Actin-binding proteins in the *Arabidopsis* genome database: properties of functionally distinct plant actin-depolymerizing factors/cofilins

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The plant actin cytoskeleton is a highly dynamic, fibrous structure essential in many cellular processes including cell division and cytoplasmic streaming. This structure is stimulus responsive, being affected by internal stimuli, by biotic and abiotic stresses mediated in signal transduction pathways by actin-binding proteins. The completion of the *Arabidopsis* genome sequence has allowed a comparative identification of many actin-binding proteins. However, not all are conserved in plants, which possibly reflects the differences in the processes involved in morphogenesis between plant and other cells. Here we have searched for the *Arabidopsis* equivalents of 67 animal/fungal actin-binding proteins and show that 36 are not conserved in plants. One protein that is conserved across phylogeny is actin-depolymerizing factor or cofilin and we describe our work on the activity of vegetative tissue and pollen-specific isoforms of this protein in plant cells, concluding that they are functionally distinct.

Keywords: actin-binding proteins; *Arabidopsis* genome; actin-depolymerizing factor; cofilin; root hairs; pollen tubes

1. INTRODUCTION

The actin cytoskeleton forms a dynamic, stimulus-responsive network in eukaryotic cells and is involved in essential functions, including cell division and cytoplasmic streaming. Actin filaments have the ability to disassemble into monomeric actin or G-actin and to reform, perhaps in another part of the cell, into filamentous or F-actin. This dynamic process occurs by depolymerization of actin from the pointed end and polymerization on the barbed end. The state of any given actin filament will depend on the balance between the polymerization and depolymerization rates and many actin-binding proteins can modulate these rates. The function of these actin-binding proteins may be controlled by numerous signals, including calcium, phosphoinositides, pH or reversible phosphorylation (Ayscough 1998).

In the first part of this paper we show the results of screening the *Arabidopsis* Genome Database with known animal and fungal actin-binding protein sequences to give some idea of the extent of usage of these proteins across phylogeny. In the second part we describe our work on one of the conserved actin-regulating proteins, ADF/cofilin with particular reference to its role in tip growing cells.

2. ACTIN-BINDING PROTEINS IN THE *ARABIDOPSIS* GENOME DATABASE

Many actin-binding proteins have been identified and studied, isolated from organisms across the evolutionary spectrum. Actin-binding proteins regulate filament assembly and disassembly (e.g. profilin, thymosin β_4 , ADF/cofilin, gelsolin, capping protein), *de novo* nucleation of actin filaments (e.g. ARP2/3, WASP), cross-link or bundle actin filaments (e.g. fimbrin, filamin, villin, α -actinin) or are involved in the formation of supramolecular structures, e.g. focal adhesions (e.g. vinculin, talin, paxillin, integrin), or other membrane interactions (e.g. the erythrocyte cortical cytoskeleton: spectrin, band 4.1, ankyrin, adducin). The list is quite extensive and is covered in many excellent reviews (Kreis & Vale 1993; Maciver 1995; Ayscough 1998; Bamberg 1999; Chen *et al.* 2000). The focus here is simply to take the most studied actin-binding proteins and to search for homologues in the *Arabidopsis* genome database (table 1). Out of 67 analysed in this way, 36 showed no close homologies in the *Arabidopsis* genome database.

Notably, there are no proteins in the *Arabidopsis* genome database that bear any resemblance to proteins required in focal adhesions (vinculin, talin, paxillin, integrin). Although there are the main proteins involved in modulating actin assembly/disassembly, ADF and profilin, there is no thymosin β_4 . In addition there are formins present that are likely to regulate profilin at least. A large villin gene family is present but there is no clear distinction between members of this family as there is between, for example, gelsolin and villin in animal cells. Interestingly, there is an ARP2 and ARP3 part of the complex required for the *de*

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Table 1. Amino acid sequences of known actin-binding proteins found in databases matched against the *Arabidopsis* database using TAIR blast (<http://arabidopsis.org/blast/>).

(Blast searches that gave a score of over 60 and which had an e-value of less than 0.1 were taken as positive searches, recorded in (a), (b), (c) and (d). Blast searches which did not give either a score of over 60 or an e-value of less than 0.1 were considered not to be good matches, recorded in (e). Score is a measure of the similarity of two sequences, which is dependent on both the proportion of conserved residues between the two sequences and the length of the sequence containing similarities. The e-value is a measure of the likelihood of this match occurring by chance. An e-value approaching zero indicates that the probability of this match having arisen by chance is virtually zero. Conversely, an e-value of unity indicates that one might expect to see a match with a similar score simply by chance. The e-values given below are in the normal exponent-style computer notation. A more detailed description of scores and e-values can be found in Attwood & Parry-Smith (1999). The *Arabidopsis* sequences referred to can be obtained by entering the identity number into 'Entrez protein browser' (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=protein>).)

protein	identity no.	score	e-value
actin-binding proteins in <i>Arabidopsis</i>			
ADF	T1N6.11	85	4e-17
AIP1	F10A8.21	385	e-107
annexin	T31P16.210	126	9e-179
ARP2	T13D8.30	126	9e-30
ARP3	F3F19.20	442	e-124
auxilin	F3A4.190	84	4e-16
CamBP	K12B20.160	616	e-176
CAP	T4L20.70	236	2e-62
capping protein	gblAAF64531	577	e-165
EF1 α	T6D2214	786	0
fimbrin	F10M23.40	432	e-121
formin	F18019.9	1462	0
myosin I	BAB03161	474	e-133
myosin II	T1G11.14	552	e-157
nuclear matrix protein	F7A10.5	66	1e-10
PK1	F17014.19	201	8e-52
PK2	T4B21.15	173	4e-43
profilin	F6F22.21	275	1e-74
protein 4.1	BAB32952.1	504	e-143
villin	T11A7.16	1748	0
proteins for which the closest match is villin			
ezrin	F9N11.10	1899	0
fragmin	T11A7.16	228	7e-60
gCAP39	T11A7.16	192	4e-49
gelsolin	T11A7.16	192	2e-49
scinderin	T8H10.10	270	2e-72
severin	T11A7.16	191	6e-49
proteins for which the closest match is myosin			
integrin	K16L.22.7	185	3e-46
MT-actin cross-linking protein	F22D22.1	116	3e-25
proteins for which the closest match is fimbrin			
plastin	F2818.21	416	e-116
SAC6	BAB09267.1	432	e-121
spectrin	GblAAB97844.1	67	2e-10
proteins for which there is no good match in the <i>Arabidopsis</i> database			
ABPI		36	0.1
ABP120 chainB		50	e-006
actobindin		29	0.65
adducin 9		32	0.7
α -actinin (matches calmodulin)		69	2e-011
caldesmon		39	e-0.01
coronin		59	2e-008
DBP/Gc		31	2.1
dematin		47	2e-005
drebrin (matches ADF only)		85	3e-017
dystrophin		54	6e-007
fascin		31	3.4
filamin		43	4e-004
gelation factor		59	1e-008
hisactophillin		38	0.004
insertin		36	0.056
MARCKS		31	0.65
MAP2		46	3e-004

Table 1. (Continued.)

protein	identity no.	score	e-value
myomesin: nuclear matrix			
constituent protein 1	46		2e-004
nebulin	45		5e-004
nuclear actin-binding protein	33		0.26
ponticulin	no hits		
radixin	32		1.1
synapsin	31		e-2.4
tau	32		0.49
talin	39		0.033
tensin (matches tyrosine phosphatase)	102		2e-021
thymosin	27		1.6
titin	50		6e-006
tropomodulin	40		0.38
tropomyosin	43		8e-005
troponin (matches calmodulin)	133		6e-032
twincillin	39		0.005
vinculin	40		0.008
WASP	31		3.2

*nov*o initiation of actin filament assembly but no WASP. Fimbrin is present as a small gene family, and this identifies spectrin as a human homologue as expected because it is a member of this family of proteins but there is no clear spectrin homologue. Intriguingly, there is a homologue of band 4.1 that is likely to be involved in the plant plasma membrane and actin cytoskeleton interaction, but no ankyrin or adducin.

Plant cells make adhesions and actin accumulates at these adhesions (Smertenko *et al.* 2001). Actin is present in the three unique plant cytoskeletal assemblies, the phragmoplast, the pre-prophase band and the interphase cortical cytoskeleton (Schmit 2000). Coupled to the fact that many plant cells have specialized architecture and activity and that key processes (e.g. guard cell movement, root hair and pollen tube tip growth) within these cells are coordinated by actin, it is possible that, in addition to the basic machinery shared amongst animals, plants, fungi and protists, plants have a tailored set of actin-binding proteins. The only evidence we have of this to date is the tobacco 190 kDa polypeptide that has both actin- and microtubule-binding properties and no obvious homologues in other organisms (Igarashi *et al.* 2000). In addition, other biochemical procedures have been used to identify plant actin-binding proteins, with promising results, but no sequence is yet available (Hu *et al.* 2000; Nakayasu *et al.* 1998).

3. REGULATION OF THE ACTIN NETWORK BY ADF/COFILIN

In general the ADF or cofilin group of proteins binds both G-actin and F-actin and modulates its dynamics. It does this by severing actin filaments, thereby providing more filament ends for polymerization, and by increasing the dissociation rate constant at the pointed end and providing more monomer for assembly at the barbed ends (Carrier 1998; Maciver 1998; Bamburg 1999; Chen *et al.*

2000) (figure 1). Plant ADFs have been cloned and sequenced from various sources, including lily (Kim *et al.* 1993; Smertenko *et al.* 2001), maize (Rozycka *et al.* 1995; Lopez *et al.* 1996), *Arabidopsis* (Carrier *et al.* 1997; Dong *et al.* 2001a,b), petunia (Mun *et al.* 2000) and wheat (Oullet *et al.* 2001). Chua and colleagues describe *Arabidopsis* ADF in this issue (Kost *et al.* 2002) and below we describe the work on vegetative or pollen-expressed ADFs in maize and lily.

(a) Pollen and vegetative ADFs

We have been working on four ADFs, three from maize, ZmADF1, -2 and -3 (Rozycka *et al.* 1995; Lopez *et al.* 1996), and one from lily, LIADF1 (Kim *et al.* 1993; Smertenko *et al.* 2001). ZmADF1/2 and LIADF1 are expressed solely in pollen whereas ZmADF3 is expressed only in vegetative tissues. The three pollen ADFs show greater identity with each other (70–94% identity) than with the maize vegetative ADF (54–60% identity), suggesting a common role for an evolutionarily conserved ADF in pollen development and function. This structural dissimilarity between pollen and vegetative ADFs is further emphasized by the fact that a polyclonal antiserum raised to ZmADF3 does not cross-react with pollen ADFs (Jiang *et al.* 1997a). In conclusion there appears to be a structural and immunological distinction between pollen ADFs and the maize vegetative ADF, ZmADF3.

(b) Biochemical characteristics of the maize vegetative ADF, ZmADF3

ZmADF3, in biochemical studies, behaves like mammalian ADF/cofilins. It binds G- and F-actin and increases actin dynamics in a manner similar to mammalian ADF. There have been extensive studies on the relation of structure to the activity of ADF (see Bamburg 1999; Bowman *et al.* 2000; Pope *et al.* 2000). Using mutant ZmADF3 proteins, we were able to demonstrate that the ZmADF3 binding of F-actin can be spatially dis-

Figure 1. Schematic diagram for the general activity of ADF/cofilin. ADF/cofilin severs actin filaments and enhances the dissociation of actin monomer from the pointed end and provides monomer for assembly at the barbed end.

tinguished from that of G-actin (Jiang *et al.* 1997b). In this study two mutant maize ADF3 proteins were made: *zmadf3-1* in which Tyr-103 and Ala-104 (equivalent to destrin Tyr-117 and Ala-118) were replaced by phenylalanine and glycine, respectively, and *zmadf3-2* in which both Tyr-67 and Tyr-70 (equivalent to destrin Tyr-82 and Tyr-85, respectively) were replaced by phenylalanine. The mutant *zmadf3-1* bound G- and F-actin weakly. However, *zmadf3-2* showed an affinity for G-actin similar to ZmADF3 but F-actin binding was abolished. Using the tertiary structures of the available ADFs it was possible to conclude that Tyr-82 plays a critical role in stabilizing the tertiary structure that is essential for F-actin binding. It was proposed that the tertiary structure is maintained as a result of a hydrogen bond between the hydroxyl of Tyr-82 and the carbonyl of Tyr-117, which is located in the long α -helix (Leu-111 to Phe-128) previously known to be involved in G- and F-actin binding.

ZmADF3 exhibits a pH-sensitive activity (Gungabissoon *et al.* 1998). In this regard ZmADF3 is more similar to the metazoan ADF/cofilins than to the protist ADF (actophorin) from *Acanthamoeba*, which is not pH sensitive (Maciver *et al.* 1998). At pH values below 7.0 ZmADF3 binds F-actin and at pH 8.0 it depolymerizes it. The crossover point (the point at which half the actin is depolymerized) is at pH 7.7 and this is higher than that for human ADF (pH 7.3),

reflecting the fact that the resting pH of plant cells is more alkaline than that of animal cells.

The activity of ZmADF3, like several other actin-binding proteins, is specifically inhibited by the phosphoinositides PIP and PIP₂ (Gungabissoon *et al.* 1998). In addition, ZmADF3 inhibits the breakdown of PIP₂ by phospholipase C. This raises the possibility that this protein is regulated by or in part by the phosphoinositide signal transduction pathway.

Also we have shown that ZmADF3 can be phosphorylated on Ser-6 by a calmodulin domain-like protein kinase (CDPK) and that this can regulate its activity (Smertenko *et al.* 1998; Allwood *et al.* 2001). CDPKs are a class of kinases unique to plants and protozoa. Metazoan ADFs are phosphorylated on Ser-3 (the equivalent to plant ADF Ser-6) by LIM kinases, which are themselves regulated by the Rho family of GTPases (Arber *et al.* 1998; Yang *et al.* 1998). These LIM kinases do not phosphorylate plant ADF (Bamburg 1999) and there are no plant LIM kinases in current databases. Phosphorylation inhibits the activity of ADF in both animals and plants (Smertenko *et al.* 1998; Ressad *et al.* 1998). These data imply that plant ADF exhibits a level of control different from that of metazoan ADF in that calcium may indirectly control the plant ADFs. Calcium's role as a second messenger in signalling processes in plant cells is well established (Trewavas 1999). Calcium ion fluxes have been correlated with a large number of stimuli (cold, light, hormones, touch, fungal elicitors). Consistent with this is the fact that many cellular processes require calcium (guard cell movement, pollen tube growth, root hair growth, cytoplasmic streaming) and all these processes require the activity of a dynamic actin cytoskeleton.

ZmADF3 enhances the ability of ZmEF-1 α to increase the viscosity of actin solutions (Gungabissoon *et al.* 2001). EF-1 α is an abundant eukaryotic protein whose principal function appears to be to bind aminoacyl-tRNA to the ribosome. However, it is also known that EF-1 α can bind and bundle microtubules and actin filaments (Moore & Cyr 2000; Gungabissoon *et al.* 2001). A similar activity for an ADF was previously observed with actophorin (the ADF/cofilin from *Acanthamoeba*) and the cross-linking/bundling protein, α -actinin *in vitro* (Maciver *et al.* 1991). Here, it was proposed that shorter filaments resulting from ADF/cofilin severing/depolymerizing activity possessed increased mobility compared with longer filaments, allowing them to rotate and become laterally orientated, thereby permitting them more readily to form a more rigid meshwork of interconnected microfilament bundles. It is likely that ZmADF3 has a similar bundle enhancement mechanism in the presence of ZmEF-1 α . This type of cooperativity between actin severing and bundling proteins may occur *in vivo* as microinjection or overexpression of ADF/cofilins causes the extensive formation of actin bundles (Aizawa *et al.* 1996; Hussey *et al.* 1998; Dong *et al.* 2001b).

In conclusion, ZmADF3 is likely to be a tightly controlled protein *in vivo* where its activity at any particular time or place will be the summation of the above controls. Moreover, ZmEF-1 α can cooperate with ZmADF3 to enhance its bundling activity. Taken together with the fact that EF-1 α is known to co-localize with actin bundles in plant cells (Clare *et al.* 1996), it is likely that ZmEF-1 α

will serve to modulate the dynamics of actin *in vivo* in conjunction with other actin-associated proteins.

(c) Biochemical characteristics of lily pollen ADF

For the biochemical analysis of pollen ADF we have used LIADF1 because it produced reasonable amounts of stable, soluble recombinant protein in *Escherichia coli*. Like ZmADF3, LIADF1 bound G- and F-actin. However, it did not have the same effect on actin dynamics. In assessing the severing activity of LIADF1 we measured the rate of nucleated actin polymerization using spectrofluorimetry. In this assay we found that LIADF1 had 20% of the activity of ZmADF3. We also measured the kinetics of depolymerization using gelsolin-capped filaments, and found that the ability of LIADF1 to activate disassembly at the pointed ends is only *ca.* 10% that of ZmADF3. When one considers the activity of the analogue of phosphorylated ZmADF3, *zmadf3-4* (in which Ser-6 is replaced by a glutamic acid residue), which does not bind G- or F-actin but has 7.5% activity in these assays, these data show that LIADF1 is very inefficient as a depolymerizing factor (Smertenko *et al.* 2001).

(d) Role of ADF in tip-growing cells

Bearing these biochemical data in mind, coupled with the immunolocalization studies described below, we can identify a role for ADF in the dramatic actin rearrangements that occur (i) during root hair formation (Jiang *et al.* 1997a) and (ii) as pollen enters and exits from dormancy (Smertenko *et al.* 2001).

(i) ADF in root hair development

Root hair formation takes place in two stages: during initiation, localized bulges (root hair primordia) form on the predetermined trichoblasts (hair-bearing epidermal cells) and during elongation the bulges extend in a unipolar direction to form a tubular root hair. This extension occurs via tip growth, a process common to both root hairs and pollen tubes, and involves addition of new membrane components at a cell's growing tip. In fact these cells provide a simple model for studying cell elongation.

In initiating root hairs, ZmADF3 is uniformly localized across the cell with no specific localization to actin cables, which are essentially longitudinal. Subsequent behaviour of the ZmADF3 is related to root hair elongation and microfilament reorganization. As the microfilament bundles redirect towards the primordium there is an apparent co-localization and concentration at the growing point. As the root hair elongates, so too do the microfilament bundles and the majority of these run parallel to the axis of root hair growth. The main ZmADF3 immunoreactivity in the elongating hair is concentrated at the tip, where the cytoplasm is most dense. However, when the hair stops growing (as marked by the position of the nucleus), there is very little ZmADF3 staining.

What we believe ADF is doing in the cytoplasm at the root hair tip is both severing actin filaments and accelerating pointed-end disassembly, providing seeds and monomer for polymerization at the membrane surface. We proposed that the hair tip is the active assembly point for actin filaments and that ZmADF3 is essential for active tip growth (Jiang *et al.* 1997a).

Figure 2. Maize root hair cells fixed and stained for fluorescence microscopy using (a) anti-ZmADF3 to identify the ADF and (b) with rhodamine-conjugated phalloidin to identify the actin filaments. In the elongating hair, ADF is concentrated at the root hair tip, a region likely to be involved in high actin dynamics (Jiang *et al.* 1997b). Asterisks show the position of the nucleus.

(ii) ADF in pollen and pollen tubes

We localized ADF in pollen and germinated pollen using the anti-ZmADF1 antiserum. This antiserum cross-reacted with ADF in pollen from a range of plants; we used daffodil pollen because this is so amenable to cell biological analysis and also because Heslop-Harrison and colleagues (Heslop-Harrison & Heslop-Harrison 1992) catalogued some amazing actin reorganizations in this system as pollen entered and exited dormancy. What was particularly striking in this study was that pollen ADF was seen to decorate the actin array in mature pollen grains (figure 3). Together with an ADF from *Caenorhabditis elegans* (Ono 2001) these are the only examples of ADF decorating F-actin in cells apart from in those cells where ADF concentration has been artificially increased through microinjection or transfection (Moriyama *et al.* 1996; Aizawa *et al.* 1996; Dong *et al.* 2001b). This decoration of F-actin by pollen ADF may reflect the F-actin binding capability of pollen ADF *in vitro* and its inefficient actin depolymerization activity, which was described in the previous sections. In dehydrated pollen, a process which occurs as the pollen grains enter dormancy, the actin-ADF network breaks down into actin and/or actin:ADF rodlets or aggregates. Presumably, upon dehydration the ADF decorating the actin array produces such torsional strain that the array breaks down into actin:ADF rodlets. These rodlets or aggregates may be the storage form of actin in readiness for germination because in germinated pollen ADF is released and is redistributed to the pollen tube primordia. At the same time the actin network reorganizes and converges on the developing tube. In elongating pollen tubes, ADF concentrates in the tube but there

Figure 3. Daffodil pollen fixed and stained for fluorescence microscopy (a) with anti-ZmADF1 to identify ADF and (b) with rhodamine-conjugated phalloidin to identify actin filaments. ADF is bound to actin in mature pollen, dehydrated pollen and at sites of pollen tube adhesion (Smertenko *et al.* 2001).

is no specific localization to the actin array in germinated pollen. However, bright spots or patches of ADF immunostaining could be seen that could be the residual aggregations of ADF–actin observed prior to germination.

These data suggest that pollen ADF serves to bind and remodel structures containing F-actin in pollen grains rather than to promote filament disassembly. In this context it is interesting to note that microinjection of the maize pollen ADF into *Tradescantia* stamen hair cells

resulted in the remodelling of the actin arrays (Hussey *et al.* 1998). In contrast, the principal activity of the maize vegetative ADF, ZmADF3, in root hairs is to increase the turnover of actin filaments and this may account for our inability to demonstrate co-localization of this ADF with F-actin in cells. Finally, the apparent release of ADF1 from F-actin in germinated pollen indicates that the F-actin binding activity is tightly regulated during development and our current work is addressing this issue.

4. ADF–ACTIN-INTERACTING PROTEINS

In addition to the controls of ADF activity described above, it has been found that a WD repeat protein can also modulate ADF activity. This WD repeat protein has been identified by genetic screens for extragenic suppressors of yeast cofilin mutants, two-hybrid screens using ADF/cofilin as bait and by affinity purification of ADF interacting proteins from yeast (Rodal *et al.* 1999; Iida & Yaharra 1999) and *Xenopus* (Okada *et al.* 1999). This protein is AIP1, which was first identified in yeast two-hybrid screens with actin as bait (Amberg *et al.* 1995).

WD repeat motifs were first identified in the β subunit of the heterotrimeric G protein, and are now known to exist in a variety of proteins involved in signal transduction, gene regulation, vesicular traffic and organization of the cytoskeleton (Neer *et al.* 1994). The WD repeat in AIP1 has been shown to be essential for activity (Iida & Yaharra 1999).

Although AIP1 has been shown to bind to and depolymerize actin filaments *in vitro* (albeit weakly), in the presence of ADF, AIP1 increases the rate of F-actin depolymerization significantly. Similarly, AIP1 is unable to sever actin filaments itself, but in the presence of ADF, actin severing is markedly increased. So AIP1 may function to enhance the activity of ADF. At pH 6.8 AIP1 binds to *Xenopus* ADF/cofilin-saturated actin filaments at a maximum ratio of one AIP1 to two actin subunits. Similar results have been obtained for the yeast AIP1 (Rodal *et al.* 1999). AIP1 binds to domains three and four of actin, which are in the core of the actin fibre (Amberg *et al.* 1995), unlike ADF, which binds on the outer edge of the actin filament (McGough *et al.* 1997). AIP1 co-localizes with ADF *in vivo*. For example, AIP1 and ADF co-localize in the cleavage furrow of the first division cycle of *Xenopus* (Okada *et al.* 1999). Also, yeast mutants, in which AIP1 is not present, show co-localization of ADF to actin cables, rather than just to cortical actin patches (Rodal *et al.* 1999).

Comparison of an *Arabidopsis* homologue of AIP1 (AtAIP1) with other AIP1s in the database shows that the AtAIP1 has 63%, 34% and 31% identity at the amino acid level with rice, *Xenopus* and yeast AIP1, respectively, and it contains nine WD repeat motifs (P. J. Hussey, unpublished data). Bearing in mind the animal and fungal data described above, it is likely that AtAIP1 will play an important role in the regulation of plant ADF.

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GLOSSARY

- ADF: actin-depolymerizing factor
 AIP: actin-interacting protein
 AIP1: actin-interacting protein 1
 CDPK: calmodulin domain-like protein kinase
 EF: elongation factor
 LIADF1: lily ADF1
 LIM: a cysteine- and histidine-rich, zinc binding motif comprising two tandemly repeated zinc fingers, which is involved in mediating protein–protein interactions; named for lin11, IsI1 and mec3 transcription factors
 PIP: phosphatidylinositol-4-phosphate
 PIP₂: phosphatidylinositol-4,5-bisphosphate
 WASP: Wiskott–Aldrich syndrome protein
 WD: Tryptophan–aspartate repeat; a sequence motif *ca.* 31 amino acids long, which encodes a structural repeat
 ZmADF 1 α : maize ADF 1 α
 ZmEF: maize elongation factor