

Protein family review

The ADF/cofilin family: actin-remodeling proteins

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Summary

The ADF/cofilins are a family of actin-binding proteins expressed in all eukaryotic cells so far examined. Members of this family remodel the actin cytoskeleton, for example during cytokinesis, when the actin-rich contractile ring shrinks as it contracts through the interaction of ADF/cofilins with both monomeric and filamentous actin. The depolymerizing activity is twofold: ADF/cofilins sever actin filaments and also increase the rate at which monomers leave the filament's pointed end. The three-dimensional structure of ADF/cofilins is similar to a fold in members of the gelsolin family of actin-binding proteins in which this fold is typically repeated three or six times; although both families bind polyphosphoinositide lipids and actin in a pH-dependent manner, they share no obvious sequence similarity. Plants and animals have multiple ADF/cofilin genes, belonging in vertebrates to two types, ADF and cofilins. Other eukaryotes (such as yeast, *Acanthamoeba* and slime moulds) have a single ADF/cofilin gene. Phylogenetic analysis of the ADF/cofilins reveals that, with few exceptions, their relationships reflect conventional views of the relationships between the major groups of organisms.

Actin-binding proteins modulate the actin-based cytoskeleton; together, they form, destroy and reform the vast array of actin-rich structures that exist in eukaryotic cells. The actin-depolymerizing factors (ADFs, also known as destrins) and the cofilins are a single family called the ADF/cofilins. They are abundant and essential in almost every eukaryotic cell type, with the possible exception of red blood cells and sperm cells (see [1,2] for comprehensive reviews). Chromosomal locations of selected ADF/cofilin genes are shown in Table 1.

Gene organization and evolutionary history

An analysis of the available ADF/cofilin sequences has been performed (Figure 1), and this agrees well with previous analyses on more limited datasets [3-5]. In general, the tree conforms to the expected relationships between the major groups; for instance, all the fungi and yeast sequences group together separately from the plants and animals and all the plant ADF/cofilin sequences group together. Relationships between

plant ADF/cofilins are complicated by the presence of many sequences from some plant species (for instance, there are 12 in *Arabidopsis*), although expected kinships, for example between the related tomato and potato sequences, can be seen.

As ADF/cofilins are probably found in all eukaryotes, are diverse in sequence, are small proteins and a large number of cDNAs are already available (Figure 1), the family is a suitable candidate for analyzing relationships between phyla. The fact that some organisms have several different ADF/cofilins is a distinct disadvantage, however. ADF/cofilin genes can be so divergent that Southern blotting reveals only one type, even though multiple forms may exist; for example, Southern blotting detects only "a few" ADF/cofilin genes in maize and lily [6,7], whereas *Arabidopsis thaliana* is known to contain 12 different sequences (although not all have been shown to be functional genes), so maize and lily would be expected to have multiple ADF/cofilin genes. From a phylogenetic point of view this

Table 1**Chromosomal locations of selected ADF/cofilin genes**

Species/gene	Chromosome location	Genomic accession number	Number of introns	Reference(s)
<i>A. thaliana</i> ADF1	Chr 3 F16L2.220 (At3g46010)	AF102173	2	TIGR database [66]
<i>A. thaliana</i> ADF2	Chr 3 F16L2.210 (At3g4600)	AL162459	2	TIGR database [66]
<i>A. thaliana</i> ADF3	Chr5 MMN10.4 (At5g59880)	AF102821	2	TIGR database [66]
<i>A. thaliana</i> ADF4	Chr5 MMN 10.8 (At5g59890)	AF102822	2	TIGR database [66]
<i>A. thaliana</i> ADF5	Chr2 T24121.11 (At2g16700)	AF102825	2	TIGR database [66]
<i>A. thaliana</i> ADF6	Chr2 F16D14.4 (At2g31200)	AF183576	?	TIGR database [66]
<i>C. elegans</i> UNC-60	Chr 5	AF024494	4	<i>C. elegans</i> sequencing consortium [67]
<i>D. discoideum</i> DCOF1	?	D37980	1	[22]
<i>D. discoideum</i> DCOF2	?	D37981	0	[22]
<i>D. melanogaster</i> <i>twinstar</i>	Chr 2	U24676	2	[11]
Human non-muscle cofilin (<i>Cfl1</i>)	Chr 11 q13.4	AC009470	3	Human Genome Project [68]
Human muscle cofilin (<i>Cfl2</i>)	Chr 14	AF242299, AF283513	4	[19,69]
Human ADF (possibly a pseudogene)	Chr 12	U47924	0	Human Genome Project [68]
Human ADF (possibly a pseudogene)	Chr 8	AC022868	0	Human Genome Project [68]
Human ADF	Chr 20	AL132765	3	Human Genome Project [68]
<i>O. sativa</i> ADF1	Chr10	AC079029	2	TIGR database [66]
<i>O. sativa</i> ADF2	Chr3	AC084320	2	TIGR database [66]
<i>S. cerevisiae</i> <i>Cof1</i>	Chr XII 39803- 40413	Z14971, D13230	1	[20,70]
<i>S. pombe</i> ADF1	Chr I	Z98600	0	[71]

presents problems, such as which of the *Arabidopsis* ADF genes best represents this plant with respect to its relationship with other plants. Such problems are especially apparent within the protists and are compounded by longbranch attraction, an artifact in which divergent species group together on a phylogenetic tree, and by the very sparse data available for many protistan groups (data is also very sparse for algae, molluscs and reptiles).

The ADF/cofilins found in each group of organisms

Animals

Most vertebrates have one ADF and two cofilins; the latter are divided into muscle and non-muscle cofilins [8]. The reported human destrin-2 gene (Genbank U72518) is most likely to be a pseudogene [9]. The frog *Xenopus* expresses two ADF/cofilins, but these appear to be more closely related to the cofilins than the ADFs; the possibility of a *Xenopus* ADF cannot presently be excluded, however. If there is no *Xenopus* ADF, this may indicate that the ADF and cofilin lineages may have diverged in the reptilian common ancestor of birds and mammals. Only one cofilin is found in chicken and this is more similar to the mouse muscle cofilin (96.4% identity) than it is to the mouse non-muscle cofilin (81.3%).

Of the invertebrates, *Caenorhabditis elegans* has one ADF/cofilin gene, *unc-60*, which encodes two different proteins, UNC-60A and UNC-60B [10] (see below). *Drosophila* has one ADF/cofilin gene, *twinstar* [11]. The first ADF/cofilin sequence to be determined, that of depactin, which was isolated from eggs of the sea star *Asterias amurensis* [12], was determined by direct amino-acid sequencing of the protein [13], and to date no supporting cDNA or gene sequence is available. Although a putative ADF/cofilin gene from another echinoderm, the sea urchin *Strongylocentrotus purpuratus*, is available, this sequence does not group with depactin. In fact, depactin is the most divergent member of the group so far discovered (see Figure 1).

Plants

A surprising finding is that plants have many more ADF/cofilin genes than animals. Using a limited data set, Mun *et al.* [3] classified the plant ADF/cofilins into four groups (I-IV); our analysis (Figure 1) supports this classification and we have also subdivided groups I and II into two subgroups and group III into three subgroups. Some indications of a separation of the plant ADF/cofilins along the lines of the major plant groups (gymnosperms, angiosperms, monocots, and dicots) is evident: group I is composed

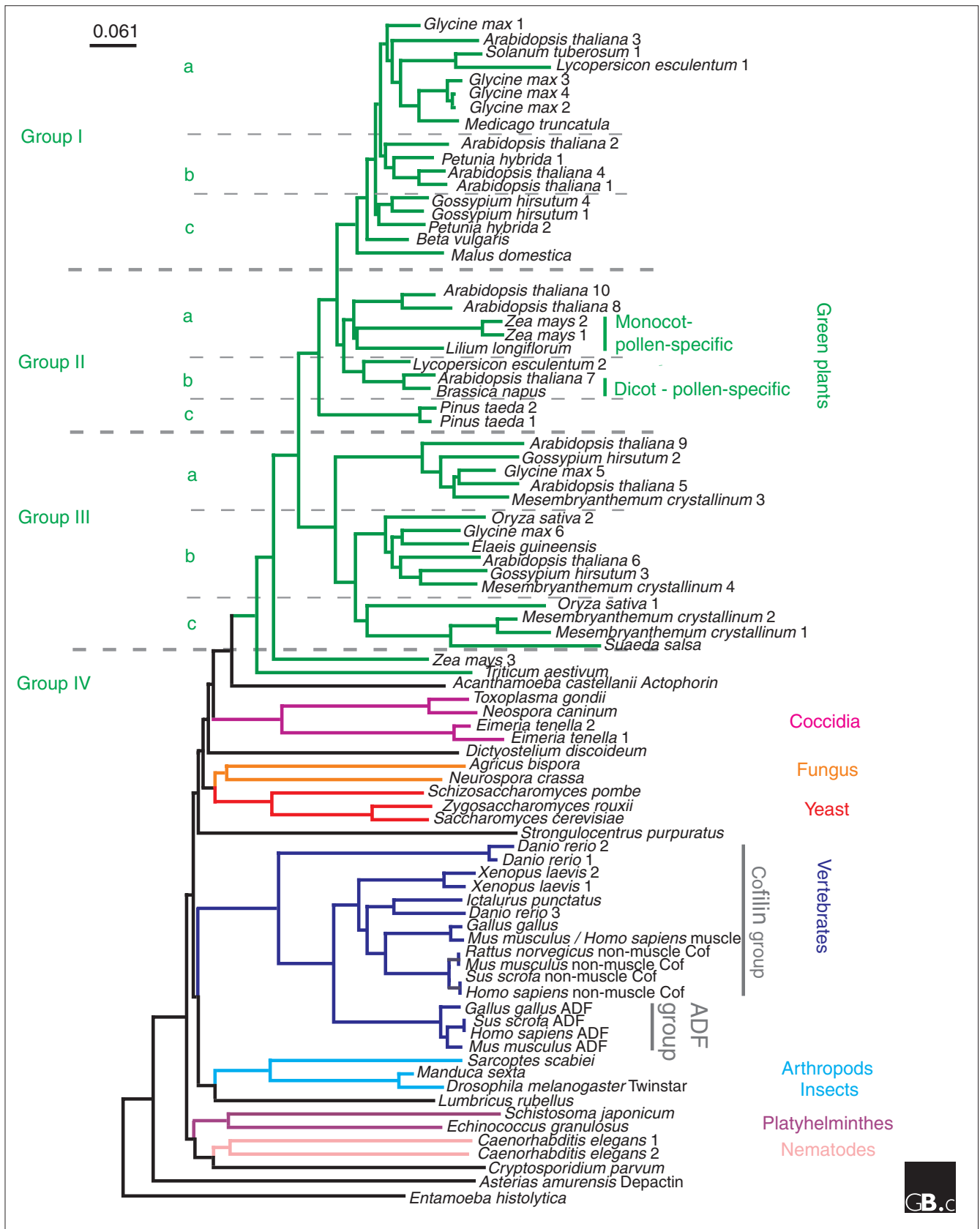


Figure 1 (see legend on the next page)

exclusively of dicots (although there is a rice gene similar to *Petunia hybrida* ADF1 on chromosome 3; GenBank accession number ACo84320), whereas group III contains both dicots and monocots. Group II contains dicots, monocots and gymnosperms, and group IV presently includes *Zea mays* ADF3 and an ADF/cofilin from wheat (some trees placed these ADF/cofilins more closely than in Figure 1). Southern blot analysis [14], probing with the wheat ADF/cofilin, reveals the presence of similar sequences in all the monocots tested, *Secale cereale*, *Avena sativa*, *Hordeum vulgare*, *Oryza sativa* and *Zea mays* (the latter sequence is presumably ADF3), whereas the dicots tested, *Medicago sativa* and *Brassica napus*, did not hybridize, indicating perhaps that group IV is exclusive to the monocots [14]. It is possible that group II is exclusively pollen-specific and that, within this group, monocots and dicots form subgroups [6,7]. Members of group IIIc (the third subgroup of group III, see Figure 1) have an insert of various lengths between sheet 6 and helix 4 (see Characteristic structural features), for no presently apparent purpose.

The *Arabidopsis thaliana* genome sequencing project is complete, so it is possible to analyze the full complement of ADF/cofilin genes from this plant. Although *Arabidopsis* has a genome size only 4% that of humans, it has 12 ADF/cofilin

genes (*AtADFs*). It is not yet clear how many of these are expressed, but cDNAs have been isolated for most [15]. Two pairs of *AtADF* gene products are very similar (*AtADF1* and *AtADF4*, and *AtADF8* and *AtADF10*), making it likely that their functions may be redundant. The phylogenetic analysis (Figure 1) predicts that *AtADF7* and perhaps *AtADF8* and *AtADF10* are pollen-specific, as maize and lily pollen-specific ADFs fall in the same grouping as these three *AtADFs*. The ADF genes of *Arabidopsis* are clustered: *AtADF3* and *AtADF4* are adjacent on chromosome 5, and a putative ADF gene is followed by *AtADF2* and *AtADF1* on chromosome 3

Other eukaryotes

Compared with animals and plants, there are relatively few ADF/cofilins characterized from other eukaryotes, which limits our interpretation of the evolution of the ADF/cofilin genes (Table 1, Figure 2b). There is only one ADF/cofilin sequence in the fully sequenced *Saccharomyces cerevisiae* genome, and there is evidence for a single ADF/cofilin gene (actophorin) in the soil amoeba *Acanthamoeba castellanii* [16]. It was previously suggested on similar evidence, however, that there was only one cofilin gene in *Dictyostelium*, but more recently the sequence of another *Dictyostelium* cofilin-like gene, *cofilin-2*, has been deposited in GenBank (accession number AB055926) by

Figure 1 (see figure on the previous page)

A phylogenetic tree of the ADF/cofilin family. The groups and subgroups of plant ADF/cofilins are separated by dotted lines. An alignment of the complete sequences was made with Clustal W; this was used to derive a phylogenetic tree with Clustal W using bootstrapping (1,000 reiterations) and the output tree was plotted using the Njplot program. The data were taken from the published literature, expressed sequence tag databases and genomic databases. *Arabidopsis thaliana* ADF1-ADF9 are named in accordance with Bowman *et al.*, 2000 [4] with an additional sequence ADF10 from GenBank (AAF78408). The *petunia* (*Petunia hybrida*) and cotton (*Gossypium hirsutum*) ADF/cofilins are numbered in accordance with Mun *et al.*, 2000 [3]. The alignment generated for this analysis and other information relating to this article and the ADF/cofilins generally is available from the authors' ADF/cofilin home page [76]. In order from top of the figure to the bottom, the sequences were derived from the following accession numbers (GB, GenBank [18]; SP, SwissProt [77]; GB, PIR, protein information resource [78]): *Glycine max* 1 (soya bean), BG725541; *A. thaliana* 3 (thale cress), GB AF360169, GB AF102821 and GB AAD09109; *Solanum tuberosum* (potato), GB BE340726; *Lycopersicon esculentum* 1 (tomato), GB BG791215; *Glycine max* 3, GB BE802250; *G. max* 4, GB BG882919; *G. max* 2, GB BG882937, GB BG882422 and GB BG882919; *Medicago truncatula* (barrel medic), GB AA660460 and GB AA660869; *A. thaliana* 2, GB U48939; *Petunia hybrida* 1 (petunia), GB AAK72617 [3]; *A. thaliana* 4, GB AF102822; *A. thaliana* 1, GB AF102173; *Gossypium hirsutum* 4 (cotton), GB AI728908; *G. hirsutum* 1, GB AF731080; *P. hybrida* 2, GB AAK72616 [3]; *Beta vulgaris* (sugar beet), GB BF011219; *Malus domestica* (apple tree), GB AF179295; *A. thaliana* 10, GB AAF78408; *A. thaliana* 8 (incomplete) [4]; *Zea mays* 2 (maize), GB X97725 [7]; *Z. mays* 1, GB X80820 [7]; *Lilium longifolium* (trumpet lily), PIR S30935, GB Z14110 [6]; *Lycopersicon esculentum* 2, GB AW218268; *A. thaliana* 7 [4]; *Brassica napus* (incomplete; rapeseed), PIR S30934 and GB Z14109 [6]; *Pinus taeda* 2 (Loblolly pine), GB AA556832; *P. taeda* 1, GB AW290013; *A. thaliana* 9 (incomplete) [4]; *G. hirsutum* 2, GB AI730337; *G. max* 5, GB BE211729; *A. thaliana* 5, AF360302, AF102825 and AF102823; *Mesembryanthemum crystallinum* 3 (ice plant or figmarigold), GB BE033507; *Oryza sativa* 2 (rice), GB AAK09235; *G. max* 6, GB BG726731; *Elaeis guineensis* (African oil palm), GB AF236068; *A. thaliana* 6 (incomplete) [4]; *G. hirsutum* 3, GB AI729046; *M. crystallinum* 4, GB BE033912; *Oryza sativa* 1, GB AAK38308; *M. crystallinum* 2, GB BE035020; *M. crystallinum* 1, GB GB035057; *Suaeda salsa* (seablite), GB AW990964; *Z. mays* 3, X97726 [7]; *Triticum aestivum* (wheat), GB U58278 [14]; *Acanthamoeba castellanii* (soil amoeba) actophorin, SP P37167 [16]; *Toxoplasma gondii* (coccidian parasite), U62146; *Neospora caninum* (apicomplexan), GB BG235118 and GB BG235281; *Eimeria tenella* 2 (coccidian parasite), GB AI756831; *E. tenella* 1, GB BG235538; *D. discoideum* (slime mould), SP P54706 [22]; *Agaricus bisporus* (cultivated mushroom), GB AVW444327; *Neurospora crassa* (incomplete; fungus), GB T49327; *Schizosaccharomyces pombe* (yeast) Cof1, GB D89939 and PIR T38120; *Zygosaccharomyces rouxii* (yeast), GB BAB18899; *S. cerevisiae* (yeast), SP Q03048 and D13230 [20,70]; *Strongylocentrotus purpuratus* (sea urchin), Contig 501 [79]; *Danio rerio* 2 (zebrafish), GB B017097; *D. rerio* 1, GB Fa96c03.Y1, GB Fa91d10.YL, GB Fb04b04.y1 and GB Fa96c03.x1; *Xenopus laevis* 2 (South African clawed toad), SP P45593 [80]; *X. laevis* 1, GB U26270 [80]; *Ictalurus punctatus* (channel catfish), GB BE470088, GB BE469308 and GB BE468299; *D. rerio* 3, GB AVW018661, GB AI658133 and GB AI794635; *Gallus gallus* (chicken) muscle cofilin, M55659 [81]; *Mus musculus* (house mouse) muscle Cof2, L29468 [8]; *Homo sapiens* (human) muscle cofilin, GB AF283513; *Rattus norvegicus* (rat) non-muscle cofilin, GB G509201; *M. musculus* non-muscle Cofilin, SP P18760; *Sus scrofa* (pig) non-muscle cofilin, GB M20866; *H. sapiens* non-muscle cofilin1, GB D00682; *G. gallus* ADF, GB J02912; *S. scrofa* ADF, GB J05290 [43]; *H. sapiens* ADF, PIR A54184 [47]; *M. musculus* ADF, NP062745; *Sarcoptes scabiei* (parasitic mite), GB BG817660; *Manduca sexta* (silkworm, insect), GB BF707432; *Drosophila melanogaster* (fruit fly) Twinstar, PIR A57569 [11,82]; *Lumbricus rubellus* (earthworm), GB BF422380; *Schistosoma japonicum* (trematode fluke causing schistosomiasis), GB AA140553; *Echinococcus granulosus* (cestode tapeworm of dogs), GB BI244320; *Caenorhabditis elegans* 1 (nematode), SP Q07750 [10]; *C. elegans* 2, SP Q07749 [10]; *Cryptosporidium parvum* (apicomplexan), GB AA224644; *Asterias amurensis* (starfish) depactin, SP P20690; *Entamoeba histolytica* (dysentery-causing amoeba), contig ENTFF06TR [83].

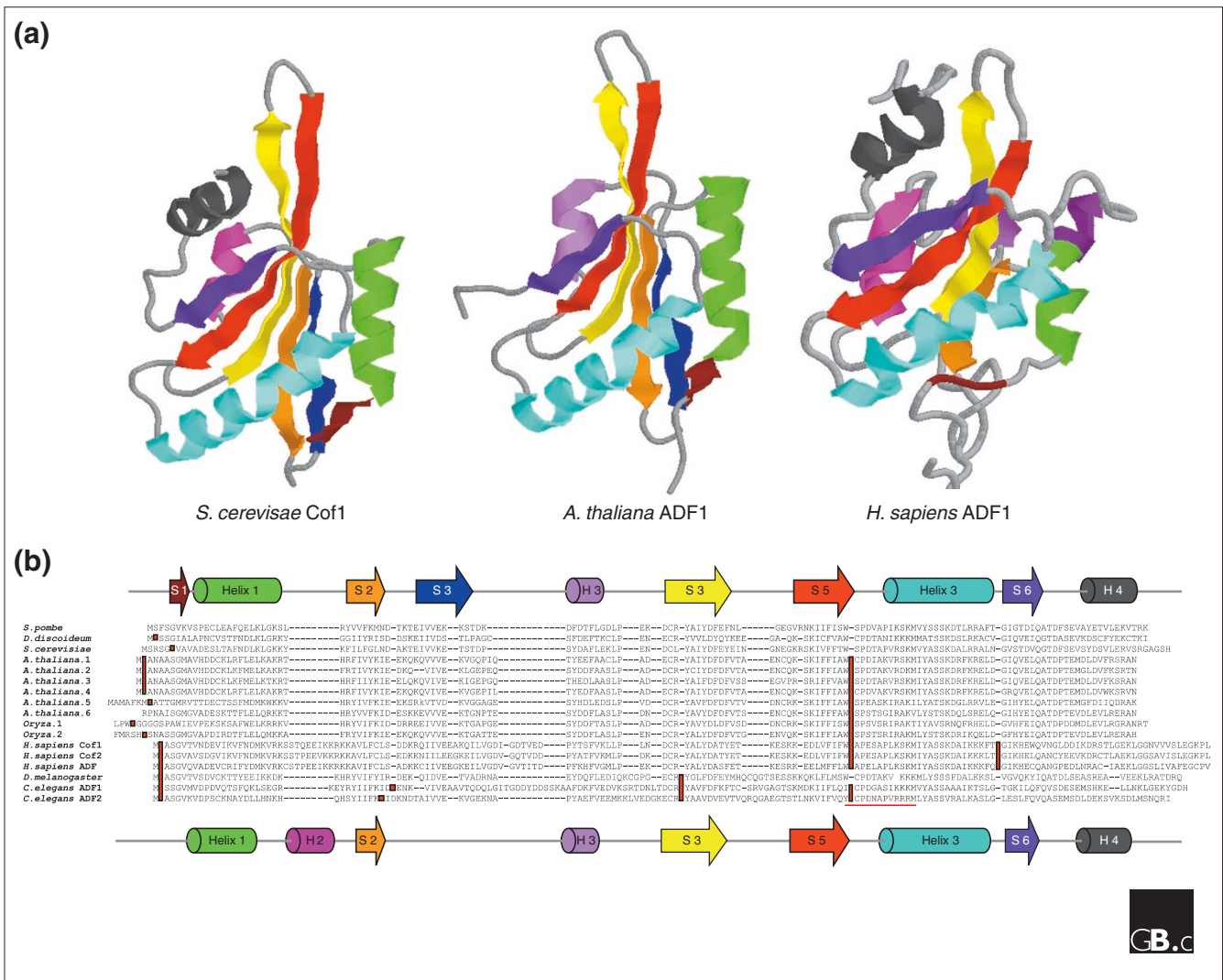


Figure 2
 The structure of ADF/cofilins. **(a)** The three major groups of ADF/cofilins identified in Figure 1 (plants, fungi and vertebrates) are each represented by a structure. The predominant structural features (α helices and β sheets) are shown in colors that correspond to those used in **(b)**, which shows the genomic organization of ADF/cofilins superimposed on the amino-acid sequence, with secondary structures highlighted. The red squares or bars indicate the positions of introns interrupting the deduced amino-acid sequences. Red underlining represents the PIP₂/actin-binding site [30].

the same group that cloned *cofilin-1*. The inclusion of this sequence in our phylogenetic analysis has the effect of removing the *cofilin-1* sequence from its present position within the tree to an outlying group with *cofilin-2*. As the *cofilin-2* gene has this effect and because it has not been verified as being an ADF/cofilin member, it has not been included in our analysis. *Acanthamoeba* actophorin most closely resembles the plant ADF/cofilins of the limited number of phyla included in the study; a kinship between *Acanthamoeba* and plants is suggested in many (but by no means all) ribosomal DNA analyses.

The coccidians, including the bird parasite *Eimeria tenella* and the cat and human parasite *Toxoplasma gondii*, appear

to have two ADF/cofilins; only one ADF/cofilin has been reported in *Toxoplasma gondii* [17], but at least two differentially spliced forms are found in expressed sequence tag (EST) databases (GenBank BG658910, BG659044 [18]). (Although the actin-binding function of the *Eimeria* ADF/cofilin protein has not been published, it is similar to *Toxoplasma* ADF/cofilin, which is a confirmed ADF/cofilin member in terms of its interaction with actin.) The ADF/cofilin sequence from *Cryptosporidium parvum* is a puzzle, because being from another protozoan (an apicomplexan), it would be expected to group with *T. gondii*, but instead, it appears in our analysis to group loosely with the nematode *C. elegans*. Some trees generated in our analysis do suggest a relationship between *Toxoplasma* and *Cryptosporidium*. More

sequences are of course needed to resolve this puzzle. A partial sequence from another apicomplexan, *Sarcocystis neurona* (GenBank BE636150, not included in our analysis), is related to mammalian cofilins, adding to the confusion. This sequence may have been 'picked up' at some point by horizontal transfer as the parasite moved between hosts.

Gene structure

The intron-exon boundaries often provide information on the ontogeny and evolution of genes. As expected, there are several such boundaries within ADF/cofilin genes, and these are preserved across the phyla. A remarkable tendency for ADF/cofilin genes is for the first amino acid (or the first few) to be encoded by a separate exon (Figure 2b). The human muscle cofilin gene (*Clf2*) produces two different mRNAs that encode identical polypeptides by the use of two alternative first exons encoding the methionine and upstream untranslated region; these mRNAs presumably differ in their localization and/or stability [19]. The opposite is true for the muscle ADF/cofilin of the nematode *C. elegans*: two different ADF/cofilin proteins are produced from one gene, although the only exon to be shared is that encoding the initiating methionine. The *S. cerevisiae Cof1* gene contains one exon in the region encoding the amino terminus of the protein [20], as does one of the two genes encoding identical proteins in *Dictyostelium discoideum*. Several ADF/cofilin genes, for example those from *Schizosaccharomyces pombe*, *Entamoeba histolytica* and *Strongylocentrotus purpuratus*, have no introns, but some of these have yet to be shown to be functional genes. Genes that contain no introns are likely to be pseudogenes [21,22], so those ADF/cofilin genes identified solely on the basis of their genomic sequence (such as those from *E. histolytica* and *S. purpuratus*) must be verified by cDNA cloning. This rule also appears to hold for human ADF genes; a number of pseudogenes homologous to ADF/cofilin genes lacking introns are suspected (such as those with GenBank accession numbers AC009498 (chromosome 2) and AL132765 (chromosome 20)). As far as can currently be determined, plant ADF/cofilin genes are organized in a similar manner, with an intron following the exon encoding the amino terminus and a conserved intron further 3'. This pattern holds for *Arabidopsis* and *Oryza sativa* ADF/cofilin genes.

Characteristic structural features

The ADF/cofilins are formed by a single folded domain, the ADF homology domain, which is also found in other actin-binding protein families, including Abp1p, drebrins [23], twinfilin [24] and coactosin [25] (Figure 3). The ADF/cofilins themselves vary in size from 113 amino acids (*E. tenella*) to 168 amino acids (both *Xenopus laevis* proteins). Despite the considerable variation in sequence and size across the ADF/cofilin family, the structures so far available (Table 2, Figure 2a) show that they share a remarkably conserved fold. The main actin-binding structure of the ADF/cofilins is the long α helix starting, for

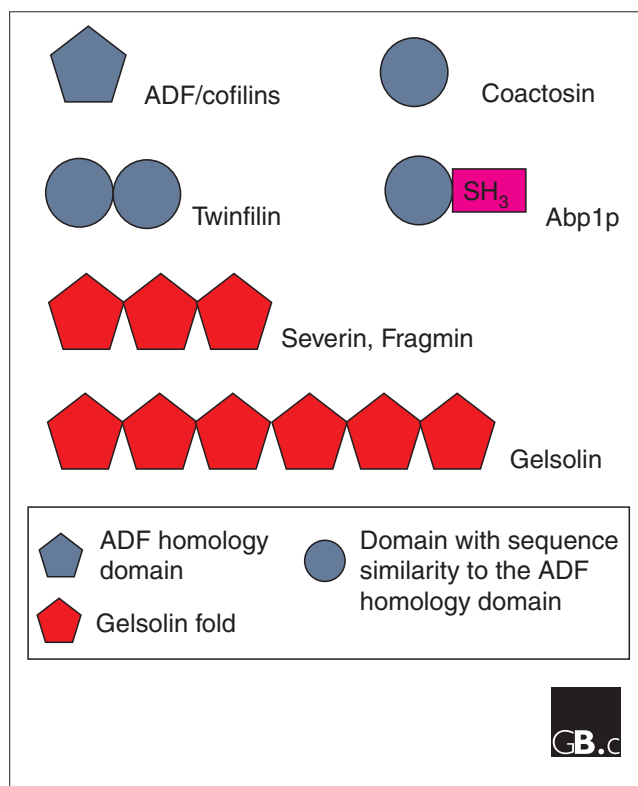


Figure 3

Relationships of ADF/cofilins with other actin-binding proteins. The ADF/cofilins are composed of a single fold (the ADF homology domain), which has sequence similarity with a domain found in drebrins, coactosin, twinfilin and Abp1p. It is not yet certain if the fold of these two domains is similar. The fold of the ADF homology domain is similar to a domain found in the gelsolin family (the 'gelsolin fold'), despite very low sequence similarity between the two.

example in human destrin, at Leu111 and terminating at Phe128. Most ADF/cofilins contain at least one nuclear-localization signal (NLS) close to the amino terminus. Interestingly, even those ADF/cofilins, such as those of *Dictyostelium* and *Zea mays*, that lack the classic bipartite NLS can still be induced to enter the nucleus when the cells are treated with either 10% dimethylsulfoxide [22] or cytochalasin D [26]. Many ADF/cofilins are known to associate with the phospholipid phosphatidylinositol-4,5-bisphosphate (PIP₂) [16,27,28], and a short sequence (Trp100-Met115; see Figure 2) has been identified that is important for binding to both actin and PIP₂ [29]. The analogous region of *Acanthamoeba* actophorin also contains overlapping sites for both actin and PIP₂, explaining the competition observed between the two ligands [30].

Localization and function

Subcellular localization

ADF/cofilins are usually localized in parts of the cell where there is a high turnover of actin filaments, such as the

Table 2

ADF/cofilins for which structures are known					
Protein name and source	Method	GenBank/SwissProt accession number	Reference	Protein Data Bank accession number	Reference
Human destrin (ADF)	NMR	P18282	[43]	I AK6/I AK7	[72]
<i>Acanthamoeba castellanii</i> actophorin	Crystal	P37167	[16]	I AHQ I CNU (phosphorylated form)	[73] [74]
<i>S. cerevisiae</i> Cof1	Crystal	Q03048	[70]	I COF	[75]
<i>A. thaliana</i> ADF1	Crystal	AAC72407		I F7S	[4]

leading edge of moving animal cells [16,31-33] and the growing tips of plant cells [26]. The main activity of ADF/cofilins has been found from *in vitro* experiments to be to increase actin-filament turnover [5,34,35]. They accomplish this by severing actin filaments and increasing the rate at which actin monomers leave the pointed end of actin filaments (see below). The rate at which actin filaments depolymerize is the rate-dependent step in the overall turnover of filaments that comes about as cells move forwards [36]. Cells lacking cofilin have impaired locomotion [37], and those over-expressing cofilins are more motile [38]. The effects are specific to certain types of actin filaments: older filaments (those at the base of leading lamellae) are 'marked' for turnover; the mark arises because they tend to contain more ADP-actin monomers and it is with these that the ADF/cofilins preferentially interact [34,35]. ADF/cofilins are also necessary for cytokinesis, depolymerizing the contractile ring between daughter cells as it contracts. ADF/cofilins localize to the contractile ring [39], and cells lacking ADF/cofilins are defective in cytokinesis [11].

In addition to their role in microfilament recycling, ADF/cofilins are also found in actin-rich, spicule-like rods found in stressed cells, in both the cytoplasm and the nucleus [26,40]. ADF/cofilins are also targeted to the nucleus upon heat shock and chemical stress. It may be that actin is taken into the nucleus in this manner so that a pool of tightly packed actin is protected from denaturation, and is then available after the stress is removed. ADF is known to inhibit actin denaturation, supporting this hypothesis [41].

The localization of ADF/cofilins in plant cells is broadly similar to that in animal and protist cells - they are primarily concentrated in regions rich in dynamic actin structures - but pollen and vegetative ADFs appear to have different properties. Pollen ADF has been seen to bind filamentous (F-) actin *in vivo* in mature pollen, dehydrated pollen and at adhesions between the tip of the pollen-tube and an adjacent substrate. Taken together with the fact that lily pollen ADF has an inefficient actin-depolymerizing activity, these data suggest that pollen ADFs serve to bind and remodel F-actin

structures, presumably in cooperation with other actin-binding proteins [42]. In contrast, given that the maize vegetative ZmADF3 localizes to the tip of growing root-hair cells, is not seen to co-localize with F-actin *in vivo* and has an effective actin-depolymerizing activity, its principal role appears to be to increase the turnover of actin filaments. In root-hair cells, the effect of increased actin dynamics at the hair tip would be to promote root-hair growth [26].

Expression

In vertebrates, a single ADF gene is expressed in most tissues [32], and ADF tends to have a reciprocal pattern of expression compared with the cofilins, with either the cofilins (generally) or ADF being more abundant. Both ADF and non-muscle cofilin are abundant in brain, both expressed at very low levels in liver and mature muscle [43]. The pattern of expression for most of the *AtADF*s has yet to be determined, but *AtADF1* and *AtADF4* are expressed in the vascular tissues in the entire plant and *AtADF5* is expressed at the tip of the root meristem [15]. *Dictyostelium* Cofilin-2 is expressed specifically at the aggregation stage of *Dictyostelium* development.

Function

The ADF/cofilins appear to have multiple functions, and this is reflected in their very complex association with monomeric and filamentous actin. They depolymerize actin filaments during, for example, cytokinesis [11,39], cell locomotion [36,37], and plant-cell elongation [26], in addition to being involved in cellular stress responses [44] and pathological situations [45]. ADF/cofilins are regulated by pH [31,46,47], polyphosphoinositides [16,27,28,31], phosphorylation [48-51], nucleotides bound by actin [36] and the presence of other actin-binding proteins [52-54]. They are, so far, unique among the actin-binding protein families in that they alter the twist of the actin filament [55]. ADFs and cofilins have very similar properties *in vitro*, but are present in varying relative concentrations in cells and, where they appear in the same cell as each other, interesting differences in behavior have been noted [31]. Rather surprisingly, the distribution of ADF but not cofilin is modulated by intracellular pH in mouse cells.

The two ADF/cofilins encoded by the *C. elegans unc-60* gene, UNC-60A and UNC-60B [10], have distinct actin-binding properties, but understanding this is further complicated by the discovery that UNC-60B behaves differently with respect to its interaction with rabbit muscle actin and actin from the nematode itself [56]. The most dramatic difference is that UNC-60A binds much more weakly to F-actin at pH 7.0 than does UNC-60B [10]. The carboxy-terminal domain of UNC-60B is essential in F-actin binding and it has been postulated to constitute a second actin-binding site [57]. The actin-binding properties of *Drosophila* Twinstar [11] have not yet been characterized.

Mechanism and regulation

The mechanism by which actin filaments are depolymerized by ADF/cofilins has been controversial and the details are still far from clear. Filaments are depolymerized by severing and by an increase in the rate at which actin monomers fall off the pointed end of the actin filament. Phosphorylation is a principal regulator of ADF/cofilin function: ADF/cofilins are phosphorylated on an amino-terminal serine (Ser3 in human non-muscle cofilin) by LIM kinases 1 and 2, TESK 1 [58] and TESK 2 [59], and maize ADF3 is phosphorylated by

a calmodulin-like domain protein kinase [50,51]. Phosphorylation by all these kinases prevents ADF/cofilins from binding actin (Figure 4).

Many ADF/cofilins, including vertebrate ADF and cofilins [28], *Acanthamoeba* actophorin [16], *Zea mays* ADF3 [27], and *Saccharomyces cerevisiae* Cof1, have been found to bind PIP₂ and, to a lesser extent, phosphatidylinositol-4-phosphate. Some of the actin-binding interfaces of ADF/cofilins partially overlap with the binding site of PIP₂ [30], explaining why PIP₂ dissociates the actin-ADF/cofilin complex. In turn, ADF/cofilins reciprocally affect the metabolism of the polyphosphoinositides. Vertebrate cofilins [29] inhibit the hydrolysis of PIP₂ by phospholipase C, as does *Zea mays* ADF3 [27]. Binding of ADF/cofilins by PIP₂, and perhaps by ion channels, may help to localize ADF/cofilins to the membrane, where they function to increase actin-filament turnover as well as to modulate PIP₂ metabolism.

Both *Acanthamoeba* actophorin [60] and sea star depactin [61] have been reported not to be pH-sensitive, although they are in other respects typical ADF/cofilins. No obvious relationship between sequence and pH sensitivity is yet

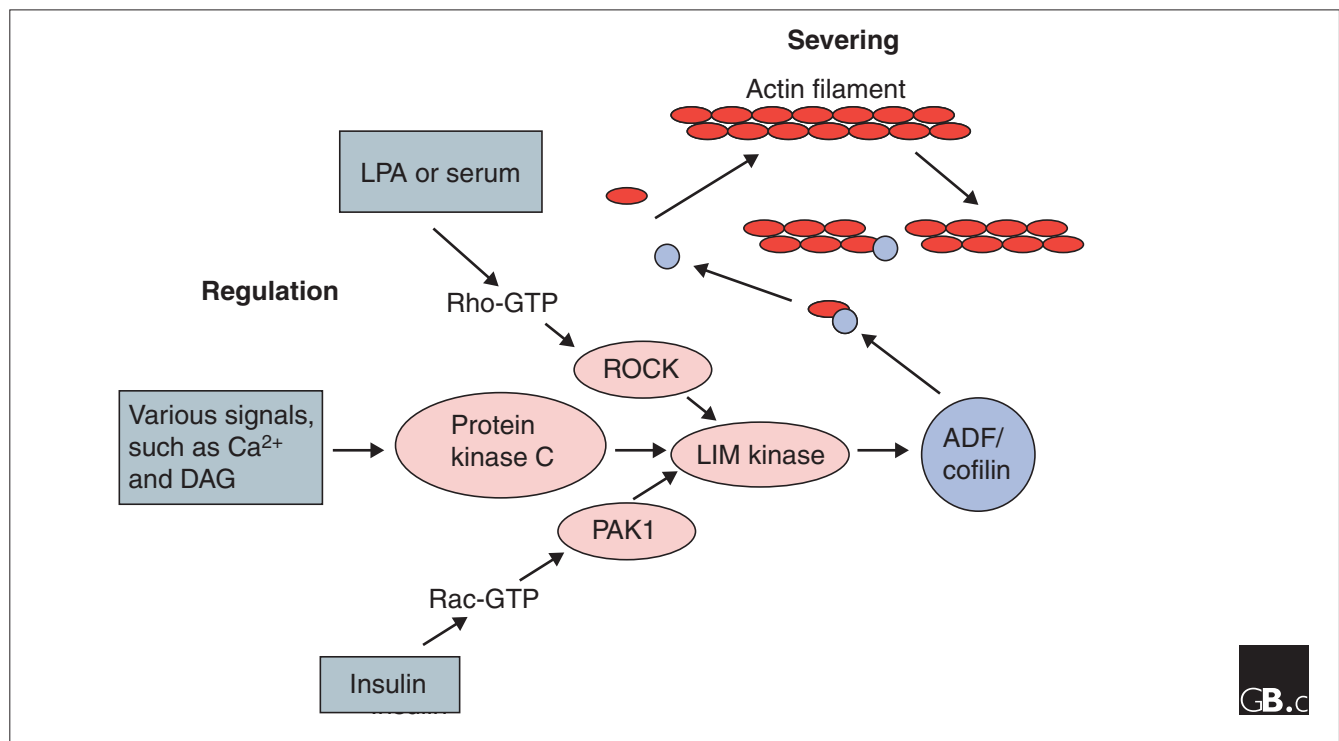


Figure 4

The regulation of ADF/cofilins through kinase and other pathways. In many cell types, the LIM kinases regulate ADF/cofilin activity by phosphorylation. LIM kinases are themselves activated by a host of upstream kinases including the Rho-activated kinase ROCK, Ca²⁺ and phospholipid-dependent kinase protein kinase C and Rac-activated kinase PAK1, which are in turn activated by small G proteins or diacylglycerol (DAG). Phosphorylated ADF/cofilins do not bind actin. Perhaps counterintuitively, the severing and depolymerization of actin filaments by ADF/cofilins is activated by phosphorylation, as this leads to dissociation of ADF/cofilin from actin, leaving it free to sever and depolymerize actin once more after it is dephosphorylated by phosphatase activity. Depolymerization would be increased further if ADF/cofilin phosphatase activity as well as LIM kinase activity were increased.

apparent, and pH dependence has been reported for many ADF/cofilins, including vertebrate ADF [41,47] and cofilins [46], *Arabidopsis thaliana* ADF1 [34], *Zea mays* ADF3 [27], and the ADF/cofilins of *Saccharomyces cerevisiae* [20], *Petunia hybrida* [3], *Triticum aestivum* [14], and the acomplexan *Toxoplasma gondii* [17].

Frontiers

Recently, some of the detail of how ADF/cofilins fit into various signaling cascades has come to light, and this continues to be a growing area of research. Another major task that is awaited is the construction of a detailed structural picture of how exactly ADF/cofilins bind and sever actin and increase the monomer release rate. It is known that the ADF/cofilins induce a remarkable (and so far unique) increase in the twist of the actin filament, but it is controversial how this is accomplished. One view is that ADF/cofilins bind between the two longitudinally associated actin monomers by binding a second actin-binding site [62], but this is in disagreement with other models in which ADF/cofilins are placed on the filament surface [63-65]. The crystallographic solution of the structure of cofilin-saturated actin filaments is an obvious but very ambitious goal that would resolve these issues.

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