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The function of actin-binding proteins in pollen tube growth

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Summary. Pollen tube growth is a key step in sexual reproduction of higher plants. The pollen tube is a typical example of tip-growing cells and shows a polarized cytoplasm. To develop and maintain polarized growth, pollen tubes need a carefully regulated actin cytoskeleton. It is well known that actin-binding proteins are responsible for the direct control of dynamic actin filaments and serve as a link between signal transduction pathways and dynamic actin changes in determining cellular architecture. Several of these classes have been identified in pollen tubes and their detailed characterisation is progressing rapidly. Here, we aim to survey what is known about the major actin-binding proteins that affect actin assembly and dynamics, and their higher-order organisation in pollen tube growth.

Keywords: Pollen tube growth; Actin cytoskeleton; Profilin; Actin-depolymerising factor; Villin/gelsolin/fragmin; Formin.

Introduction

The pollen grain represents the entire male gametophyte generation of a flowering plant. During fertilisation the pollen grain germinates and produces the pollen tube, which functions to penetrate the style and deliver the non-motile sperm cells to the ovule to accomplish the process of fertilisation. The pollen tube is a typical example of tip-growing cells and shows a polarised cytoplasm. To develop and maintain polarised growth, the pollen tube needs a carefully regulated actin cytoskeleton (Staiger 2000, Vidali and Hepler 2001). It is well known that the actin cytoskeleton undergoes dynamic changes in organisation during hydration and activation of the vegetative cells of pollen grains (Pierson and Cresti 1992). Before hydration, actin filaments exist as fusiform or spiculate structures as a storage form, but they are rearranged to form a network upon hydration (Heslop-Harrison et al. 1986, Xu et al. 2004). During pollen tube growth, a very specific arrangement of actin filaments in the shank and the tip region has been established to choreograph the arrival and fusion of secretory vesicles at the extreme apex of these cylindrical cells. According to the organisation of the actin cytoskeleton, the pollen tube can be divided into three main regions: shank, subapex, and apex (Geitmann and Emons 2000, Vidali and Hepler 2001).

In the shank, actin filaments are often organised into bundles with uniform polarity from their pointed ends to the barbed ends and serve as tracks for the tipward flow of cytoplasmic streaming, which transports the organelles and secretory vesicles through the edge of the cytoplasm to the growth axis. At the subapex, filamentous actin (F-actin) becomes less organised or forms a collarlike zone of fine filament bundles and cytoplasmic streaming reverses direction and flows back along the curved bundles of actin filaments through the central core of the tube toward the grain, giving rise to a reverse-fountain cytoplasmic streaming pattern (Hepler et al. 2001, Y. Li et al. 2001). Most recently, this subapical F-actin domain was carefully evaluated in lily pollen with modified fixation regimes (Lovy-Wheeler et al. 2005), and with the actin-stabilising reagent jasplakinolide, Cardenas et al. (2005) found that actin polymerisation in the subapex might be responsible for the promotion of reverse streaming in the tip region of the lily pollen tube. The organisation of actin at the growing apex is somewhat controversial. It was initially thought from conventionally fixed cells that there was a dense concentration of actin filaments (Derksen et al.

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1995). However, using techniques that are likely to preserve cellular structure better, such as rapid freeze fixation and green-fluorescent protein in live cell, suggested that there is limited detectable F-actin in the pollen tips (Kost et al. 1998, Gibbon et al. 1999, Vidali et al. 2001). Recently, on the other hand, a population of dynamic tip-localised F-actin, termed short actin bundles, was investigated in tobacco pollen tubes, and the dynamics of the short actin bundles was associated with that of the subapical actin ring or actin collar (Fu et al. 2001, Hwang et al. 2005), which expanded our understanding of actin function by providing evidence that there exists a dynamic meshwork of random short actin filaments at the extreme apex which are thought to organise vesicle docking and fusion (Gibbon et al. 1999, Fu et al. 2001, Vidali et al. 2001).

Taken together, the actin cytoskeleton in the pollen tube is highly ordered (especially in the shank) and dynamic (especially in the apical and subapical region). The dynamics of actin filaments like depolymerisation and polymerisation, the equilibrium between globular actin (G-actin) and F-actin pools, and the highly ordered architecture of the actin cytoskeleton are regulated spatially and temporally by several classes of actin-binding proteins whose spatiotemporal activities are in turn under the control of a variety of parameters such as Ca^{2+}, pH, phosphorylation, and phosphoinositides (for reviews, see Franklin-Tong 1999, Hepler et al. 2001, Monteiro et al. 2005). As an ideal model system for studying the plant actin cytoskeleton and the molecular mechanism regulating actin filament dynamics and spatial distribution, several members of those classes have been identified in the pollen tube and their detailed characterisation is progressing rapidly. In this review, we aim to survey what is known about the major actin-binding proteins that affect actin assembly and dynamics and its higher-order organisation in pollen tube growth (Fig. 1).

Profilin

Profilin is a low-molecular-mass (12–15 kDa), ubiquitous, soluble protein that was originally identified from calf spleen as a protein forming a high-affinity 1 : 1 complex with monomeric actin (Carlsson et al. 1977). Profilin has complex effects on actin dynamics. It acts as a simple sequestering protein by binding to actin monomers and preventing spontaneous actin nucleation and polymerisation in the presence of capped barbed ends. When filament ends are free, the profilin-actin complex can assemble at the barbed ends of microfilaments and shuttle actin into polymerisation from a pool bound to other monomeric actin-

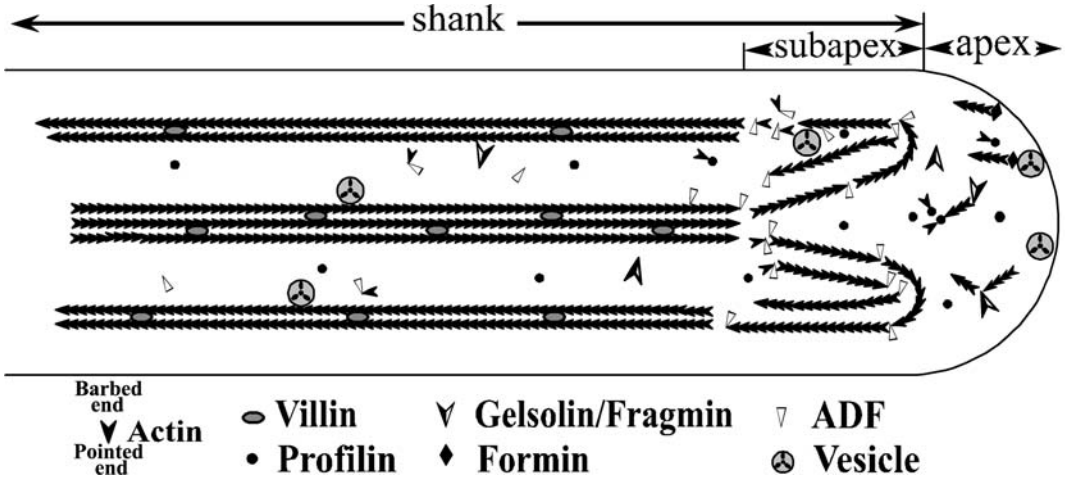

Fig. 1. Schematic drawing of F-actin organisation and the major sites of activity of actin-binding proteins in pollen tube. In the shank of the pollen tube, the actin filaments are often organised into bundles with uniform polarity from their pointed ends to the barbed ends at the edge, but from their barbed ends to the pointed ends in the inner part. These bundles might mainly be connected by villins; at the subapex, where there exists an alkaline band (reported to be approximately pH 7.5, relative to a tip pH of 6.5), F-actin becomes less organised or forms a collarlike zone of fine filament bundles. ADFs are thought to play an important role in maintaining a high level of actin cycling activity by inducing filament severing and actin filaments depolymerising at the pointed end because the binding and depolymerising activity of ADF is greater at higher pH than at lower pH; a dynamic meshwork of fine actin filaments may exist at the extreme apex, which are thought to organise vesicle docking and fusion. Gelsolin and fragmin, which have activities of Ca^{2+}-dependent severing, capping the barbed ends, and nucleating new filaments, may regulate Ca^{2+}-mediated dynamics of the actin cytoskeleton in the tip region of pollen tubes. Profilin is uniformly distributed in the pollen tube, but more actin-profilin complexes may form in the tip region, where Ca^{2+} concentration is high. Formins might be important for the nucleation of short actin filaments in the tube tip, which facilitate targeting and fusion of vesicles

binding proteins (Pollard and Cooper 1984, Pantaloni and Carlier 1993). It was also demonstrated that profilin can promote polymerisation from nucleated actin by catalysing ADP-to-ATP exchange on monomeric actin (Mockrin and Korn 1980, Goldschmidt-Clermont et al. 1991). However, this activity is absent in plants (Kovar et al. 2000). In addition to binding G-actin, profilin interacts with other specific macromolecules including phosphatidylinositides and protein with contiguous stretches of proline (Carlier and Pantaloni 1997). These features of profilin suggest that it may provide a link between signal transduction pathways and dynamic actin changes in determining cellular architecture.

Profilin was first identified in plants as an allergen that is responsible for pollen- and food-based allergies in man (Staiger et al. 1993). In higher plants, the genomes contain large multigene families coding for different profilin isoforms. For instance, profilin isoforms in maize are estimated to have six members, and at least three are expressed in pollen (Staiger et al. 1993, Gibbon et al. 1998, von Witsch et al. 1998). It has been reported that the Arabidopsis profilin family may contain up to ten members that can be divided into two groups according to tissue-specific expression patterns (Huang et al. 1996). Members of class I are expressed prominently in pollen and have a relatively low affinity for G-actin and also for poly-L-proline (PLP), whereas members of class II profilin expressed throughout the plant are more sensitive to the presence of Ca^{2+} and their ability to prevent actin polymerisation increases threefold over a physiologically relevant range of Ca^{2+} (Gibbon et al. 1998, Kover et al. 2000). Profilin is an abundant component of pollen in many plants. For example, it constitutes 0.47% of the total grain protein in lily pollen (Vidali and Hepler 1997). Although Vidali and Hepler (1997) could not detect a statistically significant increase of profilin with polyclonal antibodies against bean hypocotyl profilin during pollen germination, the results from two-dimensional differential gel electrophoresis show that the amount of profilin 1 clearly increases, but profilin 3 decreases in cultured lily pollen tubes, while the ratio of profilin 1 to profilin 3 changes during pollen development (Miki-Hirosige et al. 2004). The results indicate that although the concentrations of different profilin isoforms fluctuate, the total amount of profilin isoforms remains constant during pollen germination. They also suggest that the coexisting different profilin isoforms in pollen may have different functions in terms of regulating the actin cytoskeletal structure.

Important clues about protein function can also be gained from the knowledge of its cellular localisation. Using conventional techniques to localise profilin in pollen from *Nicotiana tabacum*, Mittermann et al. (1995) reported that profilin might be concentrated in the tip of the growing pollen tube. This work has been queried because of problems with fixation and penetration of antibodies across the cell wall. It was then convincingly shown by microinjection of fluorescently labelled profilin into growing pollen tubes that the profilin is evenly distributed 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 et al. 1998). However, in developing pollen, profilin is found at the plasma membrane (von Witsch et al. 1998), and profilin distributes with an obvious accumulation in the tip zone near the plasma membrane in another kind of tip-growing cells, root hairs (Braun et al. 1999, Baluska et al. 2000). Furthermore, profilin has also been demonstrated in association with both the vegetative and generative nuclei (Hess and Valenta 1997). These results indicate that the distribution of profilin in pollen might depend on the stage of development and the specific isoforms expressed. It has also been demonstrated that the actin-sequestering activity of profilin is Ca^{2+} regulated (Kovar et al. 2000, Snowmann et al. 2002). This is correlated with the tip-focused distribution pattern of Ca^{2+} in growing pollen tubes. Thus, even if profilin is uniformly distributed, cytosolic gradients of Ca^{2+} could dramatically alter the activity of profilin.

Given the multiplicity of interactions, it becomes a question as to which of the several properties of profilin are the key to its function in growing pollen tubes. Gibbon et al. (1998) reported that PLP affinity is critical for the effect of profilin on actin cytoarchitecture. Formins are the only PLP-containing profilin-binding proteins discovered in plants thus far (Banno and Chua 2000, Cvrčková 2000). Profilin has been biochemically characterised to be involved in some effects of plant formins on actin assembly (Michelot et al. 2005, Yi et al. 2005). However, the role of this interaction between profilin and formin in controlling actin dynamics in pollen tube growth is still unknown. In order to understand whether excess profilin regulates pollen tube growth through actin binding and/or PLP binding, McKenna et al. (2004) microinjected pollen profilin, wild-type *Schizosaccharomyces pombe* profilin, and different profilin mutants with reduced actin-binding and PLP-binding activity into growing pollen tubes. They also microinjected PLP, as well as combinations of different profilins with PLP. Their results indicated that excess profilin inhibits pollen tube growth due to its ability to bind actin. PLP has an inhibitory effect, but in their assay

system it appears to act independently of profilin. These findings are in agreement with those of Lu and Pollard (2001), who reported that the actin binding of profilin is essential for biological function in fission yeast. However, while the interaction of plant profilin with phosphoinositide lipids has been demonstrated by different experiments (Drøbak et al. 1994; Kovar et al. 2000, 2001), the data regarding the association of profilin with phosphatidylinositol 4,5-bisphosphate (PIP_2) in pollen tube is quite scant. Further evidence for a role in signal transduction of profilin comes from reports that profilins are modulated by a variety of the phosphorylation of pollen proteins and profilins from pollen can be phosphorylated in vitro (Clarke et al. 1998, Snowman et al. 2000, Limmongkon et al. 2004). Thus, it can be suggested that profilin may act in a signalling capacity involved in regulating pollen tube growth through its modulation of protein kinase or phosphatase activity.

ADF/cofilin

The actin-depolymerising factor (ADF) or cofilin is another low-molecular-weight actin-binding protein and the details of its interaction with actin are well characterised. In general, ADF interacts with both monomeric and filamentous actin and enhances actin depolymerisation by increasing the off rate of actin monomers at the pointed ends and by inducing filament severing (Carlier et al. 1997, Bamburg 1999, McGough and Chiu 1999, Chen et al. 2002, Yeoh et al. 2002). The activity of ADF can be modulated by varieties of factors. Firstly, phosphorylation of a Ser near the N terminus considerably reduces the ability of ADF/cofilin to bind actin filaments (Smertenko et al. 1998, Allwood et al. 2001). Secondly, most ADF/cofilins display pH-sensitive activity, with ADF/cofilin preferentially binding F-actin at pH 6.0 and G-actin above pH 7.4 (Yonezawa et al. 1985, Hawkins et al. 1993, Hayden et al. 1993, Gungabissoon et al. 1998, Yeoh et al. 2002). Thirdly, many ADF/cofilins are inhibited by the specific phosphoinositide PIP_2 and to a lesser extent by phosphatidylinositol 4-phosphate (PIP) (Yonezawa et al. 1990, Quirk et al. 1993, Gungabissoon et al. 1998). Moreover, the activity of ADF can be enhanced by the actin-interacting protein 1 (AIP1) (Allwood et al. 2002). This makes ADF an excellent candidate for the effective remodelling of actin architecture in tip-growing cells, either in response to signalling molecules or along gradients of calcium and pH, etc. (Hepler et al. 2001, Vidali and Hepler 2001).

In plants, ADF is encoded by a large gene family whose members can be divided into two classes differing in their patterns of expression either in reproductive or in vegetative tissues (Lopez et al. 1996). There are 12 ADF genes in *A. thaliana*. In maize, three ADF genes have been characterised; two, *ZmADF1* and *ZmADF2*, are expressed solely in pollen (Rozycka et al. 1995, Lopez et al. 1996). Bioinformatic and immunoblotting analyses show that pollen ADFs from different species have a greater degree of identity with each other than with the vegetative-tissue ADF (Allwood et al. 2002, Maciver and Hussey 2002). Moreover, all known pollen-specific ADFs fall in one clade in a phylogenetic analysis, and no ADFs known to be expressed in vegetative tissue fall within this clade (Allwood et al. 2002). These results suggest that ADF is evolutionarily conserved in pollen development and function. The potential role of a pollen-specific ADF in actin reorganisation as pollen grains enter and exit dormancy has been described by Smertenko et al. (2001). The pollen ADF binds the actin array in maturing pollen grains and is proposed to break down this array into actin/ADF rodlets, which is presumably the storage form of actin present in the vegetative cytoplasm in dehydrated pollen. As the pollen grains germinate, the ADF is apparently released from the actin and the new actin array is formed (Smertenko et al. 2001). Results of biochemical and immunological analyses showed that the pollen-specific ADF binds G- and F-actin and increases actin dynamics, but it has a much weaker effect on the enhancement of actin dynamics compared with the ADF expressed solely in vegetative tissues (Gungabissoon et al. 1998, Smertenko et al. 2001), which is more similar to human ADF in this respect (Smertenko et al. 1998). These data suggest that pollen ADF decorates actin arrays because it has a high affinity for filaments but an inefficient depolymerising activity, which has been used to explain why pollen ADF can be seen decorating F-actin in pollen grains (Allwood et al. 2002, Staiger and Hussey 2004). The release of pollen ADF from F-actin aggregates during pollen germination, however, implies that its depolymerising activity is increased (Smertenko et al. 2001). Chen et al. (2003) reported that tobacco pollen grains accumulate phosphorylated and non-phosphorylated forms of ADFs, suggesting that phosphorylation might be a regulatory mechanism for ADFs binding or releasing F-actin when pollen grains enter or exit dormancy. In addition, the weak actin-depolymerising activity can be enhanced massively by the presence of AIP1, a plant homology of actin-interacting protein. Both pollen ADF and pollen AIP1 localise to actin filament bundles in pollen grains but are mainly cytoplasmic in pollen tubes, suggesting that the two proteins may work as a pair and cooperate in vivo (Allwood et al. 2002).

In pollen tubes, increasing the level of ADF results in the disruption of the normal actin cytoskeleton organisation and in the inhibition of pollen tube growth (Vidali and Hepler 2001, Chen et al. 2002). Tobacco and lily pollen-specific ADFs tagged with GFP, GFP-NtADF1, and GFP-LlADF1 were found to predominately associate with the actin meshwork at the base of the apical clear zone in elongating tobacco and lily pollen tubes (Chen et al. 2002). The GFP-ADF-labelled subapical actin mesh spatially coincides with a region where the cytoplasm is slightly more alkaline than in the apex, and this region is known to be favourable for promoting the actin filament-severing activity of ADF (Feijo et al. 1999, Chen et al. 2002). Biochemical analysis also demonstrates that the binding and depolymerising activity of lily pollen-specific ADF, LlADF1, is greater at higher pH than at lower pH (Allwood et al. 2002). Therefore, ADFs are thought to play an important role in maintaining a high level of actin-cycling activity at the subapical region to maintain an actin organisation that is optimum for pollen tube growth (Chen et al. 2003).

It has been suggested that Ser-6 phosphorylation is a possible mechanism to modulate plant ADF activity (Smertenko et al. 1998, Allwood et al. 2001). In elongating pollen tubes, the actin-binding ability of GFP-NtADF1 is enhanced by an Ala substitution at Ser-6 (S6A), which renders this amino acid position nonphosphorylatable, but it is abolished by the phospho-mimicking S6D substitution. This finding also correlates with the observation that the mutant NtADF1(S6D) has more severe effect than the wild-type NtADF1, whereas NtADF1(S6D) has little effect on pollen tube growth (Chen et al. 2002). These observations strongly suggest that the phosphorylation at Ser-6 in NtADF1 plays an important role in regulating the actin-binding and pollen tube growth-regulating activities of NtADF1. Furthermore, Chen et al. (2003) found that NtRac/Rop GTPase is involved in modulating the actin-binding and -depolymerising activities through phosphorylation at Ser-6 in NtADF1. However, although LlADF1 contains the conserved N-terminal Ser-6, it cannot be phosphorylated under conditions at which ZmADF3 can be phosphorylated, indicating that reversible phosphorylation does not control this pollen ADF (Allwood et al. 2002). LlADF1 can be regulated by the specific phosphoinositides PIP and PIP_2, which have been found to be present in the membrane at the tip of the pollen tube (Kost et al. 1999), which may provide a fundamental link between extension stimuli and the regulation of the actin cytoskeleton in the tip region (Allwood et al. 2002).

Villin

Villin, which was originally isolated from the core actin bundles of intestinal epithelial cell microvilli (Bretscher and Weber 1979, Matsudaira and Burgess 1979), is one of the main proteins responsible for actin bundle formation. Villin belongs to a superfamily of actin-binding proteins called the villin/gelsolin family (Friederich and Louvard 1999, Sun et al. 1999). It shares structural homology with gelsolin (Kwiatkowski et al. 1986), containing six gelsolin repeats (G1–G6) that are constructed from 125- to 150-amino-acid gelsolin repeat domains. In addition to a gelsolin-like core domain, villins contain an additional C-terminal actin-binding module called the villin headpiece. The headpiece allows each molecule of villin to contact two adjacent filaments and to cross-link filaments into bundles, a property that is missing from gelsolins. In the presence of micromolar Ca^{2+}, most, but not all, villins sever actin filaments and cap filament barbed ends (Glenney et al. 1980, Northrop et al. 1986, Janmey and Matsudaira 1988).

The first biochemically identified plant villin was from pollen tubes, reported as two abundant actin-bundling proteins with apparent molecular masses of 115 and 135 kDa (P-115-ABP and P-135-ABP) in germinating lily pollen (Nakayasu et al. 1998, Yokota et al. 1998). Immunofluorescence and immunoelectron microscopic studies revealed that P-135-ABP colocalises with actin filament bundles in lily pollen tubes (Yokota et al. 1998, Vidali et al. 1999), and the protein itself binds to actin filaments and cross-links them into unipolar bundles in vitro (Yokota et al. 1999). The P-115-ABP and P-135-ABP isolated biochemically from germinating lily pollen were able to arrange F-actin filaments with uniform polarity into bundles and this bundling activity was suppressed by Ca^{2+}-calmodulin (CaM) in vitro. In further analysis, a cDNA expression library was constructed from pollen mRNA and screened with the antisera against P-135-ABP and P-115-ABP. Two full-length cDNA clones obtained from this screen were then sequenced, revealing unequivocally that P-135-ABP is a member of the villin family (Vidali et al. 1999), and P-115-ABP deduced from the clone showed high homology with P-135-ABP and four isoforms of *A. thaliana* villin (AtVLN), especially AtVLN4, indicating that P-115-ABP can also be classified as a plant villin (Yokota et al. 2003). Yokota et al. (2000) have shown that the bundling activity of 135-ABP is regulated by Ca^{2+} together with calmodulin. While neither agent alone is effective, when both Ca^{2+} and calmodulin are present and above micromolar concentrations, they inhibit binding of

villin to F-actin, which prevents the formation of large actin filament bundles. The P-135-ABP is also localised along actin filament bundles in both subcortical regions and transvacuolar strands penetrating vacuoles in the root hair cells. When antiserum against P-135-ABP was microinjected into living root hair cells, thick bundles of actin filaments in transvacuolar strands were dispersed into thin bundles concomitant with the disappearance of transvacuolar strands and a dramatic alteration of the route of cytoplasmic streaming (Tominaga et al. 2000). As described above, the polarity of actin filaments in a bundle of root hair cells was uniform, consistent with the actin-filament-bundling property of isolated P-135-ABP in vitro. These results demonstrated that P-135-ABP is a factor responsible for bundling actin filaments in somatic cells of higher plants as well as pollen tubes. Furthermore, a recent study showed that P-135-ABP can form a complex with G-actin in the presence of Ca^{2+} in vitro, and this complex may act as a nucleus for the polymerisation of actin filaments. However, the nucleation activity of P-135-ABP is probably not relevant in vivo because the presence of P-135-ABP does not accelerate the assembly of G-actin saturated with profilin, a situation that mimics conditions found in pollen. P-135-ABP also enhances the depolymerisation of actin filaments during dilution-mediated disassembly. Growth from filament barbed ends in the presence of Ca^{2+}-CaM was also prevented, consistent with filament capping activity. These results suggested that lily villin is involved not only in the arrangement of actin filaments into bundles in the basal and shank region of the pollen tube, but also in regulating and modulating actin dynamics through its capping and depolymerising (or fragmenting) activities in the apical region of the pollen tube, where there is a relatively high concentration of Ca^{2+} (Yokota et al. 2005). However, functional differences between P-115-ABP and P-135-ABP and their precise function in stabilising actin bundles in the shank region need to be further elucidated.

In plants, like other actin-binding proteins, villin is also encoded by a multigene family. The *A. thaliana* genome contains sequences for five villin-like genes, *AtVLN1* to *AtVLN5* (Klahre et al. 2000, Staiger and Hussey 2004). The headpiece domain of the Arabidopsis villins AtVLN1 to AtVLN4, when fused with GFP and expressed in plant cells, also possesses the ability to bind to actin filaments (Klahre et al. 2000). Bioinformatic studies predicted that AtVLN1 has the least well-conserved type 1 and type 2 Ca^{2+} binding sites in the gelsolin core domain among the Arabidopsis villin isoforms, unlikely to be regulated by Ca^{2+}. Biochemical analysis demonstrated that the recombinant protein does indeed lack Ca^{2+}-regulated severing, nucleating, and capping activities (Huang et al. 2005). Recombinant AtVLN1 binds to actin filaments with high affinity and generates bundled filament networks in a Ca^{2+}-independent manner. In kinetic assays with ADF/cofilin, AtVLN1 appears to bind first to growing filaments and protects filaments against ADF-mediated depolymerisation but does not nucleate, cap, and sever preexisting actin filaments as human villin does. These results, together with the observation that actin cables along the length of the pollen tube seem resistant to changes in cytosolic Ca^{2+} levels, suggest that AtVLN1 is responsible for stabilising actin cables in the pollen tube shank. This activity of AtVLN1 would uncouple direct control of actin dynamics in the tip region by Ca^{2+} signalling, while maintaining the stability of actin cables along the shank of the pollen tube (Huang et al. 2005).

Gelsolin/fragmin

Gelsolin is composed of six gelsolin homology domains (G1–G6) and has Ca^{2+}-stimulated F-actin-severing activity. Gelsolin also caps the barbed ends of actin filaments and nucleates new filaments. Both gelsolin and villin contain six evolutionarily conserved actin-binding modules, and three copies of this domain are also found in severin of *Dictyostelium discoideum* (Andre et al. 1988), fragmin of *Physarum polycephalum* (Schleicher et al. 1988), and CapG of macrophages (Yu et al. 1990). Crystal structures for one or more of these gelsolin subdomains, coupled with spectroscopy and cryoelectron microscopy studies, have provided substantial insight into the molecular mechanisms of filament severing and capping (Schleicher et al. 1988). The *A. thaliana* genome contains sequences for 5 villin-like genes but no gelsolin-only genes (Staiger and Hussey 2004).

Gelsolin-like proteins have been identified by immunoblotting in maize and lily pollen (Wu and Yan 1997, Tao and Ren 2003). Furthermore, by affinity chromatography on DNase I-Sepharose, a plant gelsolin, named PrABP80, was isolated from *Papaver rhoeas* L. pollen. Mass spectrometry results indicated that PrABP80 has high homology with P-135-ABP and Arabidopsis villin but does not contain a C-terminal headpiece and therefore belongs to plant gelsolin. PrABP80 can accelerate nucleation, block barbed ends, sever filaments, and enhance profilin-mediated actin depolymerisation in a Ca^{2+}-dependent manner in vitro. Hence, PrABP80 may act as a Ca^{2+} sensor and by contributing to the creation of dynamic actin filaments in the tip of pollen tube may play a crucial role in Ca^{2+}-

mediated depolymerisation of actin during the self-incompatibility response in poppy pollen. PrABP80 is also a phosphoinositide-binding protein, but the consequences of phospholipid binding on the actin-based function of PrABP80 remain to be tested (Huang et al. 2004).

Fragmin and severin, which only contain three gelsolin homology domains, are members of the gelsolin family and they perform Ca^{2+}-dependent severing, capping, and nucleating activity (Yin et al. 1990, T'Jampens et al. 1997). A fragmin-like protein has also been identified in *Mimosa pudica*, but it has not been purified to homogeneity and little is known about its biochemical properties (Yamashiro et al. 2001). LdABP41 is a 41 kDa ABP isolated from lily pollen. Sequence determination by N-terminal sequencing and mass spectrometry revealed that LdABP41 is highly homologous with G1–G3 domains of P-135-ABP, which is related to fragmin or severin (Fan et al. 2004). Although bioinformatics of *A. thaliana* and rice (*Oryza sativa* L.) shows no G1–G3 genes in these organisms, some evidence exists to support the presence of villin splice variants. For instance, Klahre et al. (2000) suggest the presence of an *AtVLN1* splice variant. A full-length cDNA encoding a predicted polypeptide of 446 amino acids shares 100% identity with the three N-terminal gelsolin repeats of AtVLN1 (Staiger and Hussey 2004). Biochemical analysis shows that LdABP41 possesses Ca^{2+}-dependent nucleation and severing activities in vitro; purified anti-LdABP41 antibody microinjected into lily pollen grain could cause the inhibition of pollen tube elongation and increase pollen tube width. Besides, the results of immunolocalisation showed that LdABP41 localises in the cytoplasm and is abundant in the apical region of the germinated pollen tube. It is suggested that LdABP41 might be related with the short, irregular filaments in the apical region of the pollen tube and be necessary for pollen tube growth (Fan et al. 2004). On the basis of the cellular localisation and the biological characterisation in vitro, it is believed that gelsolin/fragmin superfamily members might provide alternative mechanisms used by plants to regulate Ca^{2+}-mediated dynamics of the actin cytoskeleton in the tip region of pollen tubes.

Arp2/3 complex

The Arp2/3 complex is a 220 kDa macromolecule that comprises two actin-related proteins (Arp2 and Arp3) and five other subunits that are commonly named according to their size (Pollard and Beltzner 2002). The conserved Arp2/3 complex – nucleating actin filaments and directing the formation of a new filament as a 70° branch on the side of an existing filament – is an important actin-nucleating factor in diverse eukaryotes (for reviews, see Pollard and Beltzner 2002, Mathur 2005). It has been reported that *A. thaliana* contains all seven evolutionarily conserved genes for the Arp2/3 complex (Klahre and Chua 1999, McKinney et al. 2002). Furthermore, S. Li et al. (2003) have identified the function of the putative Arabidopsis Arp2/3 complex with four T-DNA insertion mutants which play an important role in F-actin organisation and cell morphogenesis, but none of these mutants affect the viability of the plants or cause dramatic defects in pollen development. Recently, the mechanism regulating plant Arp2/3 complex activity has been revealed, and some activators (also called nucleation promoting factors) of Arp2/3 which are necessary for Arp2/3 complex's activity have been found in plants. Among these activators, the WASP (Wiskott-Aldrich syndrome protein)/WAVE (WASP Verprolin-homologous proteins) family of proteins has been intensively studied. Homologs of all mammalian WAVE complex components, including WAVE, PIR121/Sra-1, Nap1, Abi-2, and HSPC300, are present and may play an essential role in activation of the Arp2/3 complex in *A. thaliana* (Szymanski 2005, Djakovic et al. 2006). Some Arabidopsis WAVE homologs can efficiently activate vertebrate Arp2/3 in vitro (Frank et al. 2004, Basu et al. 2005). T-DNA insertion mutants of SCAR2/DISTORTED3/WAVE4 have defects of epidermal cell morphogenesis similar to those of the characterised *distorted* mutants of Arp2/3 (Basu et al. 2005). Despite there being strong evidence for the presence of a plant Arp2/3 complex and its activators and there being no doubt that plants will use Arp2/3 to regulate formation of actin arrays, no data on Arp2/3 complex location or functional properties in pollen exists at present. Therefore, formin, another nucleator, appeared to be a provocative candidate for actin filament nucleation in the tip growth of pollen tube.

Formin

Formins, formin homology (FH) proteins that form unbranched actin filament bundles, are members of a widely conserved protein family implicated in actin-organising and signalling involved in cell polarity and cytokinesis. They are characterised by the presence of two different formin homology domains, formin homology domain 1 (FH1) and formin homology domain 2 (FH2), which allow the multiple activities of these proteins on actin (Evangelista et al. 1997, 2003; Field et al. 1999; Zeller et al. 1999). The FH2 domain contains the actin binding site, whereas FH1 with its polyproline-rich stretches binds

to profilin and the profilin/actin complex (Pruyne et al. 2002, Sagot et al. 2002b, F. Li and Higgs 2003). In animals, formin mutants have been associated with limb deformity and deafness in vertebrates and with loss of oocyte polarity and cytokinesis defects in flies (Woychik et al. 1990, Castrillon and Wasserman 1994, Lynch et al. 1997). In *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, they are responsible for the formation of actin cables involved in both cell polarity and cytokinesis (Feierbach and Chang 2001, Evangelista et al. 2002, Sagot et al. 2002a).

Cvrčková (2000) reported firstly a bioinformatics analysis for plant formins. Recently, further bioinformatics data has expanded our knowledge of plant formins (Cvrčková et al. 2004). It is now estimated that the *A. thaliana* genome contains up to 23 genes encoding FH proteins. On the basis of phylogenies derived from the conserved FH2 sequences and the organisation of their N-terminal domains, Arabidopsis formins (*A. thaliana* formin homologues, or AtFHs) can be divided into two distinct classes, referred to as class I and class II formins (Deeks et al. 2002, Cvrčková et al. 2004). The N-terminal domains of both class I and class II plant formins are different from their animal and fungal counterparts. The class I plant formins share two predicted structural features. Most surprisingly, class I plant formins contain potential transmembrane segments and signal sequences that are not found in yeast or animal formins (Banno and Chua 2000, Cvrčková 2000, Deeks et al. 2002).

A few of class I AtFHs have been reported experimentally in vitro or in vivo. Overexpression of AtFH1 in pollen tubes induces the formation of prominent actin cables, pollen tube swelling, and deformation of the cell membrane at the tip of the pollen tube (Cheung and Wu 2004). Although the functional characterisation of formin in pollen tube growth is still very limited, there appears more data about Arabidopsis formin from both cell biology (Cheung and Wu 2004, Favery et al. 2004, Deeks et al. 2005, Ingouff et al. 2005, Yi et al. 2005) and biochemistry (Michelot et al. 2005, Yi et al. 2005). In vitro, recombinant AtFHs have the ability to nucleate actin filaments at the barbed end, cap the barbed end of actin filaments (Huang et al. 2005, Ingouff et al. 2005, Yi et al. 2005), sever actin filaments into short fragments (Yi et al. 2005), and bundle actin filaments (Michelot et al. 2005). Overexpression of full-length AtFH8 results in a prominent change in root hair cell development and actin organisation, indicating the involvement of AtFH8 in polarised cell growth through the actin cytoskeleton (Yi et al. 2005).

The in vitro biochemical analysis also shows that profilin binds AtFH8 through the FH1 domain, and it increases the elongation rate of actin assembly in the presence of AtFH8, indicating that profilin might facilitate addition of actin to the barbed end (Yi et al. 2005). These results correlate with the in vivo cytoplasmic situation in pollen and growing pollen tubes, where the pool of actin monomers is sequestered with profilin. However, more work is clearly needed to elucidate the interaction between profilin and formin in vivo. Most recently, it was reported that AtFH5 is localised to the growing cell plate of dividing tissues, and AtFH4 and AtFH8 are localised to the cross walls of roots, hypocotyls, and shoot tissues (Deeks et al. 2005, Ingouff et al. 2005). Although, to our knowledge, any report of class II formins is still lacking, it has been suggested that formins organise both dynamic actin meshwork and actin bundles: the class I formins, which associate with cross walls are relevant for the dense meshwork, whereas the class II formins can be expected to be important for the assembly of thick cables (Baluška and Hlavačka 2005).

Prospects

Pollen tube growth is a key process in the life cycle of all flowering plants, and on the other hand, a fascinating model of growing cells to investigate the complex interplay between actin cytoskeleton and signal transduction in controlling many cellular functions in plant cells, such as polarised vesicle transport, docking, fusion, and secretion of cell wall components. As cross-linking molecules between signal transduction pathways and actin cytoskeleton, actin-binding proteins are believed to integrate the upstream signalling cascades and to transduce them to reorganise actin filaments. Although several actin-binding proteins have been identified in pollen tubes and subsequent studies showed that they are involved in this polarised tip growth through regulating the dynamics of the actin filaments, these studies are mostly based on the proteins' biochemical properties in vitro and their cellular localisation. Continuing studies of the cell biological functions of these characterised actin-binding proteins, distinguishing the functional differences of isoforms and identifying new or pollen-specific isoforms will hopefully further reveal the role of these actin organisers in controlling the highly dynamic nature of the actin cytoskeleton and pollen tube growth. The mechanisms by which the activities of actin-binding proteins are precisely regulated remain to be further studied. It is well known that there are a number of signalling pathways that are associated with

the actin cytoskeleton and pollen tube growth, including Ca^{2+}, pH, reversible protein phosphorylation, phosphoinositides, phospholipids, and Rop GTPases. Recent clues indicate that, in addition to acting as a target, the actin-binding proteins can also act as a transducer and modulator of signal information. A major challenge in the future will be to establish a network that integrates different factors and their activities in space and time to control pollen tube growth. Pollen cytoskeleton proteomics analysis and serial analysis of gene expression to profile the transcription of the pollen during its germination and tube growth would certainly provide us with new knowledge of the role of actin-binding proteins in mediating signals to regulate the actin dynamics in pollen germination and tube growth.

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