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Actin Monomer-Binding Proteins and the Regulation of Actin Dynamics in Plants

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Abstract

Plants are uniquely adapted to respond to environmental and developmental signals for survival. Many signals result in dramatic changes of cell shape or cytoplasmic organization that are dependent on the actin cytoskeleton. The dynamic nature of the actin cytoskeleton is conferred by a wide variety of actin-binding proteins. One class of these proteins is capable of binding to free actin monomers and thereby regulates the polymerization of actin filaments. Two such proteins have been identified in plants: profilin and actin-depolymerizing factor (ADF). These proteins comprise multigene families in plants and the isoforms of each protein have unique developmentally and spatially regulated expression patterns. Biochemical analysis of the plant monomer-binding proteins indicates that they are able to both stimulate and inhibit actin polymerization *in vitro*. Furthermore, microinjection of these proteins into cells reveals that simple models for the interaction of monomer-binding proteins with actin are inadequate. The complex effects on actin *in vitro* and *in vivo* are due to the ability of profilin and ADF to interact with a number of other ligands, such as regulatory proteins and polyphosphoinositide lipids. The monomer-binding proteins also respond to changes in cytosolic Ca^{2+} and pH. Regulation of these proteins by phosphorylation adds an additional level of complexity for the study of their role in coordinating actin reorganization in plant cells. A model of actin filament assembly in tip-growing cells that incorporates the activities of profilin and ADF is presented.

Key words: Actin; Profilin; Actin-depolymerizing factor; Morphogenesis; Cytoskeleton

Biochemistry of Monomer-Binding Proteins

The actin cytoskeleton is a central regulator of cell shape in many eukaryotic organisms. This important filamentous network is required for many aspects of cell motility such as cell crawling, muscle contraction, cytoplasmic streaming, and vesicle trafficking. Actin is also required for the establishment or maintenance of cellular polarity. The key feature of the actin cytoskeleton, which allows these diverse roles, is that actin filaments form a highly dynamic network that can rearrange in response to many signals.

The dynamic nature of the actin cytoskeleton is conferred by a number of actin-binding proteins that bind to and cross-link actin filaments, bundle filaments together, sever actin filaments, cap the ends of filaments, or bind to actin monomers (Pollard and Cooper 1986). The ability of the monomer-binding proteins to regulate the transition from filamentous

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(F-actin) to free globular actin monomers (G-actin) is essential for the process of actin filament disassembly and reassembly. Additionally, these proteins have complex effects on F-actin polymerization because the actin filament is a molecule with a fast-growing end (barbed end) and a slow-growing end (pointed end). At steady state, G-actin preferentially adds to the barbed end and dissociates from the pointed end. Monomer-binding proteins alter the rates of subunit addition or removal from filament ends differently, which leads to complex effects on actin polymerization *in vitro* and *in vivo.* Two monomer-binding proteins have been identified in plants: profilin and actin depolymerizing factor (ADF; Valenta and others 1991; Kim and others 1993). In many organisms these proteins are linked to signaling events that result in reorganization of actin (Machesky and Insall 1999; Staiger 2000).

Profilin

Profilin was discovered as a major allergen in birch pollen and comprises a multigene family in many plants (reviewed by Staiger and others 1997; Gibbon and Staiger 2000). This monomer-binding protein has complex effects on actin dynamics because, in concert with other proteins, it can either inhibit or stimulate actin assembly in nonplant cells. The complex effect of profilin on actin assembly *in vivo* is most likely due to profilin interacting with contiguous stretches of proline residues and polyphosphoinositide lipids, in addition to actin.

Clearly, the ability of profilin to bind to actin monomers is the primary activity that results in the regulation of actin filament dynamics. The affinity of plant profilin isoforms for actin is similar to that described for nonplant profilins. The K_d values for plant profilin isoforms binding to plant, or vertebrate, actin range from 0.07 μM to 5 μM (Gibbon and Staiger 2000). In plants such as *Zea mays,* for which the apparent K_d values for several profilin isoforms binding to actin have been measured, the isoforms comprise two distinct classes (Kovar and others 2000). Members of class I are expressed predominantly in pollen and have a relatively low affinity for G-actin, whereas members of class II have four-fold higher affinity for G-actin and are expressed throughout the plant (Gibbon and others 1998; Kovar and others 2000). The biological significance of differences in affinity for actin is not known, but these isoform differences *in vitro* are presumed to reflect functional diversity within plant cells.

One interesting feature of nonplant profilins is that they are able to stimulate nucleotide exchange on actin *in vitro.* Plant profilin isoforms, however, do not promote nucleotide exchange on plant actin (Kovar and others 2000) or on vertebrate actin (Perelroizen and others 1996). A comparison of the ability of vertebrate or plant profilin to participate in barbed-end assembly reveals that both profilins stimulate polymerization of vertebrate actin (Perelroizen and others 1996; Ballweber and others 1998), which indicates that nucleotide exchange is not required to lower the critical concentration of polymerization. Nucleotide exchange may, however, account for the different levels of polymerization that vertebrate and plant profilin cause (Didry and others 1998). These experiments demonstrate the value of comparative biochemical analysis of many protein isoforms.

The ability to bind to contiguous stretches of proline residues is a feature shared by all profilins. Profilin is purified easily from plant extracts by poly-L-proline (PLP) affinity chromatography, confirming that plant profilins bind to PLP. This interaction can be quantified in solution by measuring changes of tryptophan fluorescence in the presence of varying amounts of PLP (Petrella and others 1996). The K_d values for plant profilin isoforms binding to PLP range from 120 μM proline residues to 290 μM proline residues (Gibbon and others 1997; Clarke and others 1998; Gibbon and others 1998; Kovar and others 2000; Schobert and others 2000). The affinity of the plant profilins for PLP is somewhat higher than measured for human profilin I. Again, maize profilin isoforms can be classified into two distinct groups. Class I isoforms bind with relatively low affinity to PLP, whereas class II isoforms bind to PLP with 2-3-fold higher affinity. This contrasts with vertebrate profilins for which the isoform with high affinity for actin has lower affinity for PLP (Lambrechts and others 1997). The differences in affinity for PLP may have a structural basis. The crystal structures of two plant profilins have been reported and there are distinct differences in the orientation of the amino terminal α-helix, which contributes hydrophobic residues involved in PLP binding. *Arabidopsis* PFN1, a 'vegetative' isoform, has a structure that is remarkably similar to profilins from vertebrates (Thorn and others 1997), whereas birch pollen profilin has an amino terminal α-helix oriented nearly perpendicular to that found in other profilins (Fedorov and others 1997). As is the case for actin binding, the practical implications of profilin isoforms with differing affinity for PLP is also not known.

Other potentially important ligands of profilin include polyphosphoinositides (Lassing and Lindberg 1985; Goldschmidt-Clermont and others 1990), and a complex of proteins containing two actin-related proteins (Arp2/3 complex) that caps the pointed end of filaments and nucleates actin assembly as a

branched network (Machesky and others 1994; Mullins and others 1998; Pantaloni and others 2000). The data regarding the association of plant profilin with polyphosphoinositides is quite limited. Recombinant birch profilin binds to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) with an apparent K_d of 24 μM (Drøbak and others 1994). Two maize profilin isoforms display differences in their ability to inhibit hydrolysis of PtdIns(4,5)P_2 by phospholipase C (Kovar and others 2000), an indirect measure of affinity for the phospholipid (Goldschmidt-Clermont and others 1991). A class I profilin (ZmPRO1) inhibited hydrolysis more strongly than a class II isoform (ZmPRO5; Kovar and others 2000). Thus, class I profilins appear to be good PtdIns(4,5)P2-binding proteins and relatively weak actin-binding proteins, whereas class II profilins are good actin-binding proteins and poor PtdIns(4,5)P2-binding proteins. Whether this is a general feature of the two classes will require characterization of additional isoforms. Presently, there are no reports of Arp2/3 complex purification from plant cells but potential homologs of proteins associated with the complex have been identified (Klahre and Chua 1999).

Actin Depolymerizing Factor

ADF was discovered more recently in plants and the details of its interaction with actin are well characterized, but knowledge about regulation of its cellular activities is limited. ADF has been cloned from lily, maize, *Arabidopsis*, wheat and petunia (Kim and others 1993; Rozycka and others 1995; Danyluk and others 1996; Lopez and others 1996; Carlier and others 1997; Mun and others 2000). Comparison of the protein structures from several organisms to create structure-based sequence alignments reveals that plant ADF proteins form a distinct group because they lack certain loops found in other organisms (Bowman and others 2000). The activity of ADF can be modulated by a number of factors including other actin-binding proteins, pH, phosphorylation, and polyphosphoinositides. These links to signaling intermediates indicate that ADF is likely to be a key regulator of actin dynamics in plant cells (reviewed by Kovar and Staiger 2000).

The effect of ADF on actin dynamics is perhaps more complex than profilin's effect, because ADF can interact with both F-actin and G-actin. *Arabidopsis* ADF increases the dissociation rate at the pointed end of the actin filament and increases the flux of actin subunits from the barbed end to the pointed end (treadmilling) of vertebrate actin (Carlier and others 1997). This effect is further enhanced by profilins *in vitro:* in the presence of plant profilin, the treadmilling rate is approximately three-fold higher than for ADF alone (Didry and others 1998). Plant ADF is also able to stimulate actin turnover in filaments even in the presence of barbed-end or pointed-end capping proteins (Ressad and others 1999), possibly due to weak severing activity. Surprisingly, under conditions where actin assembly is favored, ADF can dramatically enhance the elongation rate of Arp2/3-nucleated actin filaments (Ressad and others 1999). The increased turnover of actin caused by ADF and profilin may be required for rapid reorganization of the actin cytoskeleton in plant cells.

Regulation of ADF activity by phosphorylation is well established (Kovar and Staiger 2000). Maize ADF isoforms with mutations at Sen6 have dramatic effects on the ability to interact with actin (Smertenko and others 1998). Mutants with a substitution of Asp for Sen6, to mimic phosphorylation, do not bind to monomeric actin and do not depolymerize F-actin. Furthermore, a calcium-sensitive kinase from pollen extracts phosphorylates maize ADF specifically at Ser6, and the phosphorylation site is confirmed by a mutant with Ala at position 6 that cannot be phosphorylated. The elucidation of the signals that lead to ADF phosphorylation and the effect of phosphorylation on ADF activity in plant cells will be very interesting. It will be particularly exciting to analyze the effect of the constitutively active Ala6 mutant in living cells.

The binding of plant ADF to actin *in vitro* can also be modulated by pH. All of the plant ADF isoforms tested interact with F-actin without causing depolymerization at acidic pH (~6.0) and depolymerize actin at alkaline pH (~8.0–9.0; Carlier and others 1997; Gungabissoon and others 1998; Mun and others 2000). The proposed mechanism for depolymerization of F-actin by ADF at high pH is an increased dissociation rate at the pointed end of the actin filament, rather than sequestering of actin monomers (Carlier and others 1997). Although the wide variation of pH tested *in vitro* is unlikely to occur within cells, there are developmental or environmentally stimulated changes of cytosolic pH in plant and animal cells that can be up to 0.8 pH units (Kurkdjian and Guern 1989; Gibbon and Kropf 1994; Feijó and others 1999).

Cellular Activities of Monomer-Binding Proteins

Profilin and ADF have dramatic effects on the actin cytoskeleton in many cells. Because actin is involved in maintenance of cytoarchitecture and cell shape, it is likely that monomer-binding proteins play a role

in the elaboration of the plant body plan. Consistent with this assertion, *Arabidopsis* plants underexpressing profilin have defects in cell elongation, altered cell shapes, and flower earlier than wild-type plants (Ramachandran and others 2000). Surprisingly, no gross change in actin protein level or actin microfilament arrangement are observed, which suggests that the large number of profilin isoforms expressed in plants may be partially redundant. In addition to this recent report of altering profilin levels throughout a plant, there are considerable data concerning the effect of these proteins on the actin cytoskeleton in many types of plant cells.

Pollen Tubes and Root Hairs

The function of profilin and ADF are best characterized in the highly polarized, tip-growing cells of pollen and root hairs. The extension of these cells is intimately linked to the actin cytoskeleton, as determined by inhibitors of actin function such as cytochalasins (Herth and others 1972; Mascarenhas and LaFountain 1972; Miller and others 1999) or latrunculin B (Bibikova and others 1999; Gibbon and others 1999). Despite dramatic differences in origin, the organization of actin in these tip-growing cells is very similar. The tube-like cells have prominent bundles of actin filaments extending throughout the cytoplasm and arranged parallel to the growth axis. Vigorous cytoplasmic streaming can be observed along these bundles of actin filaments which stops and reverses direction several micrometers behind the tip (Heslop-Harrison and Heslop-Harrison 1989). The organization of actin at the growing tip of these cells consists of fine filaments that extend toward the tip of the cell and stop at the cell cortex just behind the apex (Miller and others 1996; Gibbon and others 1999; Miller and others 1999). The disappearance of these fine filaments is correlated with the cessation of growth that occurs naturally during development (Miller and others 1999), or that is induced by low concentrations of actin inhibitors (Gibbon and others 1999; Miller and others 1999).

The distribution of profilin in tip-growing cells has been difficult to ascertain. Initial reports indicated that profilin might be concentrated in the tip of growing cells (Mittermann and others 1995). Later, it was convincingly shown by microinjection of fluorescently labeled profilin into growing pollen tubes that the distribution of profilin is uniform throughout the cytoplasm (Vidali and Hepler 1997). Cell fractionation of poppy pollen shows that nearly all of the profilin is soluble and little or no profilin is associated with the microsomal fraction (Clarke and others 1998). Yet there are data that indicate some profilin is localized at the plasma membrane. In root hairs of *Lepidium sativum, Arabidopsis* and maize profilin is localized in the dome of initiating hairs and in the apex of rapidly growing hairs (Braun and others 1999; Baluska and others 2000). This localization is not likely to be due to binding of polyphosphoinositide lipids because PtdIns(4,5)P_2 was found to be distributed along the entire root hair (Braun and others 1999). This work has been criticized, however, because the chemical fixation may not have been rapid enough to adequately preserve the dynamic, vesicle-rich tip region (Emons and de Ruijter 2000). Even if this is the case, some portion of the profilin was stably associated with the cortex and was fixed. If some freely diffusable (unfixed) profilin in the cytoplasm was extracted, this may have helped to reveal the tip-localized profilin.

The localization of ADF is also somewhat controversial in plant cells. During the emergence of root hairs from the trichoblast, ADF accumulates in the bulge along with actin microfilaments (Jiang and others 1997). In elongating root hairs, ADF is preferentially distributed in the tip of the root hair and looks strikingly similar to the first reports of profilin localization in tobacco pollen tubes. However, as was found with the initial reports of profilin localization, it may be that problems with fixation and penetration of antibodies through the cell wall do not give a completely accurate representation of ADF distribution. Whether this is correct awaits further investigation.

It is well established that root hairs and pollen tubes have gradients of Ca^{2+}, oriented with the highest concentration at the tip, that are required for growth (Obermeyer and Weisenseel 1991; Rathore and others 1991; Miller and others 1992; Felle and Hepler 1997). Pollen has also been shown to have a non-uniform cytosolic pH. Fluctuations of pH that vary by as much as 0.6 pH units propagate down the pollen tube, and it is proposed that recovery of the resting pH begins in the tip region (Messerli and Robinson 1998). Consistent with this finding, an alkaline band that is situated several microns behind the tip has been described (Feijó and others 1999). Profilin is quite sensitive to the presence of Ca^{2+} and its ability to prevent actin polymerization increases three-fold over a physiologically relevant range of Ca^{2+} (Kovar and others 2000). Maize ADF is phosphorylated by a Ca^{2+}-dependent kinase activity, which would result in decreased affinity for actin. In contrast, the higher pH of the alkaline band is predicted to enhance the ability of ADF to increase actin dynamics near the tip. Thus, even if profilin and ADF are uniformly distributed, cytosolic gradients of

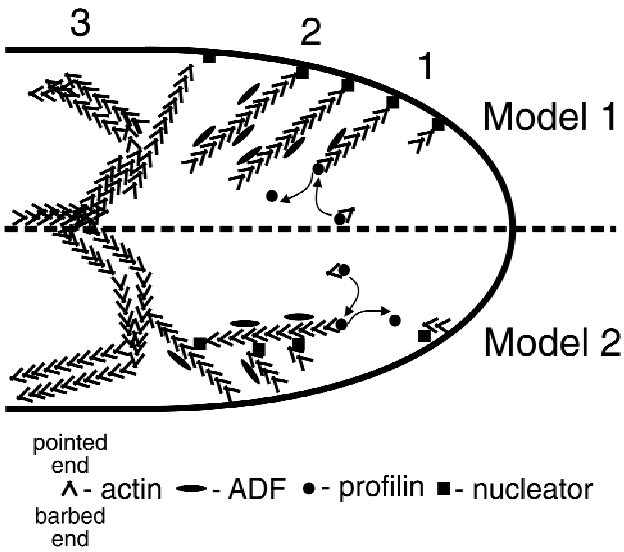

Figure 1. Models for the polymerization of actin in tip-growing cells. Two models, differing in the orientation of the fine filaments at the tip, are shown (1). In both models actin polymerization is nucleated at the plasma membrane by an unknown factor. Possible candidates include the ARP2/3 complex or profilin bound to a proline-rich protein. Actin microfilaments polymerize rapidly in a zone defined by the alkaline band described by Feijó and others (1999) (2). The primary prediction of the models is that polymerization is stimulated by the activity of ADF in the alkaline zone and by addition of profilin-actin complexes to free barbed ends. Model 1 shows the arrangement of microfilaments that results if the pointed ends of the actin filaments remain associated with the plasma membrane. Model 2 shows the actin filament orientation that is expected if the nucleation of actin polymerization is according to models proposed for the activity of the ARP2/3 complex (Borisy and Svitkina 2000; Mullins 2000). The fine subapical microfilaments are incorporated into the large bundles of actin that support streaming (Miller and others 1999; Vidali and Hepler 2000) (3), perhaps mediated by villin (not shown).

Ca^{2+} or pH could dramatically alter the activity of these proteins. It has been proposed that villin, a Ca^{2+}-sensitive severing protein, suppresses actin filament elongation in the extreme apex of pollen (Vidali and Hepler 2000) and that ADF will increase actin turnover, and therefore suppress actin filament growth in the region of the alkaline band (Feijó and others 1999).

Figure 1 presents alternative models for the effect of monomer-binding proteins on actin dynamics in the region of the alkaline band. The polarity of the fine filaments near the tip is not known, but it is possible that they originate at the subapical plasma membrane and extend toward the large actin bundles behind the tip along which cytoplasmic streaming occurs. The models comprise three stages. First, actin polymerization is nucleated at the apical or subapical plasma membrane as proposed previously (Vidali and Hepler 2000). This could be due to the activity of a nucleating factor such as the Arp2/3 complex (Mullins and others 1998), or to nucleation by profilin bound to a proline-rich protein (Jonckheere and others 1999). Second, rapid polymerization of actin occurs at the barbed ends stimulated by ADF in the region of the alkaline band, where the calcium gradient has begun to attenuate, thereby reducing the severing activity of villin. Polymerization is also stimulated by addition of profilin-actin complexes to free barbed ends (Pollard and Cooper 1984). At this point the models differ with respect to the orientation of the actin filaments. As shown in Model 1, the pointed ends remain associated with the plasma membrane and the filaments extend toward the large bundles of actin oriented with the barbed end inward. Alternatively, if polymerization occurs by a system similar to the dendritic nucleation model (Borisy and Svitkina 2000; Mullins 2000) newly formed filaments, capped at their pointed end by the ARP2/3 complex, branch from the side of adjacent filaments and extend toward the plasma membrane. The extension would drive the membrane outward, or if the cell wall is sufficiently rigid, model 2 predicts that the filaments will be displaced toward the center with the pointed end leading. Finally, farther behind the tip the fine filaments are quickly incorporated into the large actin bundles behind the tip by a bundling activity, as proposed previously (Miller and others 1999; Vidali and Hepler 2000), perhaps by villin-like proteins (Yokota and Shimmen 1999; Tominaga and others 2000).

The most important feature of both models, with respect to monomer-binding proteins, is that actin filament elongation is predicted to be maximal in the region corresponding to the alkaline band. This contrasts with recent models, which predict that the alkaline band is a region of high actin turnover and fragmentation (Feijó and others 1999; Vidali and Hepler 2000), yet still allows for suppression of polymerization by villin in the vesicle-rich apex due to extremely high levels of Ca^{2+}. Because the majority of actin filaments in plant cells are likely to be capped at their barbed end (Staiger and others 1994; Staiger and others 1997), the concentration of free actin monomers in the cytoplasm is likely to exceed the critical concentration for polymerization at the barbed ends. Therefore, the equilibrium will favor polymerization at new barbed ends. The model predicts that actin polymerization will be increased in the region of the alkaline band by stimulation of ADF activity and addition of profilin-actin com-

plexes to free barbed ends. This prediction is supported by the fact that *Arabidopsis* ADF1 increases the elongation rate of Arp2/3-nucleated actin filaments approximately three-fold (Ressad and others 1999). Furthermore, calculation of the amount of profilin-actin complex in maize pollen indicates that there is a large pool of profilin-actin complex that could participate in barbed-end assembly of new actin filaments (Gibbon and others 1999). Although it is attractive to think that actin polymerizes near the apex of tip-growing cells, there is no direct evidence of where new actin filaments are nucleated or where polymerization is maximal in plant cells. This ambiguity is a major hurdle that must be overcome to understand how actin is organized in tip-growing cells; these problems can be tested directly by measuring free barbed ends and incorporation of labeled actin monomers into filaments.

The models differ in the orientation of the fine subapical actin filaments. Recently, Staiger (2000) proposed that the region of fine actin filaments might be associated with a site of active endocytosis. If this is the case, the orientation of the microfilaments diagrammed in Model I will support movement of endocytic vesicles back to the central cytoplasm by a barbed-end directed myosin. Although a pointed-end directed class of myosin has been discovered (Wells and others 1999), plants do not seem to have a homologous class of myosins (Reichelt and Kendrick-Jones 2000). In Model 2 the orientation of the actin filaments relative to the plasma membrane is similar to current models for actin polymerization in animal systems (Mullins 2000). The orientation of actin filaments in Model 2 is also attractive because it allows for delivery of secretory vesicles toward the tip by barbed-end-directed motors. However, recent studies do not find an orderly meshwork of actin filaments immediately under the apical plasma membrane, as is clearly visible at the leading edge of migrating animal cells, so animal models of polymerization may not be fully applicable to plants. The polarity of the actin filaments in the large bundles in *Hydrocharis* has been determined and the actin filaments near the cortex, which support streaming toward the tip, are oriented with their barbed ends toward the tip, whereas the central bundles are oriented in the opposite direction (Tominaga and others 2000). Similar experiments could be performed to determine the polarity of the fine subapical actin filaments.

Monomer-Binding Proteins in Other Plant Cells

The distribution of profilin in a number of other plant cells indicates that not all profilin is freely diffusable in the cytoplasm. Birch pollen profilin expressed in vertebrate cells accumulates in the nucleus, whereas the endogenous profilin does not (Rothkegel and others 1996). Immuno-gold EM reveals a substantial amount of profilin in the nucleus of high-pressure frozen, freeze-substituted *Ledbouria socialis* pollen (Hess and Valenta 1997). Additionally fluorescently labeled profilin injected into living cells accumulates in the nucleus of the green alga *Micrasterias denticulata* (Holzinger and others 1997) and of *Tradescantia virginiana* stamen hair cells (M. von Witsch and C. J. Staiger, unpublished observation cited in Gibbon and Staiger 2000). Because plant profilin accumulates in the nucleus of diverse cell types it is likely that profilin has a specific function in the nucleus.

Much of what is known about the function of profilin and ADF in plant cells has been discovered by microinjection of native and recombinant proteins into living cells. Birch profilin injected into *Tradescantia* stamen hair cells causes transvacuolar strands to break, perturbs cytoplasmic streaming and depolymerizes actin microfilaments (Staiger and others 1994; Valster and others 1997). Likewise, profilin microinjected into tobacco mesophyll cells causes increased plasmodesmatal permeability, presumably by depolymerizing actin (Ding and others 1996). Injection of plant and *Acanthamoeba* profilin into *Micrasterias* causes transient inhibition of cell growth, similar to the effect of cytochalasin D (Holzinger and others 1997). Birch profilin injected into dividing *Tradescantia* stamen hair cells disrupts cell plate formation by depolymerizing actin filaments (Valster and others 1997). Microinjection of ADF transiently inhibits cytoplasmic streaming and, surprisingly, upon recovery the new cytoplasmic strands are oriented transverse to the growth axis (Hussey and others 1998). These experiments demonstrate that a transient increase of monomer-binding proteins depolymerizes actin filaments.

The actin-dependent positioning of the nucleus in mature *Tradescantia* stamen hair cells is a useful tool for analyzing the activity of actin-binding proteins in living cells (Gibbon and others 1997). Microinjection of actin-binding factors alters the positioning of the nucleus and the average time required for disruption can be used as an index of depolymerizing activity. Generally, the activity of profilin in the microinjection assay correlates well with the affinity of a profilin isoform for actin, regardless of the organism from which the profilin isoform is derived (Gibbon and others 1997; Clarke and others 1998; Gibbon and others 1998; Kovar and others 2000; Schobert and others 2000). However, a mutant of *Zea mays* PRO1 (ZmPRO1), with a substitution of

Phe for Tyr at position 6, has higher affinity for PLP than wild-type profilin does and has increased activity in the microinjection assay (Gibbon and others 1998). The basis for increased activity as a result of higher affinity for PLP in the living cell is not known, but is likely to involve proteins with proline-rich domains.

Signaling and Monomer-Binding Proteins

Profilin and ADF are potential targets for many sensory pathways that signal reorganization of the actin cytoskeleton. Binding to polyphosphoinositides, phosphorylation, and the small GTPases of the Rho subfamily can alter the activity of both proteins. Furthermore, as stated previously, these proteins can be affected by changes in pH or Ca^{2+} concentration. Direct evidence for the impact of signaling cascades on profilin or ADF function in plants is lacking; however, there are several reports that suggest these proteins are responsive to signal transduction cascades.

The phosphorylation of profilin has recently been shown in bean root nodules and in poppy. The profilin purifed from been root nodules is phosphorylated on a Tyr residue as determined by phosphoamino acid analysis, dephosphorylation by a Tyr phosphatase, and acid lability of the phosphate (Guillén and others 1999). It will be interesting to discover which residue is phosphorylated because the Tyr at position 6 of maize profilin is implicated in PLP binding (Mahoney and others 1997; Gibbon and others 1998) and is, consequently, an interesting target for regulation by phosphorylation. Pollen profilin can also be phosphorylated *in vitro* by protein kinase A (Snowman and others 2000). It is not known whether an endogenous kinase in poppy can phosphorylate profilin. However, profilin alters kinase activity that is involved in the self-incompatibility response, suggesting that profilin interacts with a kinase or regulatory molecule (Clarke and others 1998).

A pathway that involves the small GTPases of the Rho subfamily regulates reorganization of the actin cytoskeleton in many organisms. Profilin binds to the proline-rich formin-homology domain (FH domain) proteins, many of which also bind to Rho proteins (Imamura and others 1997; Watanabe and others 1997). Plants have a unique family of small GTPases called Rop, which are implicated in the control of actin organization (reviewed by Li and Yang 2000). Efforts to identify plant Rop-binding proteins have not resulted in the identification of an FH-domain protein (Li and Yang 2000). Recently, a putative formin-homology protein was cloned from *Arabidopsis.* This protein is unusual compared with other formins because it does not have a domain predicted to interact with Rho-like GTPases and it is predicted to have a membrane-spanning domain (Banno and Chua 2000). Additionally, the proposed proline-rich FH1 domain does not have typical proline-rich repeats but a single stretch of 9 contiguous proline residues. Further experimentation is required to demonstrate that this protein has the ability to regulate actin via profilin or Rop proteins. Rho signaling also impacts ADF function. ADF is phosphorylated by LIM kinases, which are activated by a Rho-dependent pathway (Pollard and others 2000). Plants, however, may be different in this respect because the phosphorylation of ADF by pollen cytosolic extracts is calcium dependent. These data indicate that plants have unique signaling networks to regulate actin rearrangements.

Future Prospects

The regulation of cell responses to environmental and developmental signals is very complex and plant biologists are just beginning to understand how these signaling networks are formed. As signaling events that result in, or correlate with, actin rearrangement have been reviewed in detail recently (Staiger 2000), I will not focus on this topic for future directions of investigation. There are other aspects of profilin and ADF function that warrant further consideration. First, both profilin and ADF have been observed to transit to the nucleus in plant cells, which indicates there may be a role for these proteins in nuclear function. Second, there is a large gap in our current view of profilin and ADF function *in vivo,* because few reports of whole plants over- or under-expressing monomer-binding proteins have been presented in the literature.

The role of profilin or ADF in the nucleus is not clear but there are some interesting reports that hint at a possible role in the regulation of chromatin structure. Chromosome-remodeling complexes, such as the yeast SWI2/SNF2 complexes, contain actin-related proteins (Cairns and others 1998; Peterson and others 1998). Furthermore, the SWI/SNF-like chromatin remodeling complex Brg-associated factor (BAF) binds to profilin affinity columns (Zhao and others 1998). The BAF complex comprises at least 10 polypeptides, including β-actin and an actin-related protein, which are bound by the Brg protein. Interestingly, the association of the BAF complex with chromatin is enhanced by the presence of PtdIns(4,5)P_2 (Zhao and others 1998). Polyphosphoinositides could, therefore, regulate the

association of profilin with potential nuclear ligands such as SWI or BAF complexes. There are numerous homologs of SWI2 in plants and it is likely that plants have similar chromatin remodeling complexes that include actin or actin-related proteins (D. Selinger, personal communication; also see ChromDB for updated information http://ag.arizona.edu/chromatin/chromatin.html). Profilin, therefore, may be a component of the homologous complex in plants or it may regulate the activity of the complex in some way. Together, these data hint that profilin may be a modulator of nuclear function in plant cells.

The ability to over or under express selected proteins in a whole plant is of tremendous value for understanding the role of a protein in the development or function of a particular organ or tissue. This approach has revealed differences between actin isoforms in *Arabidopsis* (Gilliland and others 1998), and should be extended to the study of actin-binding proteins. Over-expression of monomer-binding proteins in other systems led to the surprising results that profilin can increase the level of F-actin in cells (Finkel and others 1994) and that ADF can increase the motility of Dictyostelium (Aizawa and others 1996). Each of these results forced changes in the models of monomer-binding protein function, which were later confirmed in biochemical experiments. Given that plant monomer-binding proteins have unique cellular distributions and different signaling pathways that impinge on their function, reverse-genetic approaches will provide great insight into the regulation of the plant actin cytoskeleton. The presence of multiple isoforms of monomer-binding proteins may make such studies difficult; therefore over-expression of dominant mutants of the monomer binding proteins will provide particularly interesting information about the regulation of the actin cytoskeleton in plants.

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