; Cheung

and Wu, 2008). These factors exert distinct, but often overlapping effects, on actin organization and polymerization. *Monomer-binding proteins* regulate the size and activity of the actin subunit pool. *Nucleation factors* overcome the rate-limiting step for actin assembly and generate seeds that support subsequent elongation. *Capping proteins* bind with high affinity to filament ends and prevent subunit loss and addition, as well as inhibiting filament–filament annealing. *Severing proteins* create breaks in the filament backbone, generating new ends for assembly or disassembly. *Side-binding proteins* contribute to the formation of higher-order structures by stabilizing actin filaments and/or bundling and cross-linking adjacent polymers. These ABPs are also reliable sensors and transducers of signalling cascades, as their activities are almost always regulated by Ca^{2+}, pH, and phospholipids. Several excellent reviews deal with ABP function in plants and the reader is referred to these for additional information (Hussey *et al.*, 2006; Staiger and Blanchoin, 2006; Thomas *et al.*, 2009). The general properties and pollen-specific characteristics of several central regulators of actin dynamics are highlighted here.

Monomer-binding proteins and nucleation factors

The first ABP identified in angiosperm pollen, profilin, was discovered as an allergen from birch trees (Valenta *et al.*, 1991). Profilins are low molecular weight proteins that bind to G-actin with 1:1 stoichiometry and form moderate affinity profilin–actin complexes (Valenta *et al.*, 1993; Gibbon *et al.*, 1998). In pollen, profilin is present at levels equimolar with total actin and has an estimated cellular concentration of 25–200 μM (Vidali and Hepler, 1997; Gibbon *et al.*, 1999; Snowman *et al.*, 2002). Immunocytochemistry and microinjection of fluorescent analogues reveal that profilin is a uniformly-distributed, cytosolic protein (Vidali and Hepler, 1997). The high concentration of profilin and its affinity for ATP–G-actin lead to the prediction that most pollen actin will be present as profilin–actin complex (Gibbon *et al.*, 1999; Snowman *et al.*, 2002; Staiger and Blanchoin, 2006). This complex prevents spontaneous nucleation of new actin filaments and suppresses addition at filament minus-ends. These properties are likely to make a significant contribution to the low level of polymeric actin in pollen. However, when uncapped actin filaments are present, profilin shuttles actin subunits onto filament barbed-ends and contributes to elongation. In contrast, when the barbed-end of filaments is capped, profilin acts like a simple sequestering protein. Several models for actin filament turnover suggest that profilin plays an additional role, as a catalyst for nucleotide exchange on ADP–G-actin that serves to recharge subunits with ATP. Plant profilins do not have this capability, however, even when supplied with actin from a plant source (Perelroizen *et al.*, 1996; Kovar *et al.*, 2001). This might be because nucleotide exchange is not important due to the high endogenous rate of turnover on native pollen actin (Kovar *et al.*, 2001), or because other cellular factors have assumed this role (Chaudhry *et al.*, 2007).

In plants, another abundant monomer-binding protein, the adenylate cyclase-associated protein or CAP1, binds with moderate affinity to G-actin (Barrero *et al.*, 2002; Chaudhry *et al.*, 2007; Deeks *et al.*, 2007). CAP1 binds with equal affinity to ATP–G- and ADP–G-actin, which contrasts with yeast CAP (Srv2p) that has a marked preference for ADP–G-actin (Chaudhry *et al.*, 2007). Importantly, CAP1 directly enhances nucleotide exchange on actin, by more than 50-fold. It also has a weak ability to shuttle subunits onto the plus-end of filaments. Thus, plant CAP appears to have assumed several functions typically associated with profilin in other organisms and is likely to be a key regulator of actin dynamics, albeit by a molecular mechanism unique to plants. Loss-of-function *cap1* mutant *Arabidopsis* plants have significant defects in pollen germination and tube growth, consistent with a major role in regulating actin dynamics in tip-growing cells (Deeks *et al.*, 2007). In root hairs from *cap1* mutants, the normal actin cables are absent and are replaced with short actin bundles that accumulate in the cortical cytoplasm and at the apex, suggesting major defects in actin filament turnover (Deeks *et al.*, 2007). However, the nature of actin organization and dynamics in mutant *cap1* pollen, or the subcellular distribution and concentration of CAP is not presently known.

A second essential function for profilin is binding to proteins with contiguous stretches of proline residues. This presumably serves to localize profilin to specific subcellular locations where actin polymerization is required. One such class of profilin-interacting protein is formin (reviewed by Deeks *et al.*, 2002; Blanchoin and Staiger, 2008). Formins are major actin filament nucleation factors, comprised of a conserved, proline-rich formin-homology 1 (FH1) domain and an FH2 domain. The FH2 domain is essential for actin filament nucleation, whereas the FH1 domain recruits profilin–actin complexes to the assembly machine. In addition to nucleating filament formation, many formins are processive assembly motors, remaining attached to the plus-end as they supply new monomers to the elongating filament. A subset of the plant formins (Type I) have putative transmembrane domains, suggesting that they are targeted to cellular membranes where they generate new actin filaments (Cvrcková *et al.*, 2004; Blanchoin and Staiger, 2008). The best-characterized plant formin, *Arabidopsis* FORMIN1 or AtFH1, has the ability to nucleate actin filaments from actin alone or from profilin–actin complexes (Michelot *et al.*, 2005). It also bundles actin filaments and can nucleate new daughter filaments from the side of existing bundles (Michelot *et al.*, 2005, 2006). Surprisingly, time-lapse total internal reflection fluorescence microscopy (TIRFM) experiments reveal that AtFH1 is not a processive assembly motor (Michelot *et al.*, 2006). Instead, AtFH1 seems to operate by a mechanism whereby, after nucleating a new filament, it moves to the side of the mother filament where it can nucleate additional filaments or crosslink adjacent filaments into bundles (Michelot *et al.*, 2006; Blanchoin and Staiger, 2008).

In many organisms, a wealth of genetic evidence supports a role for formins in the generation of actin cables or

bundles. Consistent with this, ectopic expression of full-length AtFH1 in tobacco pollen leads to the formation of supernumerary cables of actin filaments and plasma membrane invaginations (Cheung and Wu, 2004). Similar results are obtained when AtFH8 is overexpressed in *Arabidopsis* root hairs (Yi *et al.*, 2005). Two very recent reports examine loss-of-function *formin* mutants and confirm a role for formins in regulating actin dynamics during tip growth of plant cells. The first study used a powerful RNAi strategy to knock down several type II formins in the moss, *Physcomitrella patens*, and demonstrates a function for this class of nucleator in organizing the actin cytoskeleton and maintenance of tip growth (Vidali *et al.*, 2009*b*). The type II formins are targeted to the apical domain via a PTEN-like domain located amino terminal of the FH1-FH2 domains. Moreover, TIRFM experiments reveal these moss formins to be the fastest processive assembly motors yet reported and this is a property of their FH1-FH2 domains. Although identifying and characterizing formin mutants in *Arabidopsis* was suggested to pose a significant challenge, based on the large number of formins expressed in pollen and the likelihood for functional redundancy (Blanchoin and Staiger, 2008), a second major study overcame these problems with an RNAi approach. Yang and coworkers knocked down AtFH3 in pollen tubes of *Arabidopsis* and demonstrate the requirement of this formin in the generation of actin filament cables that support streaming and polarized growth (Ye *et al.*, 2009). Indirect evidence for the ability of AtFH3 to nucleate actin assembly in pollen comes from measurements of polymer levels, which are reduced by more than half in the RNAi lines (Ye *et al.*, 2009). AtFH3 shares many biochemical features with other *Arabidopsis* formins (Ye *et al.*, 2009), however, it will ultimately be important to assess directly whether it or any of the pollen-expressed formins are processive assembly factors. If they work like AtFH1 (Michelot *et al.*, 2005, 2006), then it is probable that pollen formins will generate actin cables that have their growing plus-ends located distal to the plasma membrane, in contrast to current models that show the opposite (Ren and Xiang, 2007; Cheung and Wu, 2008).

A third actin-monomer binding protein, actin depolymerizing factor (ADF), is a central regulator of actin dynamics in numerous eukaryotic systems (for reviews see Maciver and Hussey, 2002; Staiger and Blanchoin, 2006; Bamburg and Bernstein, 2008). ADFs bind to both G- and F-actin, with a marked preference for ADP–G-actin (Carlier *et al.*, 1997; Blanchoin and Pollard, 1999), and disassemble actin filaments by a complex mechanism. This preference for ADP–actin suggests that ADF will be targetted to older filaments or regions of filaments within actin arrays. Some evidence supports a model whereby ADFs bind preferentially to ADP-loaded filaments and increase the dissociation rate of monomers from the minus-end (Carlier *et al.*, 1997). More recent data from time-lapse TIRFM demonstrates unambiguously the capacity of ADF to disassemble filaments through severing activity (Andrianantoandro and Pollard, 2006). Surprisingly, the same study shows another side of ADF. This protein can nucleate actin filaments when present at high concentrations (Andrianantoandro and Pollard, 2006); therefore, it becomes critical to know the cellular concentration of ADF under all circumstances. Plant ADFs have typical biochemical properties compared with animal and lower eukaryotic homologues (Gungabissoon *et al.*, 1998, 2001; Ressad *et al.*, 1999; Smertenko *et al.*, 2001; Chen *et al.*, 2004; Schüler *et al.*, 2005; Chaudhry *et al.*, 2007), but lily pollen ADF has rather low actin disassembly activity *in vitro* and may be preferentially associated with F-actin (Smertenko *et al.*, 2001; Allwood *et al.*, 2002).

The properties of ADF are modulated via pH, phosphorylation, phosphoinositides, and other ABPs. For example, ADF activity in plant cells is pH dependent (Gungabissoon *et al.*, 2001; Allwood *et al.*, 2002); at alkaline pH, it has high depolymerizing activity; under acidic conditions, it binds F-actin. Phosphorylation of plant ADF on a conserved, N-terminal serine residue results in a loss of actin-binding, although the endogenous kinase(s) involved has not yet been identified (Allwood *et al.*, 2001). Phosphorylated forms of ADF have been reported in both tobacco and lily pollen, and phospho-ADF accumulation depends on Rac/Rop activity (Chen *et al.*, 2003). Finally, the phosphoinositide lipid, PtdIns(4,5)P_2, binds to ADF resulting in inactivation of membrane-associated ADF; and, conversely, ADF can affect polyphosphoinositide turnover by inhibiting phospholipase C activity (Gungabissoon *et al.*, 1998). This could be an important mode of regulation for ADF at the extreme apex of pollen tubes, where PtdIns(4,5)P_2 is abundant (Kost *et al.*, 1999; Helling *et al.*, 2006).

Although the cellular concentration of ADF in pollen has not been reported, it is likely to be an abundant cytoplasmic protein, similar to the situation in *Arabidopsis* leaf and suspension-cultured cells where ADF is present at equimolar ratios with total actin (Chaudhry *et al.*, 2007). In *Narcissus* pollen grains, ADF is localized on prominent actin filament rods and cables (Smertenko *et al.*, 2001). Upon germination, however, ADF is generally cytoplasmic in the pollen tube (Smertenko *et al.*, 2001). Caution must be taken when interpreting this result, as it may be an artefact due to inadequate fixation methods (Lovy-Wheeler *et al.*, 2005). In lily and tobacco pollen, both GFP-ADF and immunocytochemistry with state-of-the art preservation methods and anti-ADF sera decorate actin filaments and show an accumulation of ADF in the cortical cytoplasm of the subapical region (Chen *et al.*, 2002, 2003; Lovy-Wheeler *et al.*, 2006; Wilsen *et al.*, 2006). ADF is recruited to this region by the oscillatory alkaline band (Lovy-Wheeler *et al.*, 2006). In the authors' model, ADF features as a central player regulating the turnover of actin filaments in the cortical fringe by enhancing polymerization at alkaline pH and destabilizing filaments under neutral or acidic pH conditions (Lovy-Wheeler *et al.*, 2006). The induction of polymerization under alkaline pH is predicted to depend upon ADF creating new filament ends by fragmentation, with addition onto those free ends coming from the large pool of actin monomers or profilin–actin complexes in pollen (see above). The mechanism of filament

destabilization is somewhat less well defined, and ignores the knowledge that actin *in vitro* polymerizes more rapidly under acidic conditions (Zimmerle and Frieden, 1988). Regardless, ADF is certain to be a key player in the oscillatory behaviour of cortical actin filaments in the apical and subapical region, but additional evidence for this will require simultaneous imaging of actin dynamics and pH oscillations *in vivo*. Moreover, much could be learned from reverse-genetic experiments with *adf* loss-of-function mutants, especially if attention is focused on the pollen-specific, class IIa genes, *ADF7* and *ADF10* (Pina *et al.*, 2005; Ruzicka *et al.*, 2007).

A simple model for the turnover of actin filaments depends on the synergistic activity of the three monomer-binding proteins: profilin, CAP, and ADF. Many years ago it was reported that ADF and profilin co-operate *in vitro* to enhance the flux of subunits through filaments by up to 75-fold (Blanchoin and Pollard, 1998; Didry *et al.*, 1998). Based on the monomer-binding and nucleotide exchange properties of CAP, it seems probable that this ABP acts as an intermediary between ADF-mediated disassembly and profilin-based shuttling of ATP-G-actin onto filament barbed-ends. Experimental support for this is provided by Chaudhry *et al.* (2007), where the three proteins are demonstrated to enhance turnover by 42-fold over actin alone.

Capping proteins

The turnover of actin filaments is also modulated by a class of proteins that bind and cap filament ends, called capping proteins. The best characterized of these proteins in plants is the heterodimeric capping protein from *Arabidopsis* (AtCP) (Huang *et al.*, 2003, 2006). AtCP binds with nanomolar affinity to filament plus-ends and prevents subunit loss and addition at those ends (Huang *et al.*, 2003). It also inhibits end-to-end annealing of filaments (Huang *et al.*, 2003) and competes with formin for binding at filament ends (Michelot *et al.*, 2005). As outlined above, in a situation where all available plus-ends are capped, profilin behaves like a simple monomer-sequestering protein (Huang *et al.*, 2003). Thus, we predict that the presence of CP in pollen is another contributing factor in providing a large monomer pool and small filament pool (Staiger and Blanchoin, 2006). Although the concentration of CP in pollen is not known, based on estimates from other plant tissues and non-plant systems, it is likely to be in the micromolar range (JC Jiménez-López *et al.*, unpublished data; Pollard *et al.*, 2000). This should be more than sufficient to cap all available filament ends, which probably exist at nanomolar concentrations considering that pollen contains just 10–15 μM actin in a filamentous form. Once bound to filament ends, CP dissociates rather slowly with a half-time for uncapping of ~40 min (Huang *et al.*, 2006). This indicates that regulation of uncapping will be an important facet of controlling actin dynamics. In other systems, several CP-interacting proteins have been identified; in some cases, their interaction leads to CP being removed from filament ends (Cooper and Sept, 2008). No such proteins from plants or pollen have been identified yet; nevertheless, AtCP is negatively regulated by phospholipids. In addition to PtdIns(4,5)P_2, AtCP binds phosphatidic acid (PtdOH) with low affinity and PtdOH releases CP from filament plus-ends (Huang *et al.*, 2003, 2006). Given the cellular abundance of PtdOH, this is likely to be the more physiologically-relevant lipid interaction. When PtdOH levels are increased artificially in pollen, actin filament levels increase in a dose-dependent manner, consistent with filament uncapping and polymerization at plus-ends from the profilin–actin pool (Huang *et al.*, 2006). PtdOH has been implicated as a regulator of pollen tube growth via modulation of vesicle trafficking, regulating the levels of phosphoinositides, and maintaining the tip-focused cytosolic Ca^{2+} gradient (Potock'y *et al.*, 2003; Monteiro *et al.*, 2005*a*, *b*). Another possibility is that lipid binding targets CP to particular membranes or organelles; this scenario is supported by immunolocalization studies on tobacco and lily pollen tubes as well as on *Arabidopsis* leaf epidermal cells (JC Jiménez-López *et al.*, unpublished data). The nature of the compartment and the role of CP association in organelle dynamics remain to be determined. Finally, CP function in pollen may be redundant with other classes of ABP that share similar biochemical properties; this is because single- and double-mutant *cp* plants show little or no disruption to pollen viability, *in vitro* germination and tube growth, or fertilization rates (X Wang *et al.*, unpublished data).

Side-binding and severing proteins

Higher-order actin filament structures, like the prominent actin cables in pollen tubes, require cross-linking or bundling proteins for their formation and stabilization. In plants, the major filament-bundling factors are fimbrins, villins, and LIM domain proteins (reviewed by Thomas *et al.*, 2009). Although fimbrins are bona fide bundling and cross-linking factors (Kovar *et al.*, 2000), there is little knowledge about their presence, abundance, and biological function in pollen. Microarray data predict that AtFIM3, FIM4, and FIM5 are expressed in pollen, with FIM4 and FIM5 being most abundant (Pina *et al.*, 2005); however, genetic evidence for their function in actin organization and pollen function has yet to be obtained. The recent identification of SB401 from *Solanum berthaultii* adds a fourth player to the collection of bundling factors in pollen (Huang *et al.*, 2007). This protein was originally identified as a microtubule-associated protein, but is capable of binding and bundling actin filaments *in vitro*. Although it predominantly decorates the cortical microtubules in pollen tubes, SB401 has the potential to be an important facilitator of microtubule–actin crosstalk in pollen.

Villins were the first actin filament-bundling proteins identified from plants, through the biochemical *tour de force* of Teruo Shimmen and Etsuo Yokota that used many grams of germinated Easter lily (*Lilium longiflorum*) pollen as starting material (Nakayasu *et al.*, 1998; Yokota *et al.*, 1998; Yokota and Shimmen, 1999). Pollen-135-ABP and

P-110-ABP were purified to homogeneity and shown to bundle actin filaments in a calcium- and calmodulin-sensitive manner (Yokota *et al.*, 1998, 2000, 2003; Yokota and Shimmen, 1999). Specific antisera facilitated the cloning of appropriate cDNAs from a lily pollen expression library, resulting in the identification of 135-ABP and 115-ABP as homologues of the conserved villin/gelsolin family of proteins (Vidali *et al.*, 1999; Yokota *et al.*, 2003). The antisera were also used to localize lily villins along actin cables in pollen tubes and root hairs (Yokota *et al.*, 1998, 2003; Vidali *et al.*, 1999; Tominaga *et al.*, 2000;). Experimental support for villin function in cable formation or maintenance comes from studies of *Hydrocharis* and *Arabidopsis* root hairs; microinjected villin antibodies disrupt the longitudinal actin filament bundles and transvacuolar strands (Shimmen *et al.*, 1995; Tominaga *et al.*, 2000; Ketelaar *et al.*, 2002). Additional villin-related polypeptides with lower molecular weights have been identified recently from *Lilium davidii* and *L. longiflorum* pollen (Fan *et al.*, 2004; Xiang *et al.*, 2007; Wang *et al.*, 2008*b*). These proteins may be splice variants of villins or proteolytically-processed villin isoforms generated from the full-length ABP. Nevertheless, they are able to disrupt actin cable maintenance, tip growth, and organization of the tip zone following overexpression by microinjection or bombardment into pollen tubes (Fan *et al.*, 2004; Xiang *et al.*, 2007; Wang *et al.*, 2008*b*).

Villins share a conserved set of domains with mammalian gelsolin, a Ca^{2+}-activated filament severing protein (reviewed by Yin, 1999; Khurana and George, 2008). The core of gelsolin, comprising six repeating units of a conserved gelsolin-homology domain (G1–G6), is capable of binding to and severing actin filaments in a Ca^{2+}-dependent fashion. Instead of creating two new free filament ends like ADF, gelsolin stably caps the filament plus-end following severing and prevents subunit loss or addition. Villins share this gelsolin core and also have a C-terminal villin headpiece (VHP) domain. The VHP contributes a second actin-binding site that allows villins to crosslink or bundle filaments. Most villins, with the notable exception of *Drosophila* QUAIL (Mahajan-Miklos and Cooley, 1994), retain the ability to bind monomeric actin, sever and cap filaments. *Arabidopsis* has five full-length *VILLIN* genes (*AtVLN1–5*) and several putative splice variants (Klahre *et al.*, 2000; Huang *et al.*, 2005). AtVLN1 appears to be a simple filament-bundling protein; its activity is not suppressed by Ca^{2+} or Ca^{2+}-calmodulin, and it stabilizes actin filaments against ADF-mediated destruction *in vitro* (Huang *et al.*, 2005). This is probably due to the poor conservation of Type I and Type II Ca^{2+}-binding sites in the *VLN1* sequence. AtVLN3, on the other hand, has five out of the eight conserved binding sites and has Ca^{2+}-activated filament severing activity *in vitro* (P Khurana *et al.*, unpublished data).

Although the presence of Ca^{2+}-mediated, actin filament severing activity in pollen had been proposed more than two decades ago (Kohno and Shimmen, 1987, 1988), an 80-kDa ABP isolated from *P. rhoeas* pollen (PrABP80) was the first example of a plant protein with *bona fide* filament severing activity (Huang *et al.*, 2004). Mass-spectrometry and antibody cross-reactivity data indicate that this protein is a member of the villin/gelsolin family. PrABP80 also does not bundle actin filaments, indicating that it contains only the gelsolin core, and hence it was named poppy gelsolin. In concert with profilin, poppy gelsolin mediates Ca^{2+}-induced depolymerization of actin filaments. These biochemical properties make it a likely candidate for SI-stimulated actin depolymerization *in vivo*, but further experimental support for this hypothesis needs to be obtained. The low molecular weight villin/gelsolins from lily pollen also sever actin filaments (Fan *et al.*, 2004; Xiang *et al.*, 2007; Wang *et al.*, *2008b*), as perhaps does ABP135 (Yokota *et al.*, 2005), suggesting that these ABPs are responsible for actin turnover in response to the oscillatory Ca^{2+} gradient at the tip of pollen tubes. In this regard, LdABP41 shows a pronounced accumulation in the tip of lily pollen, rather than along actin cables, and antibody microinjection perturbs tip growth (Fan *et al.*, 2004). Reverse-genetic analysis of the *Arabidopsis VLN* gene family, especially the pollen-specific VLN5 isovariant (Pina *et al.*, 2005), offers great potential for further understanding the role of villins in tip growth.

A third class of actin-bundling factors, with the potential to modulate actin dynamics and sense oscillatory signals in pollen, is the LIM protein family (reviewed by Thomas *et al.*, 2009). LIM domains are conserved, cysteine- and histidine-rich modules of ~55 amino acids that form a tandem zinc-finger structure. They are present in a wide variety of eukaryotic proteins and typically mediate protein–protein interactions and subcellular targeting. A pollen-specific PLIM-1 was originally discovered as a cDNA from sunflower (Baltz *et al.*, 1992), and its protein product localizes to cytoplasmic foci concentrated at the germination sites of pollen grains (Baltz *et al.*, 1999). No evidence was provided for a direct interaction between PLIM1 and actin filaments, or for actin-dependent localization in pollen. A tobacco homologue, WLIM1, is expressed throughout the plant body and comprises just two LIM domains (Thomas *et al.*, 2006*a*). Recombinant WLIM1 binds directly to actin filaments with moderate affinity, bundles filaments into higher-order structures, and stabilizes filaments against depolymerization (Thomas *et al.*, 2006*a*). A single LIM domain is sufficient to bind actin filaments, but the two domains of WLIM1 have different affinities for actin and probably cooperate to enhance binding and bundling (Thomas *et al.*, 2007). Overexpression of WLIM1 in *N. benthamiana* leaves induces massive actin cable formation, indicating that it has the potential to function as a bundling protein *in vivo*.

Another pollen-enriched LIM protein was recently identified from *L. longiflorum* (Wang *et al.*, 2008*a*). Recombinant LlLIM1 binds to actin filaments, induces bundle formation, and stabilizes actin against LatB-mediated disassembly *in vitro*. Interestingly, its F-actin binding is enhanced by low pH and low Ca^{2+} conditions. A GFP-LlLIM1 fusion protein reportedly decorates all actin filament arrays in pollen tubes, including axial bundles, the cortical actin fringe, and a network of fine filaments in the

apex. Similarly, a tobacco pollen LIM protein, NtPLIM2b, fused to GFP and stably expressed in tobacco pollen predominantly decorates the axial actin cables and a subtle subapical array (Cheung *et al.*, 2008). Transient overexpression of LlLIM1 in lily pollen results in the partial inhibition of both germination and tube growth (Wang *et al.*, 2008*a*). Moreover, pollen tube growth is sometimes abnormal with occasional multiple tubes emerging from the same grain and some swollen tubes. Overexpression of LlLIM1 also perturbs actin organization in pollen tubes, resulting in the formation of asterisk-shaped actin arrays in the subapical regions. The formation of these structures is oscillatory with their presence correlating with periods of slow growth. Moreover, ectopic overexpression of LlLIM1 disrupts the normal localization of PtdIns(4,5)P_2 and DAG on pollen membranes, causes Golgi to congregate in the subapical region, and perturbs vesicle accumulation in the apex. These phenotypes are interpreted by the authors as excessive bundling and stabilization of actin filaments in the tip region, leading to impaired vesicle trafficking. Analysis of knock-out or knock-down mutants, as well as localization of the endogenous protein in the pollen tube apical and subapical regions, would provide further compelling evidence for pollen LIM's important functions in transducing cytosolic oscillations into changes in actin dynamics and endomembrane trafficking.

Stochastic dynamics in the cortical array of epidermal cells

To understand with molecular precision how the actin cytoskeleton operates in pollen and pollen tubes, it will be necessary to translate information about the biochemical properties and regulation of ABPs into a clear picture of where each major ABP is located, where they are active, and how they behave within living cells. Coupled to this unfulfilled knowledge gap, it will also be essential to have high spatial and temporal resolution images of individual actin filaments and their turnover. Although great progress has been made toward these goals, especially using fluorescent fusion proteins to report on actin, ABP, and organelle dynamics in living pollen tubes (Kost *et al.*, 1998; Cheung, 2001; Fu *et al.*, 2001; Wilsen *et al.*, 2006; Cheung *et al.*, 2008; Wang *et al.*, 2008*a*), much remains to be learned about how actin filaments behave and are organized to accomplish their myriad functions. One major limitation has been the lack of a functional, directly-tagged fluorescent fusion protein for actin. To some extent, this has been alleviated by use of ABPs or actin-binding domains to report on the presence of actin arrays. As noted earlier, ADF, fimbrin ABD2, vertebrate talin, and LIM proteins have all been used to report actin dynamics or localization in pollen (Cheung, 2001; Fu *et al.*, 2001; Kost *et al.*, 1998; Wilsen *et al.*, 2006; Cheung *et al.*, 2008; Wang *et al.*, 2008*a*). Each probe has its limitations; however, the fluorescent fusion proteins offer the only means available at present to assess these vital processes directly. Notably, none of these previous reporters reliably appears to mark all three pollen actin arrays, and some of them lead to artefactual stabilization of actin filaments and bundles or they perturb tip growth (Wilsen *et al.*, 2006; Cheung *et al.*, 2008). Recently, a new reporter, called Lifeact, comprising the first 17 amino acids of *S. cerevisiae* ABP-140 was expressed as an EGFP fusion protein in lily and tobacco pollen and appears to decorate all of the dynamic actin arrays, including the subapical cortical fringe (Vidali *et al.*, 2009*a*). A second significant limitation of imaging actin filament dynamics *in vivo* has been the use of laser scanning confocal microscopy to examine structures which are rearranging rapidly and/or below the diffraction-limited resolution of the light microscope. For studies of microtubule dynamics *in vivo*, spinning disk confocal microscopy has come to the forefront as the technique of choice (Shaw *et al.*, 2003; Paredez *et al.*, 2006; Chan *et al.*, 2007). Even more recently, a variation on TIRF microscopy, called variable-angle epifluorescence microscopy (VAEM) has demonstrated its usefulness for imaging dynamic membrane-associated and cytoskeletal events at the plasma membrane of plant cells (Konopka *et al.*, 2008; Konopka and Bednarek, 2008*a*, *b*).

To examine actin turnover in living cells at high spatial and temporal resolution, VAEM was applied to epidermal cells from dark-grown hypocotyls of *Arabidopsis* seedlings expressing the GFP-fABD2 reporter (Staiger *et al.*, 2009). The same cell type has been used extensively for studies of microtubule dynamics, cellulose synthase translocation, and endomembrane trafficking (Shaw *et al.*, 2003; Paredez *et al.*, 2006; Gutierrez *et al.*, 2009). Further, a well-characterized developmental gradient of cell expansion (Gendreau *et al.*, 1997) allows one to select cells from the apical portion of the hypocotyl that are rapidly elongating. In elongating cells, cortical microtubules are co-aligned and oriented in arrays perpendicular to the direction of cell expansion (Shaw *et al.*, 2003). Individual microtubules exhibit dynamic instability at both plus- and minus-ends, but growth at the plus-end dominates loss of polymer at the minus-ends, leading to an overall behaviour termed 'hybrid treadmilling'. Extension rates at microtubule plus-ends during the growing phase are 3–5 μm min^{-1}. In marked contrast to the predictable organization and turnover of microtubules, the cortical actin filament array in these epidermal cells is complex and undergoes constant rearrangement. Filaments are mostly randomly arranged, with the exception of some of the massive actin cables that have a net axial orientation, and their appearance/distribution changes dramatically between time-lapse images collected at intervals of 1–3 s (Fig. 2; Staiger *et al.*, 2009).

Actin-based structures decorated with GFP-fABD2 are distributed into two classes based on fluorescence intensity, lifetime, and dynamic properties (Staiger *et al.*, 2009). The brighter, thicker structures are less dynamic, they can be tracked for long periods, are less wavy, and appear to undergo few growth or shrinkage events. We assume that these are actin filament bundles or cables that turn over slowly. By contrast, the second population of cortical actin filaments has an average fluorescence intensity that centres

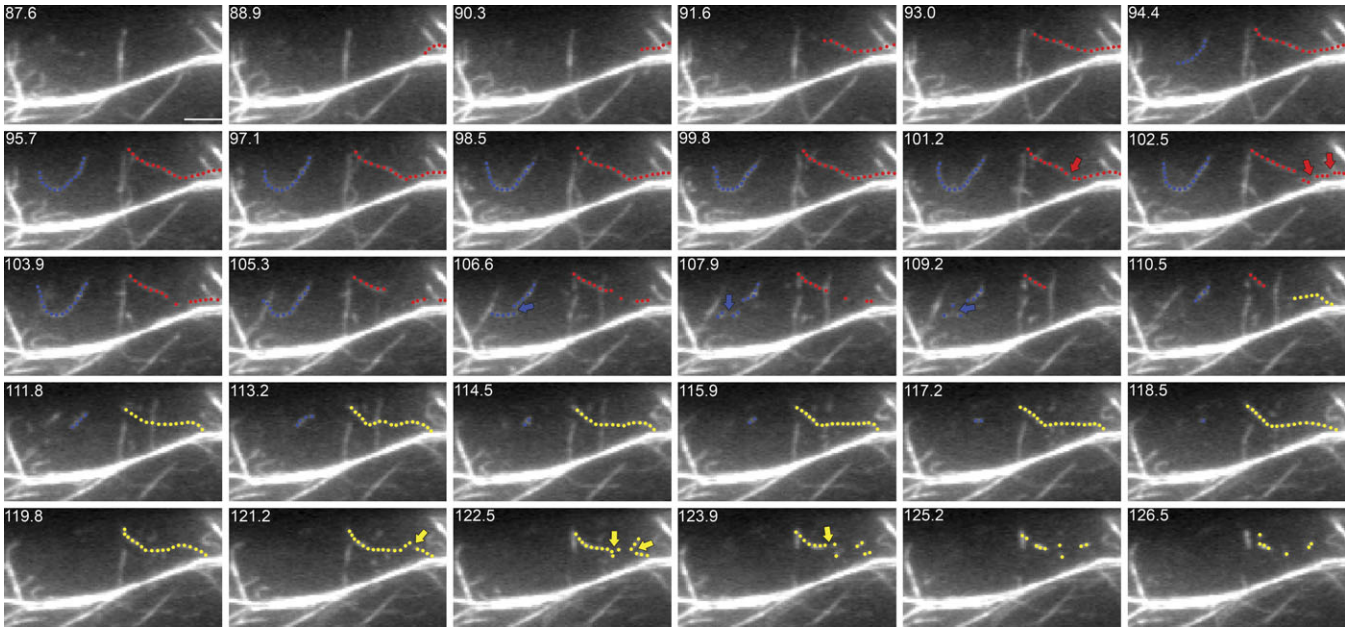

Fig. 2. Actin stochastic dynamics in hypocotyl epidermal cells. Actin filaments display dynamic instability in *Arabidopsis thaliana* hypocotyl epidermal cells as reported by GFP-fABD2 expression. A montage of successive frames at ~1.5 s intervals shows several actin filaments (marked with red, blue, and yellow dots) elongating rapidly. Each highlighted filament is then fragmented (coloured arrows) into many short pieces, with the red and blue filaments disappearing completely. See also Supplementary Video S2 at *JXB* online. Time points indicate elapsed time from start of video sequence. Bar, 5 μm.

on a value of ~1000 au, and the intensity of single filaments nearly doubles when they cross over similar structures. This population is assumed to represent individual actin filaments, based on their overall appearance and dynamic properties occuring at the ends and along their length. For quantitatively assessing parameters of actin turnover and dynamics, we focused on these putative single filaments and examined the behaviour of dozens of examples in numerous cells from multiple hypocotyls. Individual actin filaments are continously appearing and disappearing from the cortical cytoplasm, and their appearance changes dramatically over time (see Supplementary Video S2 at *JXB* online).

One prominent feature of individual filaments is continuous buckling or waving along their length. The converse activity, rapid filament straightening is also observed, albeit not as frequently as waving. To assess this property, a term, convolutedness, was defined that is the filament length divided by the length of the longest axis from a bounding rectangle. Straight filaments have a value of 1, whereas wavy filaments have convolutedness values >1. The rate of change in convolutedness for individual filaments versus actin cables was also documented. Individuals have both greater convolutedness values and rates of change in convolutedness than do the actin cables. Filament buckling and straightening events are possibly driven by myosin activity, because they are inhibited by brief treatments with 2,3-butanedione monoxime (BDM). The mechanism may involve filament–filament sliding, filament–membrane interactions, or both. Because there are questions about the specificity of BDM for plant myosins (McCurdy, 1999), it will be of interest to confirm these results with analysis of myosin mutants or the dominant-negative constructs mentioned above (Avisar *et al.*, 2008, 2009; Peremyslov *et al.*, 2008; Prokehnevsky *et al.*, 2008; Sparkes *et al.*, 2008; Yokota *et al.*, 2009).

The second significant feature of individual actin filaments is their rapid appearance and disappearance (Fig. 2; Staiger *et al.*, 2009). New filaments originate from three locations: *de novo* in the cytoplasm, from the side of pre-existing filaments or cables, and from short fragments or recently broken ends. Indeed, observations of filament breakage events that subsequently extend from that newly-created end, provide additional evidence that single actin filaments are being visualized. The three categories of filament origin, occur at nearly equal frequency, with a slight advantage to events that occur from the side of a mother filament. Filament growth is phenomenally fast, with extension occuring at rates of 1.7 μm s^{-1} (Fig. 2). (Remember, microtubule plus-ends grow at rates of just microns per minute.) We infer that this is subunit addition at filament plus-ends, because the elongation rate is inhibited in a dose-dependent manner by brief treatments with LatB. Rapid growth allows an actin filament to span the short axis of a cell in ~10 s. Filament shrinkage, by contrast, is almost an order of magnitude slower and is not an obvious feature of most filaments. Growing filaments reach an average, maximum length of ~15 μm before they disappear. Instead of depolymerization at their ends, single actin filaments are disassembled by prolific severing activity (Fig. 2). A 10 μm long filament suffers, on average, six breaks every minute. The resulting picture is of filaments that are constantly being nucleated from several different locations in the

cortical cytoplasm, they grow rapidly from one end, and are subsequently destroyed by fragmentation. What happens to the fragments and how their subunits are recycled back into the monomer pool remains a mystery, because they can only be imaged for a few frames before they disappear from the field of view. This actin turnover mechanism, with predictable and rapid filament growth balanced by random severing events, is referred to as 'stochastic dynamics'. Significantly, all of the features of actin turnover by stochastic dynamics can be re-constituted with a simple *in vitro* motility system (Michelot *et al.*, 2007). This biomimetic system comprises a processive formin attached to a plastic bead, with a pool of profilin–actin supplying monomers for rapid extension at plus-ends located on the surface of the bead. The inclusion of ADF results in populations of rapidly-growing individual filaments that are constantly trimmed at their older, presumably ADP-actin containing, regions distal to the bead.

Whether actin turnover by stochastic dynamics occurs in other plant cells remains on open question. Nevertheless, the parameters of growth rate and severing activity are indistinguishable in elongating versus non-elongating epidermal cells from the hypocotyl (Staiger *et al.*, 2009). Clearly, actin filaments in the cortical cytoplasm of tip-growing moss cells undergo constant remodelling and prominent buckling activity has been observed (Vidali *et al.*, 2009*a*). In their original studies of actin dynamics *in vivo*, Yang and coworkers report oscillations of short actin bundles (SAB) that move in and out of the cortical cytoplasm at the apex and subapical region of pollen tubes in concert with growth (Fu *et al.*, 2001; Hwang *et al.*, 2005). However, none of these studies report the imaging of single actin filaments and the parameters of growth and disappearance. Hopefully, the high spatial and temporal resolution achieved with VAEM or spinning disk confocal microscopy will permit such studies in the near future.

A model for regulation of actin turnover

Based on the known biochemical activities of the major ABPs, measurement of the size of monomer pool, and comparison with the *in vitro* biomimetic model, the following model is proposed for the regulation of stochastic dynamics in plant cells (Fig. 3). Most of the actin is present in a huge pool of monomers buffered with profilin. Profilin will prevent spontaneous nucleation of filaments and inhibits growth at filament minus-ends. New filaments with available plus-ends will be created by nucleation factors such as formins or the ARP2/3 complex. These can be generated *de novo* in the cytoplasm or along the side of mother filaments. Other filaments will grow from the end of fragments, or from recently severed filaments with free plus-ends. Once ends are available, filaments will grow at rates proportional to the size of the monomer pool and/or will be enhanced by the activity of processive formins. Filament growth can be terminated by the association of heterodimeric capping protein or other cappers. Disassembly is not

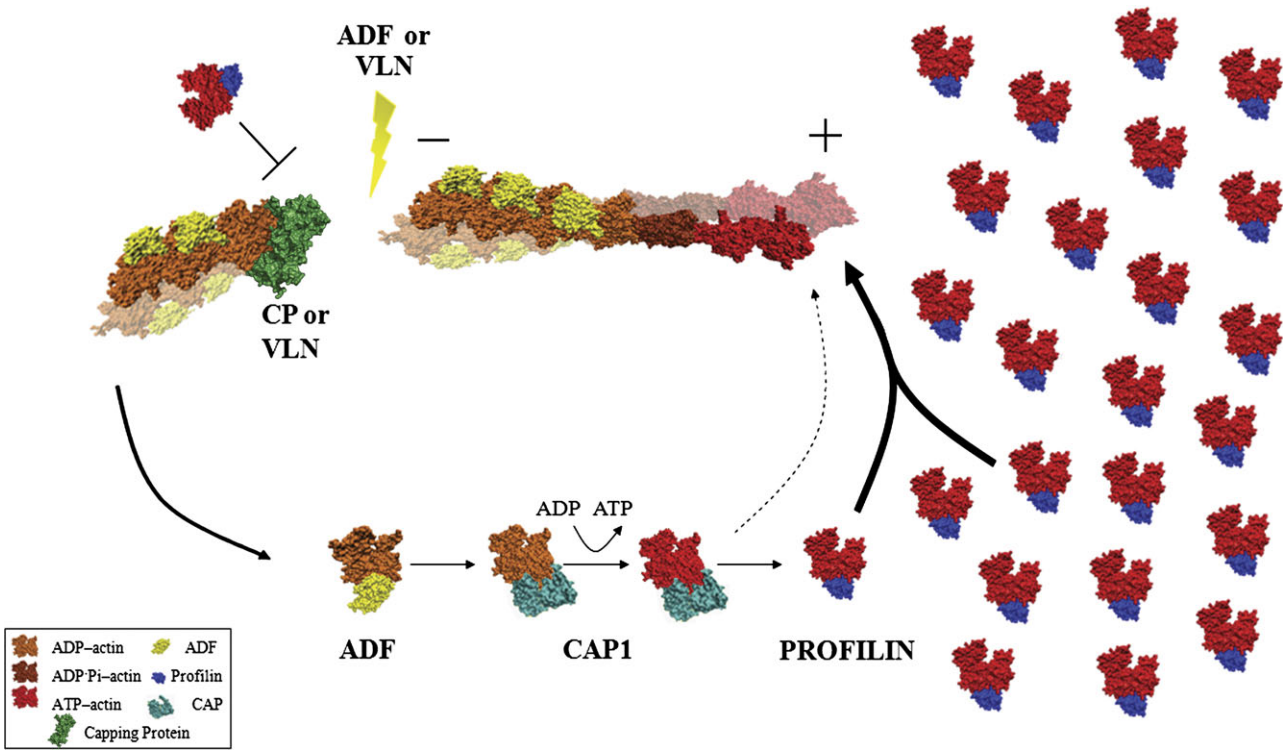

Fig. 3. A simple model for the regulation of the stochastic dynamics. This cartoon displays the major features and key molecules associated with the regulation of actin stochastic dynamics. The model is based on the cellular abundance, biochemical properties and co-operative activities of pollen actin and ABPs. See text for details.

mediated by an increased dissociation rate at the minus-ends, but rather by severing due to ADF or villin family members followed by capping and slow depolymerization. Because most filaments do not regrow after severing, it is predicted that capping occurs simultaneous with or soon after fragmentation. This could result from villin activity, the co-operative function of ADF and AIP1, or both. Monomers are recycled from fragments by the action of ADF in co-operation with CAP. This model of actin turnover is readily testable using a combination of VAEM imaging, fascile reverse-genetic strategies, and the large collection of mutants available in the *Arabidopsis* community.

Conclusions

Clearly we have learned a lot about the organization and regulation of the actin cytoskeleton and actin-binding proteins in pollen. Predominantly, this has come from a powerful combination of biochemistry, pharmacological studies, advanced imaging methods, and reverse-genetic approaches. What remains to be learned, however, are the molecular level details about how individual actin filaments are organized, where they polymerize, and how they turn over. Specifically, the question remains whether a model for actin filament turnover based on hypocotyl epidermal cells is applicable to growing pollen tubes. This issue may be resolved in the near future by the application of techniques like VAEM or spinning disk confocal microscopy and the judicious use of fluorescent fusion proteins.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Video S1. Maize pollen tube growth.

Supplementary Video S2. Actin stochastic dynamics by time lapse VAEM imaging.

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References

Allwood EG, Anthony RG, Smertenko AP, Reichelt S, Drøbak BK, Doonan JH, Weeds AG, Hussey PJ. 2002. Regulation of the pollen-specific actin-depolymerizing factor LIADF1. *The Plant Cell* **14,** 2915–2927.

Allwood EG, Smertenko AP, Hussey PJ. 2001. Phosphorylation of plant actin-depolymerising factor by calmodulin-like domain protein kinase. *FEBS Letters* **499,** 97–100.

Andrianantoandro E, Pollard TD. 2006. Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin. *Molecular Cell* **24,** 13–23.

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

Avisar D, Prokhnevsky AI, Makarova KS, Koonin EV, Dolja VV. 2008. Myosin XI-K is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. *Plant Physiology* **146,** 1098–1108.

Baltz R, Domon C, Pillay DTN, Steinmetz A. 1992. Characterization of a pollen-specific cDNA from sunflower encoding a zinc finger protein. *The Plant Journal* **2,** 713–721.

Baltz R, Schmit A-C, Kohnen M, Hentges F, Steinmetz A. 1999. Differential localization of the LIM domain protein PLIM-1 in microspores and mature pollen drains from sunflower. *Sexual Plant Reproduction* **12,** 60–65.

Bamburg JR, Bernstein BW. 2008. ADF/cofilin. *Current Biology* **18,** R273–R275.

Bamburg JR, Wiggan OP. 2002. ADF/cofilin and actin dynamics in disease. *Trends in Cell Biology* **12,** 598–605.

Barrero RA, Umeda M, Yamamura S, Uchimiya H. 2002. Arabidopsis CAP regulates the actin cytoskeleton necessary for plant cell elongation and division. *The Plant Cell* **14,** 149–163.

Blanchoin L, Pollard TD. 1998. Interaction of actin monomers with *Acanthamoeba* actophorin (ADF/cofilin) and profilin. *Journal of Biological Chemistry* **273,** 25106–25111.

Blanchoin L, Pollard TD. 1999. Mechanism of interaction of *Acanthamoeba* actophorin (ADF/cofilin) with actin filaments. *Journal of Biological Chemistry* **274,** 15538–15546.

Blanchoin L, Staiger CJ. 2008. Plant formins: diverse isoforms and unique molecular mechanism. *Biochimica et Biophysica Acta* doi: org /10.1016/j.bbamcr.2008.09.015.

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C. 1998. Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *The Plant Journal* **15,** 441–447.

Bosch M, Franklin-Tong VE. 2007. Temporal and spatial activation of caspase-like enzymes induced by self-incompatibility in *Papaver*

pollen. *Proceedings of the National Academy of Sciences, USA* **104,** 18327–18332.

Carlier M-F, Laurent V, Santolini J, Melki R, Didry D, Xia G-X, Hong Y, Chua N-H, Pantaloni D. 1997. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *Journal of Cell Biology* **136,** 1307–1322.

Chan J, Calder G, Fox S, Lloyd C. 2007. Cortical microtubule arrays undergo rotary movements in *Arabidopsis* hypocotyl epidermal cells. *Nature Cell Biology* **9,** 171–175.

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

Chen CY, Wong EI, Vidali L, Estavillo A, Hepler PK, Wu H-m, Cheung AY. 2002. The regulation of actin organization by actin-depolymerizing factor in elongating pollen tubes. *The Plant Cell* **14,** 2175–2190.

CY-h Chen, Cheung AY, Wu H-m. 2003. Actin-depolymerizing factor mediates Rac/Rop GTPase–regulated pollen tube growth. *The Plant Cell* **15,** 237–249.

Chen H, Bernstein BW, Sneider JM, Boyle JA, Minamide LS, Bamburg JR. 2004. *In vitro* activity differences between proteins of the ADF/cofilin family define two distinct subgroups. *Biochemistry* **43,** 7127–7142.

Cheung AY. 2001. Imaging elongating pollen tubes by green fluorescent protein. *Sexual Plant Reproduction* **14,** 9–14.

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

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

Cheung AY, H-m Wu. 2008. Structural and signalling networks for the polar cell growth machinery in pollen tubes. *Annual Review of Plant Biology* **59,** 547–572.

Cole RA, Fowler JE. 2006. Polarized growth: maintaining focus on the tip. *Current Opinion in Plant Biology* **9,** 579–588.

Collings DA, Harper JDI, Marc J, Overall RL, Mullen RT. 2002. Life in the fast lane: actin-based motility of plant peroxisomes. *Canadian Journal of Botany Revue Canadienne de Botanique* **80,** 430–441.

Cooper JA, Sept D. 2008. New insights into mechanism and regulation of actin capping protein. *International Reviews in Cell and Molecular Biology* **267,** 183–206.

Cvrcková F, Novotny M, Píckova D, Zársky V. 2004. Formin homology 2 domains occur in multiple contexts in angiosperms. *BMC Genomics* **5,** 44.

Deeks MJ, Hussey PJ, Davies B. 2002. Formins: intermediates in signal transduction cascades that affect cytoskeletal reorganization. *Trends in Plant Science* **7,** 492–498.

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

Dhonukshe P, Grigorieve I, Fischer R, *et al*. 2008. Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. *Proceedings of the National Academy of Sciences, USA* **105,** 4489–4494.

Didry D, Carlier M-F, Pantaloni D. 1998. Synergy between actin depolymerizing factor/cofilin and profilin in increasing actin filament turnover. *Journal of Biological Chemistry* **273,** 25602–25611.

Fan X, Hou J, Chen X, Chaudhry F, Staiger CJ, Ren H. 2004. Identification and characterization of a Ca^{2+}-dependent actin-filament severing protein from lily pollen. *Plant Physiology* **136,** 3979–3989.

Feijó JA, Sainhas J, Hackett GR, Kunkel JG, Hepler PK. 1999. Growing pollen tubes possess a constitutive alkaline band in the clear zone and a growth-dependent acidic tip. *Journal of Cell Biology* **144,** 483–496.

Foote HCC, Ride JP, Franklin-Tong VE, Walker EA, Lawrence MJ, Franklin FCH. 1994. Cloning and expression of a distinctive class of self-incompatibility (S) gene from *Papaver rhoeas* L. *Proceedings of the National Academy of Sciences, USA* **91,** 2265–2269.

Franklin-Tong VE, Gourlay CW. 2008. A role for actin in regulating apoptosis/programmed cell death: evidence spanning yeast, plants and animals. *Biochemical Journal* **413,** 389–404.

Franklin-Tong VE, Ride JP, Read ND, Trewavas AJ, Franklin FCH. 1993. The self-incompatibility response in *Papaver rhoeas* is mediated by cytosolic free calcium. *The Plant Journal* **4,** 163–177.

Fu Y, Wu G, Yang Z. 2001. Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. *Journal of Cell Biology* **152,** 1019–1032.

Gabrys H. 2004. Blue light-induced orientation movements of chloroplasts in higher plants: recent progress in the study of their mechanisms. *Acta Physiologiae Plantarum* **26,** 473–478.

Geitmann A, Snowman BN, Emons AMC, Franklin-Tong VE. 2000. Alterations in the actin cytoskeleton of pollen tubes are induced by the self-incompatibility reaction in *Papaver rhoeas*. *The Plant Cell* **12,** 1239–1251.

Geldner N, Friml J, Stierhof Y-D, Jürgens G, Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413,** 425–428.

Gendreau E, Traas J, Desnos T, Grandjean O, Caboche M, Höfte H. 1997. Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. *Plant Physiology* **114,** 295–305.

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

Gibbon BC, Zonia LE, Kovar DR, Hussey PJ, Staiger CJ. 1998. Pollen profilin function depends on interaction with proline-rich motifs. *The Plant Cell* **10,** 981–994.

Grebe M, Xu J, Mobius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B. 2003. *Arabidopsis* sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes. *Current Biology* **13,** 1378–1387.

Gu Y, Fu Y, Dowd P, Li S, Vernoud V, Gilroy S, Yang Z. 2005. A Rho family GTPase controls actin dynamics and tip growth via two

counteracting downstream pathways in pollen tubes. *Journal of Cell Biology* **169,** 127–138.

Gungabissoon RA, Jiang C-J, Drøbak BK, Maciver SK, Hussey PJ. 1998. Interaction of maize actin-depolymerising factor with actin and phosphoinositides and its inhibition of plant phospholipase C. *The Plant Journal* **16,** 689–696.

Gungabissoon RA, Khan S, Hussey PJ, Maciver SK. 2001. Interaction of elongation factor 1α from *Zea mays* (ZmEF-1α) with F-actin and interplay with the maize actin severing protein, ZmADF3. *Cell Motility and the Cytoskeleton* **49,** 104–111.

Gutierrez R, Lindeboom JJ, Paredez AR, Emons AMC, Ehrhardt DW. 2009. *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nature Cell Biology* **11,** 797–806.

Helling D, Possart A, Cottier S, Klahre U, Kost B. 2006. Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling. *The Plant Cell* **18,** 3519–3534.

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

Holdaway-Clarke TL, Feijó JA, Hackett GR, Kunkel JG, Hepler PK. 1997. Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. *The Plant Cell* **9,** 1999–2010.

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

Huang S, Blanchoin L, Kovar DR, Staiger CJ. 2003. *Arabidopsis* capping protein (AtCP) is a heterodimer that regulates assembly at the barbed ends of actin filaments. *Journal of Biological Chemistry* **278,** 44832–44842.

Huang S, Gao L, Blanchoin L, Staiger CJ. 2006. Heterodimeric capping protein from *Arabidopsis* is regulated by phosphatidic acid. *Molecular Biology of the Cell* **17,** 1946–1958.

Huang S, Jin L, Du J, Li H, Zhao Q, Ou G, Ao G, Yuan M. 2007. SB401, a pollen-specific protein from *Solanum berthaultii*, binds to and bundles microtubules and F-actin. *The Plant Journal* **51,** 406–418.

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

Hussey PJ, Ketelaar T, Deeks MJ. 2006. Control of the actin cytoskeleton in plant cell growth. *Annual Review of Plant Biology* **57,** 109–125.

Hwang J-U, Gu Y, Lee Y-J, Yang Z. 2005. Oscillatory ROP GTPase activation leads the oscillatory polarized growth of pollen tubes. *Molecular Biology of the Cell* **16,** 5385–5399.

Hwang J-U, Vernoud V, Szumlanski A, Nielsen E, Yang Z. 2008. A tip-localized RhoGAP controls cell polarity by globally inhibiting Rho GTPase at the cell apex. *Current Biology* **18,** 1907–1916.

Jedd G, Chua NH. 2002. Visualization of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding. *Plant and Cell Physiology* **43,** 384–392.

Kabsch W, Mannherz HG, Suck D, Pai EF, Holmes KC. 1990. Atomic structure of the actin:DNase I complex. *Nature* **347,** 37–44.

Kadota A, Yamada N, Suetsugu N, Hirose M, Saito C, Shoda K, Ichikawa S, Kagawa T, Nakano A, Wada M. 2009. Short actin-based mechanism for light-directed chloroplast movement in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **106,** 13106–13111.

Karpova TS, Tatchell K, Cooper JA. 1995. Actin filaments in yeast are unstable in the absence of capping protein or fimbrin. *Journal of Cell Biology* **131,** 1483–1493.

Ketelaar T, Faivre-Moskalenko C, Esseling JJ, de Ruijter NCA, Grierson CS, Dogterom M, Emons AMC. 2002. Positioning of nuclei in Arabidopsis root hairs: an actin-regulated process of tip growth. *The Plant Cell* **14,** 2941–2955.

Khurana S, George SP. 2008. Regulation of cell structure and function by actin-binding proteins: Villin's perspective. *FEBS Letters* **582,** 2128–2139.

Kiesselbach TA. 1949. *The structure and reproduction of corn*. University of Nebraska College of Agriculture, Agriculture Experiment Station.

Kim H, Park M, Kim SJ, Hwang I. 2005. Actin filaments play a critical role in vacuolar trafficking at the Golgi complex in plant cells. *The Plant Cell* **17,** 888–902.

Kim Y-W, Yamashita A, Wear MA, Maéda Y, Cooper JA. 2004. Capping protein binding to actin in yeast: biochemical mechanism and physiological relevance. *Journal of Cell Biology* **164,** 567–580.

Klahre U, Friederich E, Kost B, Louvard D, Chua N-H. 2000. Villin-like actin-binding proteins are expressed ubiquitously in *Arabidopsis*. *Plant Physiology* **122,** 35–47.

Kohno T, Shimmen T. 1987. Ca^{2+}-induced fragmentation of actin filaments in pollen tubes. *Protoplasma* **141,** 177–179.

Kohno T, Shimmen T. 1988. Mechanism of Ca^{2+}inhibition of cytoplasmic streaming in lily pollen tubes. *Journal of Cell Science* **91,** 501–509.

Konopka CA, Backues SK, Bednarek SY. 2008. Dynamics of Arabidopsis dynamin-related protein 1C and a clathrin light chain at the plasma membrane. *The Plant Cell* **20,** 1363–1380.

Konopka CA, Bednarek SY. 2008*a*. Comparison of the dynamics and functional redundancy of the *Arabidopsis* dynamin-related isoforms, DRP1A and DRP1C, during plant development. *Plant Physiology* **147,** 1590–1602.

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

Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua N- H. 1999. Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *Journal of Cell Biology* **145,** 317–330.

Kost B, Spielhofer P, Chua N-H. 1998. A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualizes the actin cytoskeleton in growing pollen tubes. *The Plant Journal* **16,** 393–401.

Kovar DR, Staiger CJ, Weaver EA, McCurdy DW. 2000. AtFim1 is an actin filament crosslinking protein from *Arabidopsis thaliana*. *The Plant Journal* **24,** 625–636.

Kovar DR, Yang P, Sale WS, Drøbak BK, Staiger CJ. 2001. *Chlamydomonas reinhardtii* produces a profilin with unusual biochemical properties. *Journal of Cell Science* **114,** 4293–4305.

Kreis T, Vale R. 1999. *Guidebook to the cytoskeletal and motor proteins*. New York: Oxford University Press.

Lee YJ, Szumlanski A, Nielsen E, Yang Z. 2008. Rho-GTPase–dependent filamentous actin dynamics co-ordinate vesicle trafficking and exocytosis during tip growth. *Journal of Cell Biology* **181,** 1155–1168.

Lichtscheidl IK, Hepler PK. 1996. Endoplasmic reticulum in the cortex of plant cells. In: Smallwood M, Knox JP, Bowles DJ, eds. *Membranes: specialized functions in plants*. Oxford, UK: BIOS Scientific Publishers, 383–402.

Lord EM, Walling LL, Jauh GY. 1996. Cell adhesion in plants and its role in pollination. In: Smallwood M, Knox JP, Bowles DJ, eds. *Membranes: specialized functions in plants*. Oxford, UK: BIOS Scientific Publishers, 21–38.

Lovy-Wheeler A, Cárdenas L, Kunkel JG, Hepler PK. 2007. Differential organelle movement on the actin cytoskeleton in lily pollen tubes. *Cell Motility and the Cytoskeleton* **64,** 217–232.

Lovy-Wheeler A, Kunkel JG, Allwood EG, Hussey PJ, Hepler PK. 2006. Oscillatory increases in alkalinity anticipate growth and may regulate actin dynamics in pollen tubes of lily. *The Plant Cell* **18,** 2182–2193.

Lovy-Wheeler A, Wilsen KL, Baskin TI, Hepler PK. 2005. Enhanced fixation reveals the apical cortical fringe of actin filaments as a consistent feature of the pollen tube. *Planta* **221,** 95–104.

Machesky LM, Insall RH. 1999. Signalling to actin dynamics. *Journal of Cell Biology* **146,** 267–272.

Maciver SK, Hussey PJ. 2002. The ADF/cofilin family: actin-remodeling proteins. *Genome Biology* **3,** 3007.1-3007.12.

Mahajan-Miklos S, Cooley L. 1994. The villin-like protein encoded by the *Drosophila quail* gene is required for actin bundle assembly during oogenesis. *Cell* **78,** 291–301.

Mathur J. 2005. Conservation of boundary extension mechanisms between plants and animals. *Journal of Cell Biology* **168,** 679–682.

Mathur J, Mathur N, Hülskamp M. 2002. Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. *Plant Physiology* **128,** 1031–1045.

McCurdy DW. 1999. Is 2,3-butanedione monoxime an effective inhibitor of myosin-based activities in plant cells? *Protoplasma* **209,** 120–125.

Messerli M, Robinson KR. 1997. Tip localized $Ca2^{+}$ pulses are coincident with peak pulsatile growth rates in pollen tubes of *Lilium longiflorum*. *Journal of Cell Science* **110,** 1269–1278.

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

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

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

Monteiro D, Coelho PC, Rodrigues C, Camacho L, Quader H, Malhó R. 2005*a*. Modulation of endocytosis in pollen tube growth by phosphoinositides and phospholipids. *Protoplasma* **226,** 31–38.

Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malhó R. 2005*b*. Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. *Journal of Experimental Botany* **416,** 1665–1674.

Nakayasu T, Yokota E, Shimmen T. 1998. Purification of an actin-binding protein composed of 115-kDa polypeptide from pollen tubes of lily. *Biochemical and Biophysical Research Communications* **249,** 61–65.

Nebenführ S, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, Meehl JB, Staehelin LA. 1999. Stop-and-go movements of plant Golgi stacks are mediated by the actin-myosin system. *Plant Physiology* **121,** 1127–1141.

Nishimura T, Yokota E, Wada T, Shimmen T, Okada K. 2003. An *Arabidopsis ACT2* dominant-negative mutation, which disturbs F-actin polymerization, reveals its distinctive function in root development. *Plant and Cell Physiology* **44,** 1131–1140.

Oda T, Iwasa M, Aihara T, Maéda Y, Narita A. 2009. The nature of the globular- to fibrous-actin transition. *Nature* **457,** 441–445.

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

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

Paredez AR, Somerville CR, Ehrhardt DW. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312,** 1491–1495.

Perelroizen I, Didry D, Christensen H, Chua N-H, Carlier M-F. 1996. Role of nucleotide exchange and hydrolysis in the function of profilin in actin assembly. *Journal of Biological Chemistry* **271,** 12302–12309.

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

Pina C, Pinto F, Feijó JA, Becker JD. 2005. Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. *Plant Physiology* **138,** 744–756.

Pollard TD. 1986. Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments. *Journal of Cell Biology* **103,** 2747–2754.

Pollard TD, Blanchoin L, Mullins RD. 2000. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annual Review of Biophysics and Biomolecular Structure* **29,** 545–576.

Potocký M, Eliáš M, Profotová B, Novotná Z, Vantová O, Žárský V. 2003. Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. *Planta* **217,** 122–130.

Poulter NS, Staiger CJ, Rappoport JZ, Franklin-Tong VE. 2010. Actin-binding proteins implicated in the formation of the punctate actin foci stimulated by the self-incompatibility response in *Papaver*. *Plant Physiology* doi: 10/1104/pp.109.152066.

Poulter NS, Vatovec S, Franklin-Tong VE. 2008. Microtubules are a target for self-incompatibility signalling in *Papaver* pollen. *Plant Physiology* **146,** 1358–1367.

Prokhnevsky AI, Peremyslov VV, Dolja VV. 2008. Overlapping functions of the four class XI myosins in *Arabidopsis* growth, root hair elongation, and organelle motility. *Proceedings of the National Academy of Sciences, USA* **105,** 19744–19749.

Quader H, Hofmann A, Schnepf E. 1987. Shape and movement of the endoplasmic reticulum in onion bulb epidermis cells: possible involvement of actin. *European Journal of Cell Biology* **44,** 17–26.

Ren H-Y, Xiang Y. 2007. The function of actin-binding proteins in pollen tube growth. *Protoplasma* **230,** 171–182.

Ressad F, Didry D, Egile C, Pantaloni D, Carlier M-F. 1999. Control of actin filament length and turnover by actin depolymerizing factor (ADF/cofilin) in the presence of capping proteins and ARP2/3 complex. *Journal of Biological Chemistry* **274,** 20970–20976.

Ruzicka DR, Kandasamy MK, McKinney EC, Burgos-Rivera B, Meagher RB. 2007. The ancient subclasses of *Arabidopsis* ACTIN DEPOLYMERIZING FACTOR genes exhibit novel and differential expression. *The Plant Journal* **52,** 460–472.

Sagot I, Pinson B, Salin B, Daignan-Fornier B. 2006. Actin bodies in yeast quiescent cells: an immediately available actin reserve? *Molecular Biology of the Cell* **17,** 4645–4655.

Schüler H, Mueller A-K, Matuschewski K. 2005. A *Plasmodium* actin depolymerizing factor that binds exclusively to actin monomers. *Molecular Biology of the Cell* **16,** 4013–4023.

Shaw SL, Kamyar R, Ehrhardt DW. 2003. Sustained microtubule treadmilling in *Arabidopsis* cortical arrays. *Science* **300,** 1715–1718.

Sheahan MB, Rose RJ, McCurdy DW. 2007. Actin-filament-dependent remodeling of the vacuole in cultured mesophyll protoplasts. *Protoplasma* **230,** 141–152.

Shimmen T. 2007. The sliding theory of cytoplasmic streaming: fifty years of progress. *Journal of Plant Research* **120,** 31–43.

Shimmen T, Hamatani M, Saito S, Yokota E, Mimura T, Fusetani N, Karaki H. 1995. Roles of actin filaments in cytoplasmic streaming and organization of transvacuolar strands in root hair cells of *Hydrocharis. Protoplasma* **185,** 188–193.

Shimmen T, Yokota E. 2004. Cytoplasmic streaming in plants. *Current Opinion in Cell Biology* **16,** 68–72.

Smertenko AP, Allwood EG, Khan S, Jiang C-J, Maciver SK, Weeds AG, Hussey PJ. 2001. Interaction of pollen-specific actin-depolymerizing factor with actin. *The Plant Journal* **25,** 203–212.

Smith LG, Oppenheimer DG. 2005. Spatial control of cell expansion by the plant cytoskeleton. *Annual Review of Cell and Developmental Biology* **21,** 271–295.

Snowman BN, Kovar DR, Shevchenko G, Franklin-Tong VE, Staiger CJ. 2002. Signal-mediated depolymerization of actin in pollen during the self-incompatibility response. *The Plant Cell* **14,** 2613–2626.

Sparkes IA, Teanby NA, Hawes C. 2008. Truncated myosin XI tail fusions inhibit peroxisome, Golgi, and mitochondrial movement in tobacco leaf epidermal cells: a genetic tool for the next generation. *Journal of Experimental Botany* **59,** 2499–2512.

Staiger CJ. 2000. Signalling to the actin cytoskeleton in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **51,** 257–288.

Staiger CJ, Blanchoin L. 2006. Actin dynamics: old friends with new stories. *Current Opinion in Plant Biology* **9,** 554–562.

Staiger CJ, Sheahan MB, Khurana P, Wang X, McCurdy DW, Blanchoin L. 2009. Actin filament dynamics are dominated by rapid growth and severing activity in the *Arabidopsis* cortical array. *Journal of Cell Biology* **184,** 269–280.

Staiger CJ, Yuan M, Valenta R, Shaw PJ, Warn RM, Lloyd CW. 1994. Microinjected profilin affects cytoplasmic streaming in plant cells by rapidly depolymerizing actin microfilaments. *Current Biology* **4,** 215–219.

Szymanski DB, Cosgrove DJ. 2009. Dynamic co-ordination of cytoskeletal and cell wall systems during plant cell morphogenesis. *Current Biology* **19,** R800–R811.

Takagi S, Takamatsu H, Sakurai-Ozato N. 2009. Chloroplast anchoring: its implication for the regulation of intracellular chloroplast distribution. *Journal of Experimental Botany* **60,** 3301–3310.

Taylor LP, Hepler PK. 1997. Pollen germination and tube growth. *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 461–491.

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

Thomas C, Moreau F, Dieterle M, Hoffmann C, Gatti S, Hofmann C, Van Troys M, Ampe C, Steinmetz A. 2007. The LIM domains of WLIM1 define a new class of actin bundling modules. *Journal of Biological Chemistry* **282,** 33599–33608.

Thomas C, Tholl S, Moes D, Dieterle M, Papuga J, Moreau F, Steinmetz A. 2009. Actin bundling in plants. *Cell Motility and the Cytoskeleton* **66,** 940–957.

Thomas SG, Franklin-Tong VE. 2004. Self-incompatibility triggers programmed cell death in *Papaver* pollen. *Nature* **429,** 305–309.

Thomas SG, Huang S, Li S, Staiger CJ, Franklin-Tong VE. 2006*b*. Actin depolymerization is sufficient to induce programmed cell death in self-incompatible pollen. *Journal of Cell Biology* **174,** 221–229.

Tominaga M, Yokota E, Vidali L, Sonobe S, Hepler PK, Shimmen T. 2000. The role of plant villin in the organization of the actin cytoskeleton, cytoplasmic streaming and the architecture of the transvacuolar strand in root hair cells of *Hydrocharis*. *Planta* **210,** 836–843.

Valenta R, Duchêne M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O. 1991. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* **253,** 557–560.

Valenta R, Ferreira F, Grote M, *et al*. 1993. Identification of profilin as an actin-binding protein in higher plants. *Journal of Biological Chemistry* **268,** 22777–22781.

Van Gestel K, Köhler RH, Verbelen J-P. 2002. Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. *Journal of Experimental Botany* **53,** 659–667.

Verbelen JP, Tao W. 1998. Mobile arrays of vacuole ripples are common in plant cells. *Plant Cell Reports* **17,** 917–920.

Vidali L, Hepler PK. 1997. Characterization and localization of profilin in pollen grains and tubes of *Lilium longiflorum*. *Cell Motility and the Cytoskeleton* **36,** 323–338.

Vidali L, McKenna ST, Hepler PK. 2001. Actin polymerization is essential for pollen tube growth. *Molecular Biology of the Cell* **12,** 2534–2545.

Vidali L, Rounds CM, Hepler PK, Bezanilla M. 2009*a*. Lifeact-mEGFP reveals a dynamic apical F-actin network in tip growing plant cells. *PLoS ONE* **4,** e5744.

Vidali L, van Gisbergen PAC, Guérin C, Franco P, Li M, Burkart GM, Augustine RC, Blanchoin L, Bezanilla M. 2009*b*. Rapid formin-mediated actin-filament elongation is essential for polarized plant cell growth. *Proceedings of the National Academy of Sciences, USA* **160,** 13341–13346.

Vidali L, Yokota E, Cheung AY, Shimmen T, Hepler PK. 1999. The 135 kDa actin-bundling protein from *Lilium longiflorum* pollen is the plant homologue of villin. *Protoplasma* **209,** 283–291.

Wada M, Kagawa T, Sato Y. 2003. Chloroplast movement. *Annual Review of Plant Biology* **54,** 455–468.

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

Wang T, Xiang Y, Hou J, Ren H- Y. 2008*b*. ABP41 is involved in the pollen tube development via fragmenting actin filaments. *Molecular Plant* **1,** 1048–1055.

Wheeler MJ, de Graaf BHJ, Hadjiosif N, Perry RM, Poulter NS, Osman K, Vatovec S, Harper A, Franklin FCH, Franklin-Tong VE. 2009. Identification of the pollen self-incompatibility determinant in *Papaver rhoeas*. *Nature* **459,** 992–995.

Wightman R, Turner SR. 2008. The roles of the cytoskeleton during cellulose deposition at the secondary cell wall. *The Plant Journal* **54,** 794–805.

Wilsen KL, Lovy-Wheeler A, Voigt B, Menzel D, Kunkel JG, Hepler PK. 2006. Imaging the actin cytoskeleton in growing pollen tubes. *Sexual Plant Reproduction* **19,** 51–62.

Xiang Y, Huang X, Wang T, Zhang Y, Liu Q, Hussey PJ, Ren H. 2007. ACTIN BINDING PROTEIN29 from *Lilium* pollen plays an important role in dynamic actin remodeling. *The Plant Cell* **19,** 1930–1946.

Yang Z. 2008. Cell polarity signalling in *Arabidopsis*. *Annual Review of Cell and Developmental Biology* **24,** 551–575.

Ye J, Zheng Y, Yan A, Chen N, Wang Z, Huang S, Yang Z. 2009. *Arabidopsis* Formin3 directs the formation of actin cables and polarized growth in pollen tubes. *The Plant Cell* doi/10.1105/tpc.109.068700.

Yi K, Guo C, Chen D, Zhao B, Yang B, Ren H. 2005. Cloning and functional characterization of a formin-like protein (AtFH8) from *Arabidopsis*. *Plant Physiology* **138,** 1071–1082.

Yin HL. 1999. Gelsolin. In: Kreis T, Vale R, eds. *Guidebook to the cytoskeletal and motor proteins*, Vol. 2. New York: Oxford University Press, 99–102.

Yokota E, Muto S, Shimmen T. 2000. Calcium-calmodulin suppresses the filamentous actin-binding activity of 135-kilodalton actin-bundling protein isolated from lily pollen tubes. *Plant Physiology* **123,** 645–654.

Yokota E, Shimmen T. 1999. The 135-kDa actin-bundling protein from lily pollen tubes arranges F-actin into bundles with uniform polarity. *Planta* **209,** 264–266.

Yokota E, Takahara K-i, Shimmen T. 1998. Actin-bundling protein isolated from pollen tubes of lily. *Plant Physiology* **116,** 1421–1429.

Yokota E, Tominaga M, Mabuchi I, Tsuji Y, Staiger CJ, Oiwa K, Shimmen T. 2005. Plant villin, lily P-135-ABP, possesses G-actin binding activity and accelerates the polymerization and depolymerization of actin in a Ca^{2+}-sensitive manner. *Plant and Cell Physiology* **46,** 1690–1703.

Yokota E, Ueda T, Tamura K, Orii H, Uchi S, Sonobe S, Hara-Nishimura I, Shimmen T. 2009. An isoform of myosin XI is responsible for the translocation of endoplasmic reticulum in tobacco cultured BY-2 cells. *Journal of Experimental Botany* **60,** 197–212.

Yokota E, Vidali L, Tominaga M, Tahara H, Orii H, Morizane Y, Hepler PK, Shimmen T. 2003. Plant 115-kDa actin-filament bundling protein, P-115-ABP, is a homologue of plant villin and is widely distributed in cells. *Plant and Cell Physiology* **44,** 1088–1099.

Zimmerle CT, Frieden C. 1988. Effect of pH on the mechanism of actin polymerization. *Biochemistry* **27,** 7766–7772.