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Regulation of Actin Dynamics in Pollen Tubes: Control of Actin Polymer Level

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Actin cytoskeleton undergoes rapid reorganization in response to internal and external cues. How the dynamics of actin cytoskeleton are regulated, and how its dynamics relate to its function are fundamental questions in plant cell biology. The pollen tube is a well characterized actin-based cell morphogenesis in plants. One of the striking features of actin cytoskeleton characterized in the pollen tube is its surprisingly low level of actin polymer. This special phenomenon might relate to the function of actin cytoskeleton in pollen tubes. Understanding the molecular mechanism underlying this special phenomenon requires careful analysis of actin-binding proteins that modulate actin dynamics directly. Recent biochemical and biophysical analyses of several highly conserved plant actin-binding proteins reveal unusual and unexpected properties, which emphasizes the importance of carefully analyzing their action mechanism and cellular activity. In this review, we highlight an actin monomer sequestering protein, a barbed end capping protein and an F-actin severing and dynamizing protein in plant. We propose that these proteins function in harmony to regulate actin dynamics and maintain the low level of actin polymer in pollen tubes.

Key words: actin-binding proteins; actin dynamics; pollen tube.

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Pollen tube growth is the restricted step of reproduction in flowering plants. It provides passage for sperm cells to reach the embryo sac and eventually fuse with egg cell and central cell to achieve double fertilization. It is a well-documented typical tip-growing system, which forms the orderly and highly polarized architecture, and the growth is restricted to a dome-shaped tip domain as compared with the diffuse growth of other cells. To accomplish this process, directed intracellular trafficking is essential, such as cytoplasmic streaming, exocytosis, endocytosis, etc. The actin cytoskeleton has been shown to be a prominent structure in the cytoplasm of pollen tube, which is demonstrated to be involved in the organization of cytoplasm and is assumed to power these trafficking processes.

The pollen tube is divided into three distinct regions: the apical region, sub-apical region and the shank region. Each of them has a distinct pattern of cytoplasm organization. The actin cytoskeleton is organized into distinct structures in these regions based on the observation of various pollen tubes. It was generally accepted that each actin structure carries out a distinct function. In the shank of the pollen tube, the actin cytoskeleton forms actin bundles parallel to the axis of the pollen tube, which is assumed to provide a track for delivering the Golgi-derived vesicles to the apical region of the pollen tube. Various actin structures, including the ring (Kost et al. 1998),

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collar (Gibbon et al. 1999), mesh (Chen et al. 2002), and fringe (Lovy-Wheeler et al. 2005), or funnel-like structures (Geitmann and Emons 2000) are found in the subapical region of the pollen tube. The studies through labeling actin cytoskeleton of the living cells by GFP-mTalin and GFP-NtADF1 probes revealed that the apical region contains some short actin filaments and the actin is highly dynamic in this region (Kost et al. 1998; Fu et al. 2001; Chen et al. 2002; Cheung et al. 2008). Pharmacological treatment, together with genetic manipulation of the actin cytoskeleton, indicates that the actin cytoskeleton is essential for pollen tube growth (Gibbon et al. 1999; Vidali et al. 2001; Cheung and Wu 2004; Xiang et al. 2007). The actin cytoskeleton exists in equilibrium between a monomeric form (G-actin) and a polymeric form (F-actin). Rapid conversion of G-actin to F-actin or F-actin to G-actin is required to carry out its function. Alteration of this ratio is believed to affect the function of actin cytoskeleton. Indeed, alteration of G-actin and F-actin ratio by latrunculin B treatment or microinjection of profilin arrested pollen tube growth (Gibbon et al. 1999; Vidali et al. 2001).

One striking difference noted for the actin cytoskeleton in the pollen tube is its surprising low amount of F-actin. The results from maize and *Papaver rhoeas* (field poppy) indicate that filamentous actin occupies less than 10% of the total actin of pollen tubes (Gibbon et al. 1999; Snowman et al. 2002). By comparison, the majority of actin is in filamentous form for budding yeast cells (Karpova et al. 1995). These results hint that actin dynamics in pollen might be quite different. It seems that the phenomenon of low F-actin level could extend to other plant cell types. The level of F-actin in tobacco suspension cells is even lower, which only represents 1–2% of total actin (Wang et al. 2005). The total actin concentration in pollen tube is quite high, which can reach more than 200 μM (Vidali and Hepler 1997; Gibbon et al. 1999; Snowman et al. 2002). Considering the low content of F-actin ($<$10%), G-actin concentration in pollen tube could be as high as 100–200 μM. In addition, previous measurement results indicate that profilin is equimolar with the total actin pool (Vidali and Hepler 1997; Gibbon et al. 1999; Snowman et al. 2002), and when taken together with the low apparent binding constant determined for profilin and pollen actin (Gibbon et al. 1998; Kovar et al. 2000), it leads us to predict that the unpolymerized actin in pollen is bound to profilin (Figure 1). It could be expected that almost all actins would be in the F-actin form since actin-profilin could elongate the barbed end as well as actin alone (Pantaloni and Carlier 1993), though it inhibits spontaneous actin nucleation. If that is the case, the cytoplasm of the plant cell will be choked by the presence of high actin polymer. However, that does not happen for the pollen tube. How the pollen tube precisely regulates the ratio of F-actin to G-actin remains an open question.

Within cells, actin dynamics are regulated by more than 70 classes of actin-binding proteins (Pollard et al. 2000). Based on their functions, they can be divided into several categories, including the monomer binding proteins that regulate the size of the G-actin pool; side binding proteins that stabilize actin filaments and allow the formation of higher order actin structures, for example, actin bundles and network; capping proteins that control the behavior of actin filament ends for subunit loss or addition; and nucleation factors that promote actin polymerization to eliminate the lag phase of actin assembly. Developing a full understanding of the properties of actin-binding proteins will enrich our knowledge of the regulation of actin dynamics in pollen tubes and in plant cells in general. Several excellent recent reviews summarize the research progress on actin-binding proteins in plants (McCurdy et al. 2001; Staiger and Blanchoin 2006; Higaki et al. 2007; Ren and Xiang 2007). In the present review, we highlight a monomer binding protein, an F-actin severing and dynamizing protein and a barbed end capping protein. We also discuss the potential role of these proteins in regulating actin dynamics and maintaining the ratio of F-actin to G-actin in pollen tube.

The Role of Profilin in Maintaining the Pool of Actin Monomer

Profilin was originally isolated from calf spleen as a protein forming high affinity complex with G-actin (Carlsson et al. 1977). It is a small (molecular weight 12–15 kDa) ubiquitous cytosolic protein. It is now accepted that profilin is a major regulator of actin dynamics and is crucial for cellular growth, morphogenesis and cytokinesis. Profilin was reported to be essential for the viability of flies (Verheyen and Cooley 1994), fission yeast (Lu and Pollard 2001), and mice (Witke et al. 2001). The disruption of profilin severely impacts the growth of *Dictyostelium* (Haugwitz et al. 1994) and budding yeast (Haarer et al. 1990). In addition to binding to actin, profilins bind to other ligands, for example, stretch of poly-proline (PLP) and proline-rich proteins, and phospholipids.

Binding to proline-rich proteins could be a major function of profilins. In particular, increasing evidence has shown the importance of formins, which use profilin-actin to drive actin assembly *de novo*. In yeast cells, genetic evidence suggest that the ability of profilin binds to PLP sequence is important (Ostrander et al. 1999; Lu and Pollard 2001). A site-directed mutant of profilin that lacks PLP binding but has normal actin binding does not complement the growth defect of *Dictyostelium* profilin mutants (Lee et al. 2000). Biochemical evidence suggested that the proline-binding ability is different among profilin isoforms, and the nuclear displacement experiments suggested that the differential ability of the binding of profilins to PLP correlates with its ability to affect actin-based structures (Kovar et al. 2000; Kovar et al. 2001a). The differential preference for profilin isoforms of formin indirectly supports the importance of the binding of profilin to PLP (Neidt et al. 2009). Together with the preliminary evidence that *Arabidopsis* formins have preference for different profilin isoforms (Deeks et al. 2005),

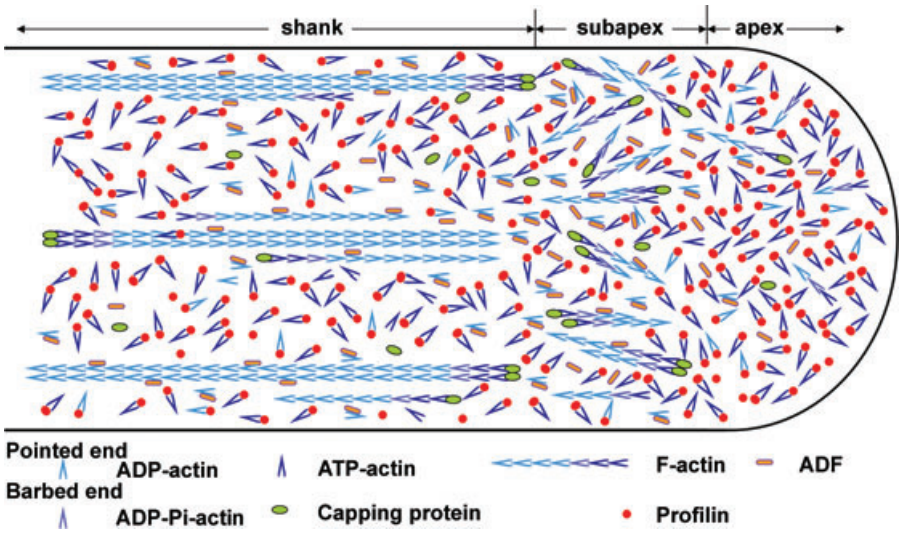

Figure 1. The majority of actin is in G-actin form and sequestered by profilin in the pollen tube.

The pollen tube is divided into three characteristic regions: apical region, subapical region and shank region. Each of them has a distinct pattern of actin cytoskeleton organization. According to previous measuring results, total actin and profilin are roughly present at a 1:1 molar ratio, and F-actin occupies less than 10% in the pollen tube. Therefore, it is assumed that the majority of G-actin is in profilin-actin complex form in the pollen tube. We propose that profilin, capping protein (CP) and actin depolymerizing factor (ADF) function coordinately to control the dynamic equilibrium between G-actin and F-actin pools in the pollen tube.

it strongly supports the notion that the ability of profilin binding to proline-rich proteins is important. Another binding ligand of profilin is phospholipids. The binding of profilin to phospholipids links its potential role to vesicle trafficking. Indeed, mutation of profilin in *Dictyostelium* causes defects in phagocytosis, endocytosis and maccropinocytosis (Haugwitz et al. 1994; Lee et al. 2000; Janssen and Schleicher 2001).

Profilin was first identified in plants as birch allergen (Valenta et al. 1991). They are encoded by small multigene families. In *Arabidopsis*, there are five genes encoding profilin (Kandasamy et al. 2002). Profilins show distinct expression patterns in plants, and they are divided into two groups: vegetative profilins and reproductive profilins. The *in vitro* biochemical data suggested that different profilin isoform functions distinctly (Gibbon et al. 1998; Kovar et al. 2000), which supports the isovariant dynamics model. The alteration of profilin expression level reveals the physiological role of profilin in actin-based processes. Overexpression of profilin in transgenic *Arabidopsis* plants results in longer roots and root hairs (Ramachandran et al. 2000). On the contrary, the reduction of profilin expression in transgenic anti-sense *Arabidopsis* induced a dwarf phenotype with short hypocotyls. However, *PRF1* T-DNA insertion mutant has elongated hypocotyls and longer roots and an increased number of root hairs (McKinney et al. 2001). The reason for the different outcome between the T-DNA insertion and anti-sense approach is not clear. Regardless, neither study provides evidence of alteration of actin organization in these mutants. Therefore, it is not known whether phenotypes directly correlate with its actin binding.

Structurally, plant profilins look overall similar to profilins from yeast and vertebrate, though the identity of primary amino acid sequence is only about 30% (Fedorov et al. 1997; Thorn et al. 1997). As shown in Figure 2, *Arabidopsis* profilin 1

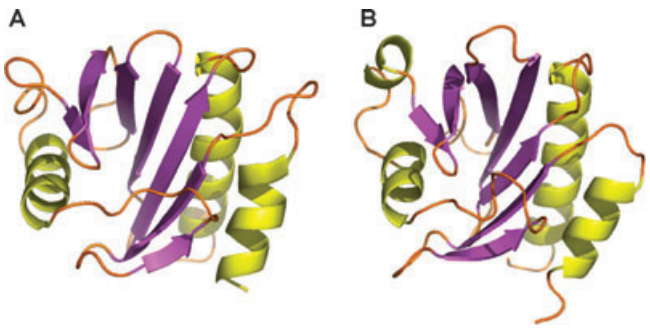

Figure 2. The structure of *Arabidopsis* profilin 1 looks overall similar to bovine profilin.

Structures of profilins used in this essay have been downloaded from the protein data bank (PDB) database and displayed by Pymol software (DeLano Scientific LLC, San Carlos, CA, USA). The PDB IDs for *Arabidopsis* profilin 1 and bovine profilin are 1a0k and 1pne, respectively. α-helixes, β-strands and loops are colored with yellow, violet, and orange, respectively.

mainly consists of three α-helixes and seven β-strands, which is similar to that of profilins from other species, implicating profilins conduct conserved functions throughout different kingdoms. Indeed, the residues involved in actin-binding and PLP-binding are well conserved between plant profilin and vertebrate profilin (Figure 3). This has been demonstrated experimentally for Zmpro5 (Kovar et al. 2001a). The actin-binding and PLP-binding motifs separate spatially, which explains the ability of profilin to bind both ligands simultaneously. However, many of the positive charged residues implicated in binding PtdIns(4,5)P_2 are not conserved, suggesting that the PtdIns(4,5)P_2 mediated disruption of the profilin–actin complex in plant may occur by a different mechanism (Fedorov et al. 1997). *Zmpro5D8A* mutant enhances the binding to PtdIns(4,5)P_2 (Kovar et al. 2001a), but has not been tested for actin binding. In addition, several other differences are also noticeable, for example, a specific solvent-filled pocket is discovered in *Arabidopsis* profilin 1 which locates near the actin-binding surface (Figure 4A). But for bovine profilin, this pocket is relatively compact (Figure 4B). The residues that make up the pocket are well conserved among plant profilins, implicating that the pocket is a likely structural characteristic of plant profilins (Thorn et al. 1997). Plant profilins do not enhance the exchange of adenosine 5′-diphosphate (ADP) to adenosine 5′-triphosphate (ATP) on actin monomer (Perelroizen et al. 1996; Kovar et al. 2001b). It might be explained by the substitution of key amino acids on plant profilin. The residue Y79 on fission yeast profilin is potentially important for nucleotide exchange (Lu and Pollard 2001). Fission yeast profilin mutant Y79R lacks nucleotide exchange activity, but all plant profilins known to lack nucleotide exchange activity have R at this position. As shown in Figure 4C, the corresponding residue is R84 in *Arabidopsis* profilin 1. In addition, previous structural studies indicated that binding of profilin to the C-terminus of actin could trigger the conformational change of nucleotide cleft in actin (Crosbie et al. 1994), and consequently may affect the nucleotide exchange on actin. The structure of bovine profilin-beta-actin shows that His119 in bovine profilin has a close contact with the C-terminus of actin (Chik et al. 1996). However, the corresponding residue in *Arabidopsis* profilin 1 is Threonine at position 111 (Thr111) (Figure 4C), which to some extent suggests the binding of plant profilin might cause a possible structural change in the neighboring C-terminus of actin, hence inducing a distinct conformation in the nucleotide cleft and somehow inhibiting the ATP exchange on actin (Thorn et al. 1997).

The diverse actin phenotypes associated with profilin mutants suggest that the function of profilins should be analyzed case by case. Indeed, overexpression of a cotton profilin in BY-2 suspension cell induces actin polymerization and promotes cell elongation (Wang et al. 2005), suggesting that profilin functions as an actin polymerization promoting factor in this regard. However, microinjection of profilin into stamen hair cells depolymerizes actin cytoskeleton, which supports the role of profilin in sequestering actin (Staiger et al. 1994). The well studied recombinant profilins are from maize. The biochemical characterization of maize profilins places them into two distinct classes: class I profilins and class II profilins. Class I profilins bind to PtdIns(4,5)P_2 much stronger than class II profilins, whereas class II profilins have stronger affinity for actin and PLP. These correlate with the stronger ability of class II profilins to disrupt actin cytoplasmic architecture in stamen hair cell (Gibbon et al. 1998; Kovar et al. 2000).

Plant profilin was reported to localize in the cytoplasm of pollen tube uniformly (Hess et al. 1995; Vidali and Hepler 1997). However, it was shown very recently that GFP-AtPRF1 and GFP-AtPRF2 localized differentially in *Arabidopsis* epidermal cell (Wang et al. 2009), which emphasizes the necessity to determine the localization of each profilin isoform in pollen tube carefully. However, if profilins localize uniformly in the cytoplasm of pollen tube, in consideration of the existence of calcium gradient in pollen tube and the regulation of profilin's sequestering activity by calcium (Kovar et al. 2000), the existence of a gradient of total sequestering activity of profilin in the pollen tube is expected. The results of measurement of various pollens showed that profilin was present in a 1:1 ratio with actin (Vidali and Hepler 1997; Gibbon et al. 1999; Snowman et al. 2002). The *in vitro* biochemical analysis (Gibbon et al. 1998) and microinjection experiment (Staiger et al. 1994) suggest that plant profilin functions as a simple sequestering protein. Taking these data together, it can be concluded that plant profilin forms a complex with G-actin, suppresses spontaneous actin nucleation and maintains the large G-actin pool in the pollen tube.

The Role of Capping Protein in Controlling the Availability of Actin Barbed End for the Addition of Profilin-Actin Complex

Capping protein (CP) is one of the best characterized capping factors. It is a heterodimer consisting of α- and β- subunits, with a molecular weight ranging from 28 to 36 kDa. CP, which was called β-actinin, was initially purified and characterized from muscle (Maruyama 1965, 1966; Maruyama et al. 1977). Nonmuscle CP was first purified from *Acanthamoeba* and demonstrated to cap barbed ends (Isenberg et al. 1980). It was shown to be conserved among various species (Cooper and Sept 2008). Recent observation of single actin filaments with total internal reflection fluorescence microscopy (TIRFM) unambiguously demonstrated that the presence of CP at the barbed end prevents the addition and loss of actin subunits (Kim et al. 2007). Simultaneous expression of both subunits in bacteria produces large quantities of soluble active protein; this facilitates the biochemical studies of CP *in vitro* (Soeno et al. 1998). Benefiting from that, the crystal structure of chicken CapZ was resolved recently (Yamashita et al. 2003). The study reveals that α- and β-heterodimer forms a compact structure resembling a mushroom with pseudo-twofold

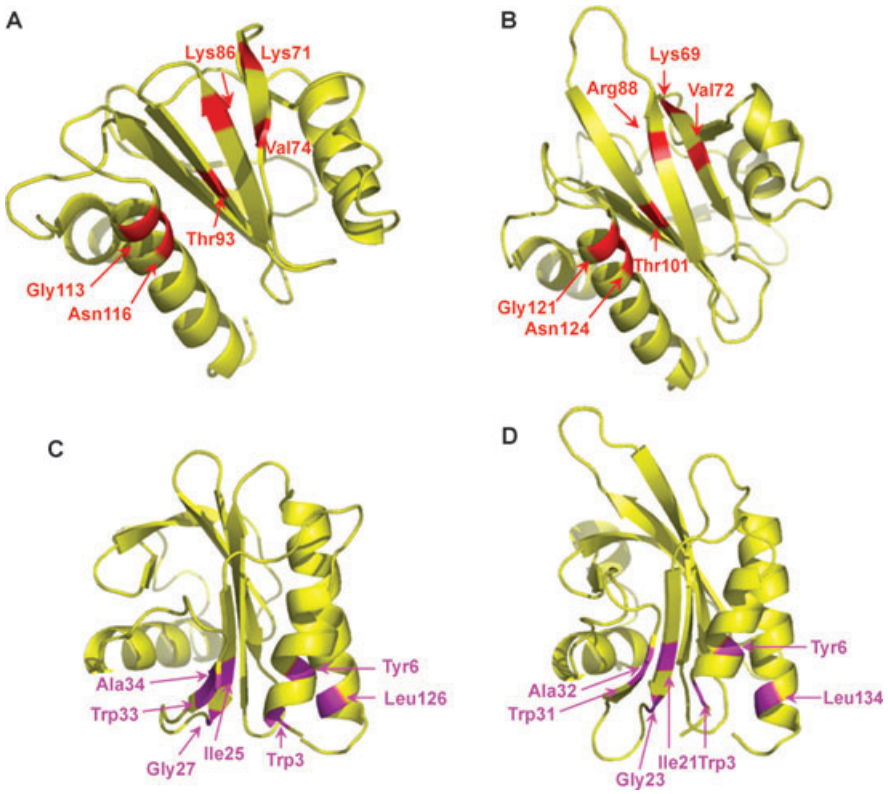

Figure 3. The residues involved in actin-binding and poly-proline (PLP)-binding are highly conserved between *Arabidopsis* profilin 1 and bovine profilin.

Residues conserved for actin-binding of *Arabidopsis* profilin 1 **(A)** and bovine profilin **(B)** are highlighted in red. They are Lys71, Val74, Lys86, Thr93, Gly113, Asn116 in *Arabidopsis* profilin and Lys69, Val72, Arg88, Thr101, Gly121, Asn124 in bovine profilin, respectively. Arg88 in bovine profilin is replaced with Lys86 in *Arabidopsis* profilin, but both are basic. Residues conserved for PLP-binding of *Arabidopsis* profilin 1 **(C)** and bovine profilin **(D)** are highlighted in magenta. They are Trp3, Tyr6, Ile25, Gly27, Trp33, Ala34, Leu126 in *Arabidopsis* profilin, and Trp3, Tyr6, Ile21, Gly23, Trp31, Ala32, Leu134 in bovine profilin, respectively.

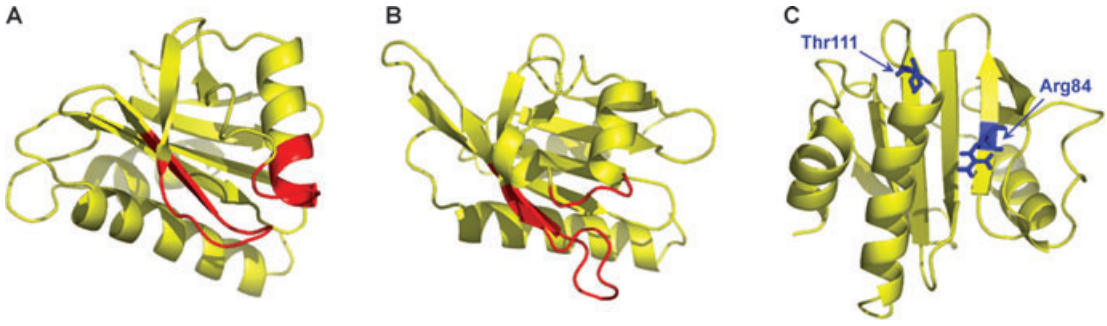

Figure 4. *Arabidopsis* profilin 1 has a unique solvent-filled pocket and several distinct residues that may contribute to its inhibitory effect on nucleotide exchange on actin.

Residues making up the pocket of *Arabidopsis* profilin 1 **(A)** (residues 52–58, 75–85) and bovine profilin **(B)** (residues 52–56, 73–87) are highlighted in red. Either in a freezing state where glycerol acts as a cryoprotectant, or in room-temperature state where water acts as the major surroundings, the pocket is filled with glycerol or water, respectively. But for bovine profilin, this pocket is relatively compact **(B)**. Based on the data of mutagenesis analysis on fission yeast profilin (Lu and Pollard 2001), it is implied that Arginine at position 84 in *Arabidopsis* profilin 1 **(C)** may explain why *Arabidopsis* profilin lacks nucleotide exchange activity on actin. In addition, Threonine at position 111 in *Arabidopsis* profiling 1 **(C)**, corresponding to Histidine at position 119 in bovine profilin, may also contribute to the lack of nucleotide exchange activity of *Arabidopsis* profilin 1 on actin.

rotational symmetry. Each of two C-terminal regions contains a short stretch of amphipathic α-helix. These are predicted to form a flexible "tentacle" that makes contact with the barbed end of the actin filament. The experimental evidence from Cooper's lab supports this model and gives slightly greater importance to the α-subunit (Wear et al. 2003; Kim et al. 2004; Wear and Cooper 2004; Kim et al. 2007).

Capping protein has been demonstrated to be a major actin regulator, for example, it has been shown to be one of five actin-binding proteins that regulate actin polymerization and depolymerization to generate "comet-tail" motility *in vitro* (Loisel et al. 1999). Recent studies with the reconstituted system demonstrated that CP promotes Arp2/3-dependent nucleation and branching rather than increasing the rate of filament elongation (Akin and Mullins 2008). CP is essential for basic cellular processes. Loss-of-function mutation of CP in *Dictyostelium* reduced F-actin level, and consequently affected cell motility (Hug et al. 1995). The results in yeast support the notion that CP stabilizes actin filaments *in vivo*, since actin filaments are not stable in the mutants of yeast CP (Amatruda et al. 1990, 1992; Karpova et al. 1995). However, CP is essential for the viability of *Drosophila* (Hopmann et al. 1996).

In the *Arabidopsis* genome, both α- and β-subunits are encoded by a single gene. The α-subunit of *Arabidopsis* capping protein (AtCP) (AtCPA) shares 29–35% amino acid sequence identity with α-subunits from amoeba, yeast and vertebrate. The β-subunit of AtCP (AtCPB) shares relative higher amino acid identity ($\sim$50%) with β-subunits of *Dictyostelium* and vertebrate. As shown in Figure 5A, the structure of AtCP resembles a mushroom and is overall similar to that of chicken CP (Yamashita et al. 2003). In particular, the C-terminal sequence of each subunit has a short region of amphipathic α-helix as the proposed "tentacle", though the C-terminal is not very conserved when comparing AtCP to CPs from other organisms. The purified recombinant AtCP eliminates the initial lag period for actin polymerization and increases the maximum rate of polymerization. AtCP prevents addition of profilin-actin into barbed ends during a seeded elongation reaction and prevents dilution-mediated depolymerization, conferring its barbed capping activity. It binds to actin filaments barbed ends with K_d values of 12–24 nM, it is somewhat higher than that of muscle CapZ (Schafer et al. 1996), but it is not too much different from non-muscle capping protein (Sauterer et al. 1991; DiNubile et al. 1995; Maun et al. 1996; Kuhlman and Fowler 1997) and recombinant *Schizosaccharomyces pombe* CP (Kovar et al. 2005). AtCP also inhibits the annealing of actin filaments, which could be relevant to the role of AtCP in the maintenance of short actin filaments population in a particular region of the cell, for example, the tip of the pollen tube. The divergence of the primary amino acid sequence at the C-termini of both subunits may account for these differences in ability to nucleate, and capping (Hug et al. 1992). However, it should be noted that the dissociation rate constant of AtCP from the barbed end is extremely low,

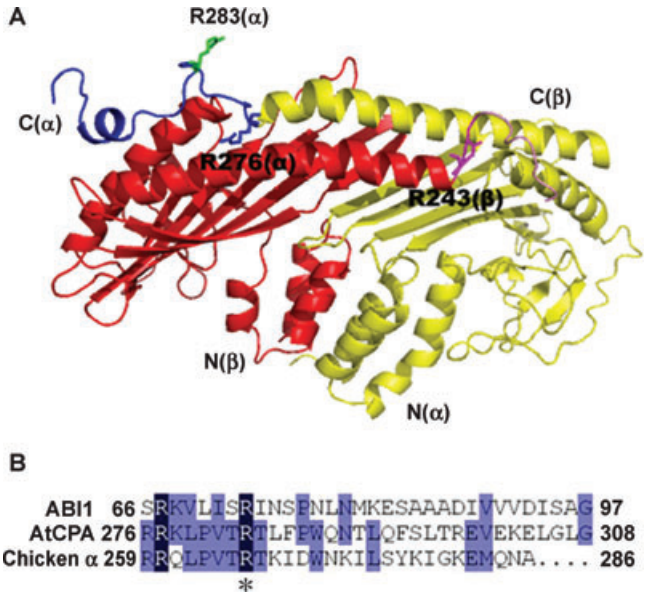

Figure 5. A model structure of *Arabidopsis* capping protein (AtCP) is mushroom-like, but the C-terminal "tentacle" is not very conserved.

(A) A model structure for AtCP.

This homology modeling was done with SWISS-MODEL (Guex and Peitsch 1997) using the chicken CP structure (protein data bank (PDB) code: 1IZN) as a template. AtCPA and AtCPB are colored yellow and red, respectively, except with their C-terminus colored blue and pink, respectively. However, CPA residues 1–12, 298–308, CPB residues 246–256 could not be modeled. Therefore, CPA residues 1–12 and 298–308 do not present in the structure. CPB residues 246–256 are replaced with the corresponding sequence of chicken β1, which is indicated by a light pink line. The proposed phosphatidic acid (PA) binding sequence is within the C-terminal "tentacle" of AtCPA, highlighted in blue. The arginine, which is important for the binding of ABI1 to PA is conserved in AtCPA at position 283 (R283(α)). In addition, two conserved arginines are also found in AtCP (Arg276(α) and Arg243(β) respectively), which were shown to be important for the "tentacle" to make contact with the barbed end of actin filaments (Kim et al. 2004).

(B) A sequence from the C-terminal "tentacle" domain of AtCPA shares reasonable similarity to a PA binding sequence from ABI1 (Zhang et al. 2004). The gene accession numbers of protein used for alignment are as follows: ABI1 (NM_118741), AtCPA (NM_111425) and chicken α1(M25534). The sequence from AtCPA shares 45% similarity with ABI1 PA binding sequence, whereas the sequence from chicken shares 30% similarity with the ABI1 PA binding sequence. This may explain why mammalian CP binds to PA so poorly. The Arginine (R73), which was shown to be important for the binding of ABI1 to PA (Zhang et al. 2004), is also conserved in AtCPA, indicated by the "star" below the sequence. It is demonstrated that this sequence is sufficient to bind to PA, and the indicated Arginine is important for the binding (Huang and Staiger, unpubl. data, 2006).

corresponding to a half-time of dissociation of approximately 38 min (Huang et al. 2006), which is roughly in the same time scale of the muscle CapZ (Schafer et al. 1996).

As stated above, it exists in a very high concentration of the profilin-actin complex in the pollen tube, and it suppresses spontaneous nucleation, but it should elongate actin filaments if their barbed ends are free. Therefore, maintenance of the large pool of actin monomers requires the combination of CP and profilin. Indeed, in the presence of CP, the addition of profilin-actin into the barbed end is prevented (Huang et al. 2003), making profilin a simple sequestering protein, consistent with previous reports (Pollard and Cooper 1984; Kang et al. 1999). To understand this hypothesis precisely, the cellular AtCP concentration needs to be determined in the future. It should be possible with the availability of AtCP specific antibodies (Huang et al. 2003).

Given the low dissociation rate constant for AtCP from the actin barbed end, the regulation of its activity is obviously needed, especially under some stimulating conditions, which need rapid actin polymerization. AtCP activity is not affected by calcium, but its activity is inhibited by PtIns(4,5)P_2 (Huang et al. 2003). In addition, AtCP also binds to phosphatidic acid (PA) with moderate affinity (Huang et al. 2006). Due to the low similarity between the identified PA binding sequences, making the sequence-based prediction of PA binding motif on a new PA-binding protein rather impractical (Testerink and Munnik 2005). However, we found that the C-terminal region of the AtCP α-subunit is quite similar to a PA binding sequence from the *Arabidopsis* protein phosphatase 2C, ABI1 (Zhang et al. 2004), which shares 45% amino acid similarity over 33 amino acids (Figure 5B; (Huang et al. 2006)). Indeed, several basic amino acids are absolutely conserved, including one arginine (R73) that was demonstrated to be necessary for PA binding by ABI1 (Figure 5B). We demonstrated that this sequence containing 33 amino acids is sufficient to bind to PA and the conserved arginine is important for the binding (Figure 5B; Huang and Staiger, unpubl. data, 2006). Therefore, it is reasonable to speculate that the binding of PA to the C-terminus of AtCPA would hinder the binding of AtCP to the barbed end of actin filament, consequently preventing capping and causing uncapping of AtCP as well.

The interaction of CP with PA alters the activity of AtCP. In the presence of PA, AtCP cannot bind to the barbed end. The AtCP-capped barbed end can also be uncapped by the addition of PA, allowing actin assembly from the pool of profilin-actin. Exogenous PA application elevates the actin polymer level in *Arabidopsis* suspension cell and poppy pollen (Huang et al. 2006). This verified the observation carried out by Lee et al., in soybean suspension cell (Lee et al. 2003). To explain this, one simple model has been proposed, in which PA dissociates CP from the actin filament barbed end, allowing profilin–actin complex to add into the free barbed ends and promote filament elongation (Huang et al. 2006). The biochemical analysis of the interaction of CP with PA, together with the results of F-actin elevation after exogenous PA application, strongly supports the role of CP in maintaining the level of F-actin. Taking these data into consideration, it is fair to speculate that CP functions in concert with profilin to maintain the low level of actin polymer in pollen tube and plant cell in general. However, the direct genetic evidence is urgently needed to support this hypothesis firmly.

The Role of Actin Depolymerizing Factors in Dissociating Actin Monomers from Pointed End

Increasing evidence suggests that actin depolymerizing factor (ADF) is another major actin regulator in plants, which binds to both G-actin and F-actin with a small mass (15–22 kDa). ADFs have been shown to be stimulus-responsive proteins, whose activity is regulated by pH, phospholipids and reversible phosphorylation. The potential role of ADF in maintaining the low F-actin pool could be due to the following functions: increasing the dissociation rate of actin subunits from the pointed end of filaments (Carlier et al. 1997); creating new filament ends by severing (Maciver et al. 1991; Andrianantoandro and Pollard 2006); increasing the rate of phosphate release following actin polymerization, and marked preference for binding to regions of actin filaments that contain ADP-actin subunits (Carlier et al. 1997).

Actin depolymerizing factors were first identified in plants during a search for pollen specific transcripts in *Lilium longiflorum* (Kim et al. 1993), and are present as a small multigene family in maize and *Arabidopsis*. Like profilins, plant ADFs can be separated into two phylogenetic classes: vegetative ADFs and reproductive ADFs (Kandasamy et al. 2007; Ruzicka et al. 2007). Plant ADFs belong to a relatively large gene family. It contains 12 isoforms in *Arabidopsis*, among which *Arabidopsis* ADF1 (Carlier et al. 1997; Bowman et al. 2000) is well studied biochemically. The deduced amino acid sequences of plant ADFs share only 28–35% identity with vertebrate ADF sequences. Residues for putative actin binding and a presumed phosphorylation residue share greatest similarity with the corresponding site of mammalian ADFs (Moon and Drubin 1995).

Recombinant AtADF1 binds G-actin in a 1:1 complex, but with 100-fold preference for ADP-bound actin versus ATP-bound actin (Carlier et al. 1997). AtADF1 also binds to F-actin, with a preference for the ADP-bound form. However, AtADF1 interacts with F-actin with less pH-dependent manner compared with nonplant ADFs. AtADF1 increased the initial rate of actin polymerization. This could be due to the combination of nucleation activity and severing activity. Indeed, it was reported that several nonplant ADFs have nucleation activity at their higher concentration and sever actin filaments efficiently (Andrianantoandro and Pollard 2006). Direct visualization of the effect of AtADF1 on actin dynamics with TIRFM will provide insight into the detailed action mechanism of AtADF1. In

particular, it has been shown very recently that the dynamics of plant cortical actin filaments is dominated by severing activity (Staiger et al. 2009). Future trial will be finding out if ADF is a major player here. An analysis of the kinetics of AtADF1-induced actin polymerization and depolymerization in the presence of blocked filament ends demonstrates that AtADF1 increases the depolymerization rate by 22-fold at the pointed end and increases the polymerization rate by 12-fold at the barbed end. In addition to considering the severing activity here, another explanation could be that AtADF1 binds to ADP-F-actin and alters the association between two adjacent actin monomers and increases the rate of actin monomer dissociation from the pointed end. Taking the biochemical data together, it is reasonable to imagine that ADF accelerates the rate of treadmilling of actin filaments and increases the depolymerization rate of capped actin filaments in the pollen tube. The activity of ADF is highly dependent on the presence of other actin-binding proteins, for example, the presence of AtVLN1 suppresses the depolymerization activity of AtADF1 (Huang et al. 2005). Whereas Aip1, another actin-interacting protein, containing 7–10 tryptophan-aspartate (WD repeats), enhances the depolymerization activity of ADF *in vitro*. In the absence of ADF/cofilin, Aip1 interacts with actin filaments weakly; whereas in the presence of ADF/cofilin, Aip1 caps filament barbed ends and binds weakly along the sides of filaments (Okada et al. 1999, 2002, 2006; Ono et al. 2004). Although recombinant AtAip1-1 enhances the weak depolymerizing activity of lily ADF1 *in vitro* (Allwood et al. 2002), the precise molecular mechanism underlying the enhancement of ADF severing activity by Aip1 needs further analysis.

To understand the role of ADF in maintaining the dynamics of actin filaments and controlling the ratio of F-actin to G-actin precisely, the determination of its cellular concentration in pollen tube is needed. It could be expected that ADF might be a very abundant protein in the pollen tube, since the previous measuring result from Staiger's laboratory shows that ADF is present at a 1:1 molar ratio with total actin in *Arabidopsis* suspension cells and in a roughly 1:3 ratio in leaf tissues (Chaudhry et al. 2007).

Conclusion

The picture emerging is that ADF, profilin and CP are key players in regulating actin dynamics and controlling the level of actin polymer in the pollen tube. In particular, ADF, probably together with Aip1, accelerates the treadmilling rate of actin filaments and increases the dissociation rate of actin monomers from the pointed end of actin filaments. Profilin forms a complex with G-actin and functions as a simple sequestering protein to maintain the large G-actin pool. AtCP binds to actin filament and prevents the addition of profilin-actin complex into the barbed end of the actin filaments to achieve the low level of F-actin in the pollen tube. To draw this picture more precisely, determination of the cellular concentration and intracellular localization of these players in pollen tube is urgently needed. To firmly support this hypothesis, genetic evidence with the alteration of the expression of these players is definitely required. In addition, considering these players in the cellular context, to determine how they may coordinate with other actin-binding proteins to modulate actin dynamics is also necessary.

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