Protein family review

The ADF/cofilin family: actin-remodeling proteins

Sutherland K Maciver* and Patrick J Hussey†

Addresses: *Genes and Development Interdisciplinary Group, Department of Biomedical Sciences, University of Edinburgh, George Square, Edinburgh EH8 9XD, Scotland, UK. †The Integrative Biology Laboratory, School of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK.

Correspondence: Sutherland K Maciver. E-mail: SKM@srv4.med.ed.ac.uk

Published: 26 April 2002

Genome ***Biology*** 2002, **3(5)**:reviews3007.1–3007.12

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2002/3/5/reviews/3007

© BioMed Central Ltd (Print ISSN 1465-6906; Online ISSN 1465-6914)

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)
A. thaliana ADF1	Chr 3 F16L2.220 (At3g46010)	AF102173	2	TIGR database [66]
A. thaliana ADF2	Chr 3 F16L2.210 (At3g4600)	AL162459	2	TIGR database [66]
A. thaliana ADF3	Chr5 MMN10.4 (At5g59880)	AF102821	2	TIGR database [66]
A. thaliana ADF4	Chr5 MMN 10.8 (At5g59890)	AF102822	2	TIGR database [66]
A. thaliana ADF5	Chr2 T24121.11 (At2g16700)	AF102825	2	TIGR database [66]
A. thaliana ADF6	Chr2 F16D14.4 (At2g31200)	AF183576	?	TIGR database [66]
C. elegans UNC-60	Chr 5	AF024494	4	*C. elegans* sequencing consortium [67]
D. discoideum DCOF1	?	D37980	1	[22]
D. discoideum DCOF2	?	D37981	0	[22]
D. melanogaster twinstar	Chr 2	U24676	2	[11]
Human non-muscle cofilin (*Cfl1*)	Chr 11 q13.4	AC009470	3	Human Genome Project [68]
Human muscle cofilin (*Cfl2*)	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]
O. sativa ADF1	Chr10	AC079029	2	TIGR database [66]
O. sativa ADF2	Chr3	AC084320	2	TIGR database [66]
S. cerevisiae Cof1	Chr XII 39803- 40413	Z14971, D13230	1	[20,70]
S. pombe 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

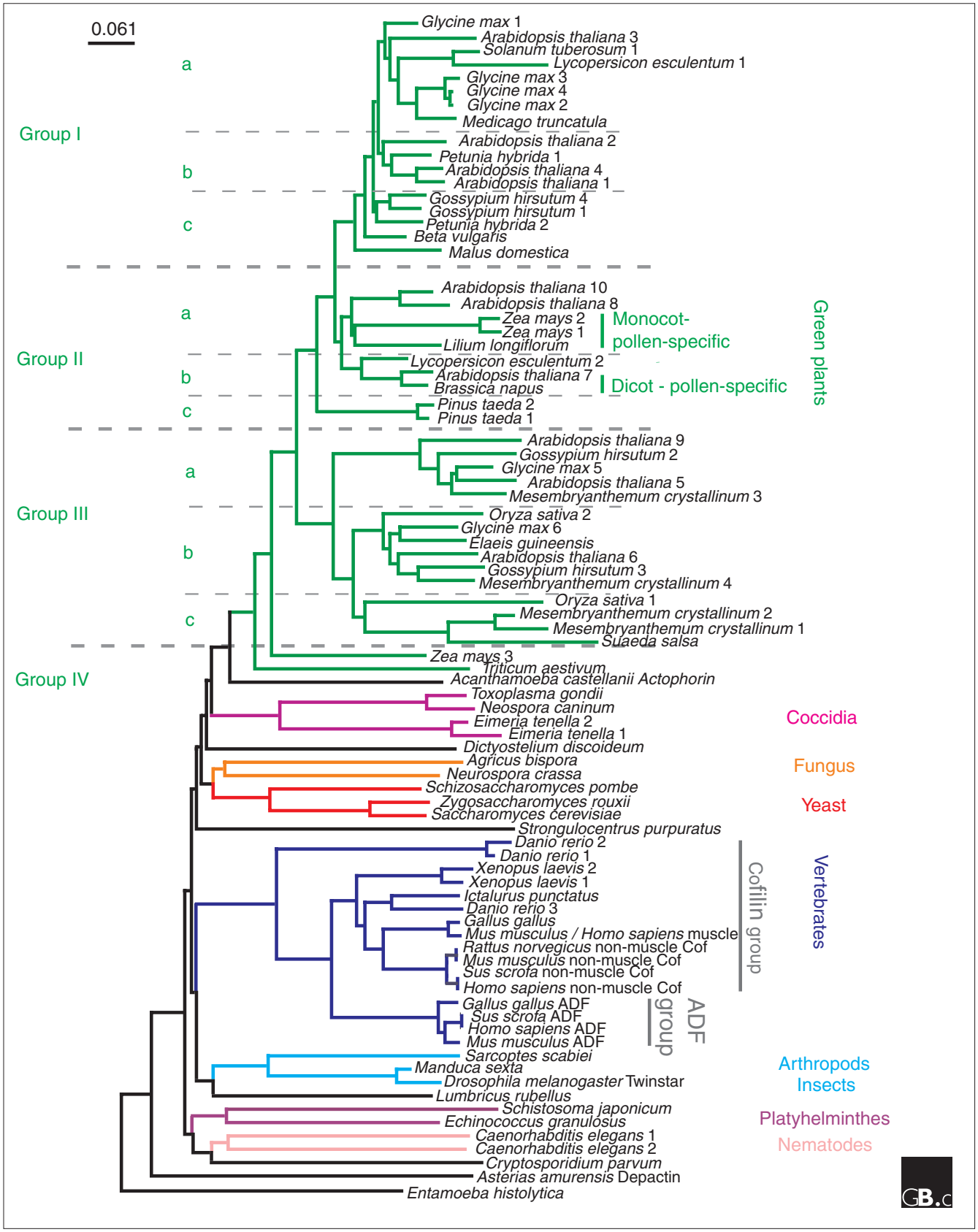

Figure 1 *(see legend on the next page)*

exclusively of dicots (although there is a rice gene similar to *Petunia hybrida* ADF1 on chromosome 3; GenBank accession number AC084320), 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 (*AtADF*s). 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 AW444327; *Neurospora crassa* (incomplete; fungus), GB T49327; *Schizosaccharomcyes 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 AW018661, 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].

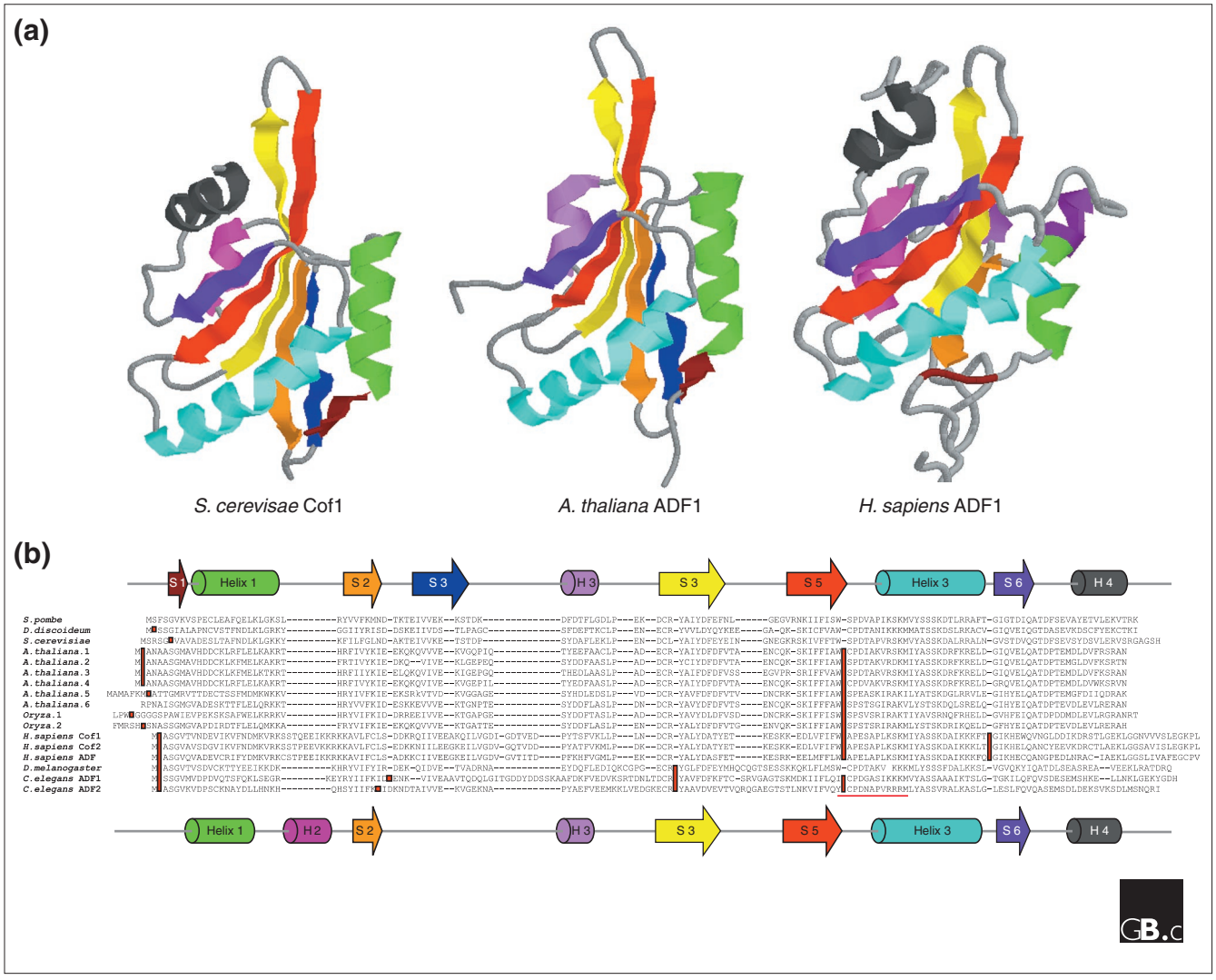

Figure 2
The structure of ADF/cofilins. **(a)** The three major groups of ADF/cofilins identified in Figure 1 (plants, fungi and vertebrates) are each represented by a structure. The predominant structural features (α helices and β sheets) are shown in colors that correspond to those used in **(b)**, 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 PIP_2/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 gene 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 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 (PIP_2) [16,27,28], and a short sequence (Trp100-Met115; see Figure 2) has been identified that is important for binding to both actin and PIP_2 [29]. The analogous region of *Acanthamoeba* actophorin also contains overlapping sites for both actin and PIP_2, explaining the competition observed between the two ligands [30].

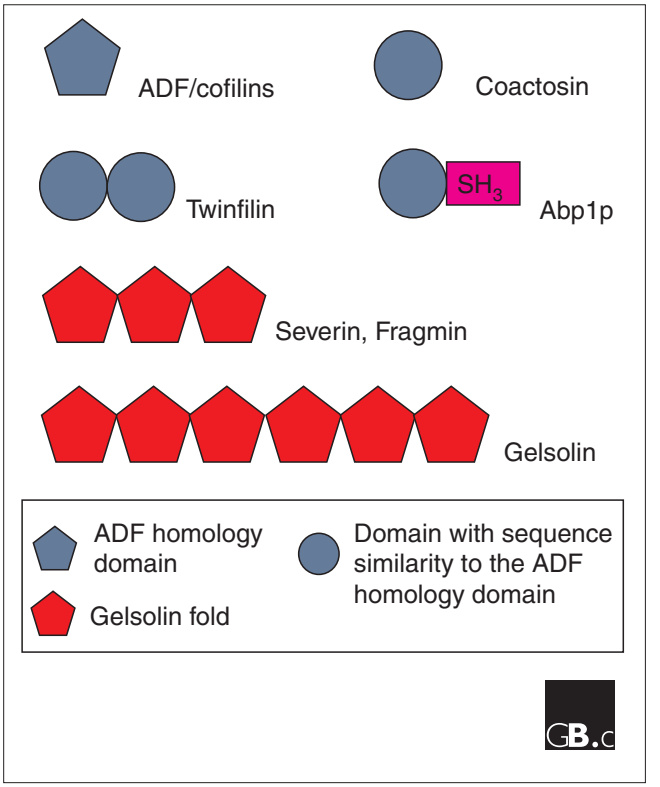

Figure 3
Relationships of ADF/cofilins with other actin-binding proteins. The ADF/cofilins are composed of a single fold (the ADF homology domain), which has sequence similarity with a domain found in drebrins, coactosin, twinfilin and Abp1p. It is not yet certain if the fold of these two domains is 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.

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]	1AK6/1AK7	[72]
Acanthamoeba castellanii					
actophorin	Crystal	P37167	[16]	1AHQ	[73]
				1CNU (phosphorylated form)	[74]
S. cerevisiae Cof1	Crystal	Q03048	[70]	1COF	[75]
A. thaliana ADF1	Crystal	AAC72407		1F7S	[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 locates 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 PIP_2 and, to a lesser extent, phosphatidylinositol-4-phosphate. Some of the actin-binding interfaces of ADF/cofilins partially overlap with the binding site of PIP_2 [30], explaining why PIP_2 dissociates the actin-ADF/cofilin complex. In turn, ADF/cofilins reciprocally affect the metabolism of the polyphosphoinositides. Vertebrate cofilins [29] inhibit the hydrolysis of PIP_2 by phospholipase C, as does *Zea mays* ADF3 [27]. Binding of ADF/cofilins by PIP_2, 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 PIP_2 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

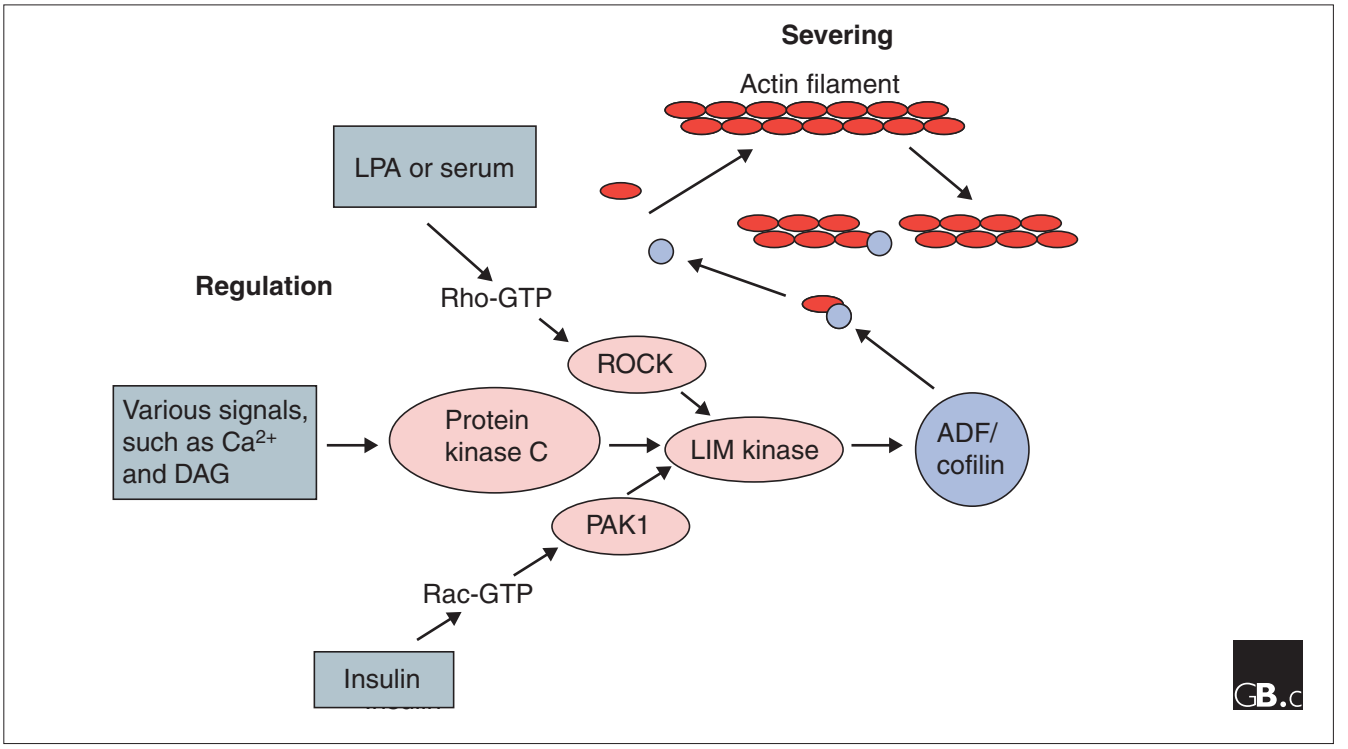

Figure 4
The regulation of ADF/cofilins through kinase and other pathways. In many cell types, the LIM kinases regulate ADF/cofilin activity by phosphorylation. LIM kinases are themselves activated by a host of upstream kinases including the Rho-activated kinase ROCK, Ca^{2+} and phospholipid-dependent kinase protein kinase C and Rac-activated kinase PAK1, which are in turn activated by small G proteins or diacyglycerol (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.

Acknowledgements

Work in the authors' labs is supported in part by Amoebics Ltd., Edinburgh (S.K.M.), and by the BBSRC (P.J.H.).

References

1. Bamburg JR: **Proteins of the ADF/cofilin family: essential regulators of actin dynamics.** *Annu Rev Cell Dev Biol* 1999, **15:**185-230.
   A very comprehensive review of the properties and function of the ADF/cofilin family.
2. Pollard TD, Blanchoin L, Mullins RD: **Molecular mechanisms controlling actin filament dynamics in nonmuscle cells.** *Annu Rev Biophys Biomol Struct* 2000, **29:**545-576.
   A very comprehensive review of how the multitude of actin-binding proteins, including the ADF/cofilin family, work together to modulate the actin cytoskeleton.
3. Mun J-H, Yu H-J, Lee HS, Kwon YM, Lee JS, Lee I, Kim S-G: **Two closely related cDNA encoding actin-depolymerizing factors of *Petunia* are mainly expressed in vegetative tissues.** *Gene* 2000, **257:**167-176.
   Cloning of two ADF/cofilin cDNAs from *Petunia hybrida* and their expression in bacteria. The recombinant proteins were found to be pH-sensitive with respect to their interaction with F-actin. Both cDNAs were widely expressed in the plant, but neither was expressed in pollen. A phylogenetic analysis was presented that showed evidence of several ADF/cofilin sub-groups within green plants.
4. Bowman GD, Nodelman IM, Hong Y, Chua N-H, Linberg U, Schutt CE: **A comparative structural analysis of the ADF/Cofilin family.** *Proteins* 2000, **41:**374-384.
   A review of the available ADF/cofilin structures and the determination the crystal structure of ADF1 from *A. thaliana* (the first structure from a plant ADF/cofilin).
5. McGough A, Pope B, Weeds A: **The ADF/Cofilin family: accelerators of actin reorganization.** *Results Probl Cell Differ* 2001, **32:**135-154.
   A review of the ADF/cofilin family function with a phylogenetic analysis of some of the members.
6. Kim S-R, Kim Y, An G: **Molecular cloning and characterization of anther-preferential cDNA encoding a putative actin-depolymerizing factor.** *Plant Mol Biol* 1993, **21:**39-45.
   The first identification of ADF/cofilin genes in a plant.
7. Lopez I, Anthony RG, Maciver SK, Jiang C-J, Khan S, Weeds AG, Hussey PJ: **Pollen specific expression of maize genes encoding actin depolymerizing factor-like proteins.** *Proc Natl Acad Sci USA* 1996, **93:**7415-7420.
   Demonstration that plant ADF/cofilin-like genes encoded proteins that behaved like ADF/cofilins. *Zea mays* has at least three ADF/cofilin genes some of which (*ZmADF1* and *2*) are expressed only in pollen.
8. Ono S, Minami N, Abe H, Obinata T: **Characterization of a novel cofilin isoform that is predominantly expressed in mammalian skeletal-muscle.** *J Biol Chem* 1994, **269:**15280-15286.
   Description of a muscle-specific cofilin.
9. Ansari-Lari MA, ShenY, Muzny DM, Lee W, Gibbs RA: **Large-scale sequencing in human chromosome 12p13: experimental and computational gene structure determination.** *Genome Res* 1997, **7:**268-280.
   This regions contains an ADF/cofilin gene, *destrin-2*, which is probably a pseudogene.
10. Ono S, Benian GM: **Two *Caenorhabditis elegans* actin depolymerizing factor/cofilin proteins, encoded by the *unc-60* gene, differentially regulate actin filament dynamics.** *J Biol Chem* 1998, **273:**3778-3783.
    The two *C. elegans* ADF/cofilins differ in their ability to depolymerize actin filaments: UNC-A depolymerizes filaments and inhibits polymerization, and UNC-B binds stably to F-actin without depolymerizing it.
11. Gunsalus KC, Bonaccors S, William E, Vern F, Gatt M, Goldber ML: **Mutations in *twinstar*, a *Drosophila* gene encoding a cofilin/ADF homologue, result in defects in centrosome migration and cytokinesis.** *J Cell Biol* 1995, **131:**1243-1259.
    This study was amongst the first to show that ADF/cofilin function is necessary for cytokinesis. This and [82] were also the first identification of an ADF/cofilin in insects.
12. Mabuchi I: **Purification from starfish eggs of a protein that depolymerizes actin.** *J Biochem* 1981, **89:**1341-1344.
    The first description of depactin.
13. Takagi T, Konishi K, Mabuchi I: **Amino acid sequence of starfish oocyte depactin.** *J Biol Chem* 1988, **263:**3097-3102.
    The first available sequence of an ADF/cofilin, which it turned out to be one of the more divergent members of the family. The sequence was determined solely by direct amino-acid sequencing.
14. Danyluk J, Carpentier E, Sarhan F: **Identification and characterization of a low-temperature regulated gene encoding an actin-binding protein from wheat.** *FEBS Lett* 1996, **389:**324-327.
    Cloning of a wheat ADF/cofilin and a study of the expression of the gene during cold acclimatization.
15. Dong C-H, Kost B, Xia G, Chua N-H: **Molecular identification and characterization of the *Arabidopsis* AtADF1, AtADF5 and AtADF6 genes.** *Plant Mol Biol* 2001, **45:**517-527.
    Transgenic *Arabidopsis* plants expressing sense or antisense cDNA encoding ADF1 were used to modulate the concentration of the protein *in vivo*. Overexpressing lines produced thick actin cables (like *Dictyostelium* [38]); reduced ADF1 expression also produced thick actin bundles by a different mechanism. Both resulted in malformation of the resulting plants.
16. Quirk S, Maciver SK, Ampe C, Doberstein SK, Kaiser DA, Van-Damme J, Vandekerckhove JS, Pollard TD: **Primary structure of and studies on *Acanthamoeba* actophorin.** *Biochemistry* 1993, **32:**8525-8533.
    Cloning and sequencing of actophorin, interaction with PIP_2, and immunolocalization to the leading edge of moving amoebae.
17. Allen ML, Dobrowolski JM, Muller H, Sibley LD, Mansour TE: **Cloning and characterization of actin depolymerizing factor from *Toxoplasma gondii*.** *Mol Biochem Parasitol* 1997, **88:**43-52.
    Cloning of a very small ADF/cofilin (118 amino acids) from the parasite *Toxoplasma gondii*. The recombinant protein was shown to bind to F-actin and there was an indication of pH sensitivity of the interaction.
18. **Searching GenBank** [http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html]
    One of the main sequence databases.
19. Thirion C, Stucka R, Mendel B, Gruhler A, Jaksch M, Nowak KJ, Binz N, Laing NG, Lochmuller H: **Characterization of human muscle type cofilin (CFL2) in normal and regenerating muscle.** *Eur J Biochem* 2001, **268:**3473-3482.
    Cloning of a human muscle-specific cofilin cDNA and the characterization of the gene on chromosome 14. It is concluded that this cofilin has a role in regenerating muscle cells.

20. Iida K, Moriyama K, Matsumoto S, Kawasaki H, Nishida E, Yahara I: **Isolation of a yeast essential gene, COF1, that encodes a homologue of mammalian cofilin, a low-M_r actin-binding and depolymerizing protein.** *Gene* 1993, **124:**115-120.
Reports the cloning of an ADF/cofilin gene from *S. cerevisiae* and that the recombinant protein binds F-actin in a pH-sensitive manner. The gene was also cloned independently [70].
21. Lee MGS, Lewis SA, Wilde CD, Cowan NJ: **Evolutionary history of a multigene family: An expressed human β-tubulin gene and three processed pseudogenes.** *Cell* 1983, **33:**477-487.
This paper demonstrates that pseudogenes tend to lack introns.
22. Aizawa H, Sutoh K, Tsubuki S, Kawashima S, Ishii A, Yahara I: **Identification, characterization, and intracellular distribution of cofilin in *Dictyostelium discoideum*.** *J Biol Chem* 1995, **270:**10923-10932.
The purification of *Dictyostelium* cofilin, its cloning and its immunolocalization to regions of rapid actin turnover such as the ruffling membrane at the leading edge of the cell. Like other ADF/cofilins, *Dictyostelium* cofilin was discovered to enter the nucleus with actin and to form rods upon addition of DMSO.
23. Peitsch WK, Grund C, Kuhn C, Schnölzer M, Spring H, Schmelz M, Franke WW: **Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells.** *Eur J Cell Biol* 1999, **78:**767-778.
A review of the actin-binding protein family the Drebrins, which contain a region with homology to the ADF/cofilin family.
24. Lappalainen P, Kessels MM, Cope MJTV, Drubin DG: **The ADF homolog (ADF-H) domain: a highly exploited actin-binding module.** *Mol Biol Cell* 1998, **9:**1951-1959.
A review of the various proteins, most of which bind actin, that share the ADF homology domain: the ADF/cofilins themselves, coactosin [25], twinfilin, drebrins and others.
25. de Hostas EL, Bradtke B, Lottspeich F, Gerisch G: **Coactosin, a 17 kDa F-actin binding protein from *Dictyostelium discoideum*.** *Cell Motil Cytoskel* 1993, **26:**181-191.
A description of a protein with sequence homology to the ADF/cofilins but that binds actin in a different manner.
26. Jiang C-J, Weeds AG, Hussey PJ: **The maize actin-depolymerizing factor, ZmADF3, redistributes to the growing tip of elongating root hairs and can be induced to translocate into the nucleus with actin.** *Plant J* 1997, **12:**1035-1043.
This was the first study to show that a plant ADF/cofilin enters the nucleus and forms actin-ADF/cofilin-rich bundles of filaments upon stress. It also shows that plant ADF/cofilins redistribute to the tips of growing root-hair cells.
27. Gungabissoon RA, Jiang C-J, Drøbak B-K, Maciver SK, Hussey PJ: **Interaction of maize actin-depolymerising factor with actin and phosphoinositides and its inhibition of plant phospholipase C.** *Plant J* 1998, **16:**689-696.
Interaction between ZmADF3, an ADF/cofilin expressed in most tissues of *Zea mays* (except pollen), and actin was stronger in the presence of ADP than ATP, in common with other ADF/cofilins [34,36]. ZmADF3 also increased actin dynamics and bound F-actin in a pH sensitive manner. The interaction of ZmADF3 with actin was inhibited by PIP_2, and PIP_2 hydrolysis by plant phosphoinositide phospholipase C was inhibited by ZmADF3.
28. Yonezawa N, Nishida E, Iida K, Yahara I, Sakai H: **Inhibition of the interactions of cofilin, destrin, and deoxyribonuclease-I with actin by phosphoinositides.** *J Biol Chem* 1990, **265:**8382-8386.
The first report of an interaction between ADF/cofilins and PIP_2.
29. Yonezawa N, Homma Y, Yahara I, Sakai H, Nishida E: **A short sequence responsible for both phosphoinositide binding and actin binding activities of cofilin.** *J Biol Chem* 1991, **266:**17218-17221.
This paper provides an explanation of the observation that ADF/cofilins can bind PIP_2 or actin but not both, as the sites are overlapping.
30. Van Troys M, Dewitte D, Verschelde J-L, Goethals M, Vandekerkhove J, Ampe C: **The competitive interaction of actin and PIP_2 with actophorin is based on overlapping target sites: design of a gain-of-function mutant.** *Biochemistry* 2000, **39:**12181-12189.
This report pins down a region on the long helix in which a mutation increases PIP_2 binding but not actin binding, thus demonstrating that the two sites are probably very close but distinct.
31. Bernstein BW, Painter WB, Chen H, Minamide LS, Abe H, Bamburg JR: **Intracellular pH modulation of ADF/Cofilin proteins.** *Cell Motil Cytoskel* 2000, **47:**319-336.
Showed that ADF/cofilin pH sensitivity *in vitro* is reflected in the behavior of the ADF/cofilins in cells. ADF/cofilins were translocated to alkaline-induced ruffling membranes; surprisingly, cofilin was found to be less pH-sensitive than ADF in the cells.
32. Bamburg JR, Bray D: **Distribution and cellular localization of actin depolymerizing factor.** *J Cell Biol* 1987, **105:**2817-2825.
A survey of ADF expression in mammalian tissues and the first localization of the protein in cells: it is enriched at the leading edge of fibroblasts and in the growth cones on neurons.
33. Yonezawa N, Nishida E, Koyasu S, Maekawa S, Ohta Y, Yahara I, Sakai H: **Distribution among tissues and intracellular localization of cofilin, a 21kDa actin-binding protein.** *Cell Struct Funct* 1987, **12:**443-452.
A similar study to [32] that also showed that ADF/cofilins were generally localized to dynamic actin structures in the cell.
34. Carlier MF, Laurent V, Santolini J, Melki R, Didry D, Xia G-X, Hong Y, Chua N-H, Pantaloni D: **Actin depolymerizing factor (ADF/Cofilin) enhances the rate of filament turnover: implication in actin-based motility.** *J Cell Biol* 1997, **136:**1307-1323.
ADF/cofilins increase the release rate of actin monomers from the pointed end of filaments.
35. Maciver SK: **How ADF/cofilin depolymerizes actin filaments.** *Curr Biol Cell Biol* 1998, **10:**140-144.
A hypothesis explaining how ADF/cofilins increase the actin monomer release rate at the pointed end of filaments and how they sever actin filaments; both processes are proposed to result from a common action.
36. Maciver SK, Weeds AG: **Actophorin preferentially binds monomeric ADP-actin over ATP-bound actin: consequences for cell locomotion.** *FEBS Lett* 1994, **347:**251-256.
This study was the first to demonstrate that ADF/cofilin binds to actin tighter if ADP rather than ATP is bound to the actin. This has important implications for which filaments are targeted for depolymerization and is the basis for the acceleration of actin treadmilling by ADF/cofilins at the leading edge of cells.
37. Chen J, Godt D, Gunsalus K, Kiss I, Goldberg M, Laski FA: **Cofilin/ADF is required for cell motility during *Drosophila* ovary development and oogenesis.** *Nature Cell Biol* 2001, **3:**204-209.
An involvement of ADF/cofilin in the *in vivo* locomotion of cells was established in this study.
38. Aizawa H, Sutoh K, Yahara I: **Overexpression of cofilin stimulates bundling of actin filaments, membrane ruffling and cell movement in *Dictyostelium*.** *J Cell Biol* 1996, **132:**335-344.
Dictyostelium amoebae over-expressing cofilin move faster and have actin bundles in their cytoplasm; the analysis was complicated by the fact that these cells were larger and expressed increased amounts of actin.
39. Nagaoka R, Abe H, Kusano K, Obinata T: **Concentration of cofilin, a small actin-binding protein, at the cleavage furrow during cytokinesis.** *Cell Motil Cytoskel* 1995, **30:**1-7.
In agreement with [11], this study indicates an involvement of ADF/cofilin with filament dynamics at the cleavage furrow in rodents; it was found to accumulate there in a number of different cell types and to persist until midbody formation. Immunofluorescence studies were backed up by introduction of fluorescently labeled cofilin to living cells.
40. Nishida E, Iida K, Yonezawa N, Koyasu S, Yahara I, Sakai H: **Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells.** *Proc Natl Acad Sci USA* 1987, **84:**5262-5266.
The first report of the presence of ADF/cofilin in the nucleus of stressed cells in association with actin. These bundles of filaments do not stain with phalloidin.
41. Hayden SM, Miller PS, Brauweiler A, Bamburg JR: **Analysis of the interactions of actin depolymerizing factor (ADF) with G- and F-actin.** *Biochemistry* 1993, **32:**9994-10004.
Chick ADF was discovered to protect actin against denaturation and to bind it in a pH-sensitive manner, in good agreement with [47].
42. Smertenko AP, Allwood EG, Khan S, Jiang C-J, Maciver SK, Weeds AG, Hussey PJ: **Interaction of pollen-specific actin-depolymerizing factor with actin.** *Plant J* 2001, **25:**203-212.
This paper reports the existence of ADF/cofilin-decorated F-actin in cells that had not been stressed. ADF/cofilin was present in F-actin-containing rodlets in pollen during dormancy. ADF/cofilin was localized generally within the growing pollen tube, concentrated in regions where the pollen tube adhered to the substrate.

43. Moriyama K, Nishida E, Yonezawa N, Sakai H, Matsumoto S, Iida K, Yahara I: **Destrin, a mammalian actin-depolymerizing protein, is closely related to cofilin.** *J Biol Chem* 1990, **265:**5768-5773.
The first sequence of vertebrate (pig) destrin (ADF), which is 71% identical to pig cofilin.
44. Minamide LS, Streigl AM, Boyle JA, Meberg PJ, Bamburg JR: **Neurodegenerative stimuli induced persistent ADF/cofilin-actin rods that disrupt distal neurite function.** *Nat Cell Biol* 2000, **2:**628-636.
This paper and [45] indicate the involvement of ADF/cofilins in human neuronal pathology. This paper suggests that bundles of actin and cofilin block communication in the processes of neurons, leading to neurodegeneration.
45. Maciver SK, Harrington CR: **Two actin-binding proteins, actin depolymerizing factor and cofilin, are associated with Hirano bodies.** *Neuroreport* 1995, **6:**1985-1988.
Hirano bodies are actin-rich deposits found in the cytoplasm of neurons in a number of pathological situations, especially Alzheimer's disease. These deposits were thought likely to be a result of collateral damage to the cell and not to be a cause of the neurodegeneration itself, but the results of [44] indicate that such deposits may be the cause rather than merely the effect of neuronal degeneration.
46. Yonezawa N, Nishida E, Sakai H: **pH control of actin polymerization by cofilin.** *J Biol Chem* 1985, **260:**14410-14412.
The original description of the marked effect of pH plays on the interaction of typical ADF/cofilins and actin filaments. The relative concentration of monomeric actin was found to increase as pH was increased, and the effect was fully reversible by lowering the pH.
47. Hawkins M, Pope B, Maciver SK, Weeds AG: **Human actin depolymerizing factor mediates a pH-sensitive destruction of actin filaments.** *Biochemistry* 1993, **32:**9985-9993.
A further study of the effect of pH showing full 1:1 binding of ADF/cofilin to filaments at low pH and binding of monomeric-actin only at pH 8.0.
48. Morgan TE, Lockerbie RO, Minamide LS, Browning MD, Bamburg JR: **Isolation and characterization of a regulated form of actin depolymerizing factor.** *J Cell Biol* 1993, **122:**623-633.
The first report of an ADF/cofilin being regulated by phosphorylation.
49. Agnew BJ, Minamide LS, Bamburg JR: **Reactivation of phosphorylated actin depolymerizing factor and identification of the regulatory site.** *J Biol Chem* 1995, **270:**17582-17587.
The site of phosphorylation was determined to be serine-3 in vertebrate ADF. It was also shown that substitution of serine-3 with glutamine-3 mimicked the action of phospho-ADF.
50. Smertenko AP, Jiang C-J, Simmons NJ, Weeds AG, Davies DR, Hussey PJ: **Ser6 in the maize actin-depolymerizing factor, ZmADF3, is phosphorylated by a calcium-stimulated protein kinase and is essential for the control of functional activity.** *Plant J* 1998, **14:**187-193.
Zea mays ADF3 (ZmADF3) was found to be phosphorylated on serine 6, which is equivalent to vertebrate ADF/cofilins serine-3.
51. Allwood EG, Smertenko AP, Hussey PJ: **Phosphorylation of plant actin-depolymerising factor by calmodulin-like domain protein kinase.** *FEBS Lett* 2001, **499:**97-100.
In addition to the LIM and TESK kinases known to phosphorylate ADF/cofilins in mammalian cells, this reports suggests that plants have an alternative pathway for regulating ADF/cofilins. The ADF/cofilin was found to be phosphorylated by calmodulin-like domain protein kinase (CDPK), a kinase unique to plants and some protists.
52. Bernstein BW, Bamburg JR: **Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin depolymerizing factor (ADF).** *Cell Motil* 1982, **2:**1-8.
The first report to suggest that the activity of the ADF/cofilins can be modulated by other actin-binding proteins. It is likely that the physical stability of the filament that tropomyosin produces might inhibit the severing action of ADF/cofilins.
53. Mabuchi I: **Effects of muscle proteins on the interface between actin and actin-depolymerizing protein from starfish oocytes.** *J Biochem* 1982, **92:**1439-1447.
Competition for F-actin binding between echinoderm depactin and myosin was demonstrated. The presence of depactin inhibited the myosin-actin ATPase rate.
54. Iida K, Yahara I: **Cooperation of two actin-binding proteins, cofilin and Aip1, in *Saccharomyces cerevisiae*.** *Genes to Cells* 1999, **4:**21-32.
Yeast cofilin was discovered to interact genetically with AIP1, another actin-binding protein, to remodel the actin cytoskeleton in yeast.
55. McGough A, Pope B, Chiu W, Weeds A: **Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function.** *J Cell Biol* 1997, **138:**771-781.
Actin filaments are helical structures and the ADF/cofilins increase the helicity of the filament by increasing the twist. This may explain the extreme co-cooperativity observed in F-actin binding [47].
56. Ono S: **Purification and biochemical characterization of actin from *Caenorhabditis elegans*: its difference from rabbit muscle actin in the interaction with nematode ADF/cofilin.** *Cell Motil Cytoskel* 1999, **48:**128-136.
A salutary lesson that despite actin's notorious conservation, the standard rabbit muscle actin does not always behave like other actins with respect to the binding of ADF/cofilins. This study also reports the different depolymerizing activities of the two *C. elegans* ADF/cofilins.
57. Ono S, McGough A, Pope BJ, Tolbert VT, Bui A, Pohl J, Benian GM, Gernert KM, Weeds AG. **The C-terminal tail of UNC-60B (Actin Depolmerizing Factor/Cofilin) is critical for maintaining its stable association with F-actin and is implicated in the second actin-binding site.** *J Biol Chem* 2001, **276:**5952-5958.
Two actin-binding sites are proposed for F-actin binding of the ADF/cofilins, in agreement with [62]. It has been established that the primary actin-binding site in both G-and F-actin is around the long helix [49], and this work suggests that the second is based around the carboxyl terminus.
58. Toshima J, Toshima JY, Amano T, Yang N, Narumiya S, Mizuno K: **Cofilin phosphorylation by protein kinase TESK1 and its role in integrin-mediated actin reorganization and focal adhesion formation.** *Mol Biol Cell* 2001, **12:**1131-1145.
The TESK1 kinase can phosphorylate human non-muscle cofilin at Ser3. Unlike LIMK, however, TESK1 was not stimulated by ROCK or PAK, showing that this is yet another pathway by which ADF/cofilins can be regulated in cells.
59. Toshima J, Toshima JY, Takeuchi K, Mori R, Mizuno K: **Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular Sertoli cells.** *J Biol Chem* 2001, **276:**31449-31458.
TESK2 is thought to have a distinct role in ADF/cofilin phosphorylation; TESK1 is cytoplasmic but TESK2 is mainly nuclear.
60. Maciver SK, Pope BJ, Whytock S, Weeds AG: **The effect of two ADF/cofilins on actin filament turnover: pH sensitivity of F-actin by human ADF, but not of *Acanthamoeba* actophorin.** *Eur J Biochem* 1998, **256:**388-397.
This study shows that not all the ADF/cofilins bind actin in the same manner: actophorin is not pH regulated but behaves at all pHs as ADF and cofilin do at pH 6.5. This study also shows that ADF/cofilins increase the rate at which monomers leave the pointed end, in agreement with [34].
61. Mabuchi I: **An actin-depolymerizing protein (depactin) from starfish oocytes: properties and interaction with actin.** *J Cell Biol* 1983, **97:**1612-1621.
The biochemical isolation of an ADF/cofilin from starfish (*Asterias amurensis*) oocytes. Like other ADF/cofilins, it accelerates the late stages of polymerization and severs actin filaments.
62. Renoult C, Ternent D, Maciver SK, Fattoum A, Astier C, Benyamin Y, Roustan C: **The identification of a second cofilin binding site on actin suggests a novel, intercalated arrangement of F-actin binding.** *J Biol Chem* 1999, **274:**28893-28899.
This paper proposes a new model of ADF/cofilin-F-actin interaction in which the ADF/cofilin lies between subdomain 1 of one actin monomer in the filament and slides 'behind' the longitudinally associated monomer immediately towards the barbed end of the filament, binding a helix at the upper rear surface on subdomain 1.
63. McGough A, Chiu W: **ADF/cofilin weakens lateral contacts in the actin filament.** *J Mol Biol* 1999, **291:**513-519.
An electron microscopy study that shows the 'unwinding' by ADF/cofilin of the actin filament by loosening of the weak diagonal or lateral bonds between the actin molecules across the centre of the filament.
64. Pope BJ, Gonsior SM, Yeoh S, McGough A, Weeds AG: **Uncoupling actin filament fragmentation by cofilin from increased subunit turnover.** *J Mol Biol* 2000, **298:**649-661.
This paper reports that a mutation of human non-muscle cofilin (S3D) increases the filament twist and severs filaments but does not increase the off rate.
65. Galkin V E, Orlova A, Lukoyanova N, Wriggers W, Egelman EH: **Actin depolymerization factor stabilizes an existing state of F-actin and can change the tilt of F-actin subunits.** *J Cell Biol* 2001, **153:**75-86.
A possible explanation of how ADF/cofilin alters the apparent twist in the actin filament [55] by merely stabilizing the extreme twist that

spontaneously occurs in the filament without ADF/cofilins. A more controversial claim of this paper is that a second ADF/cofilin binds the filament at another site, but this may be an artifact resulting from disulfide-bridge formation.

66. **The TIGR *Arabidopsis thaliana* database** [http://www.tigr.org/tdb/e2k1/ath1/]
A collection of information on *Arabidopsis* genome research at the Institute for Genomic Research.
67. **The *C. elegans* genome project** [http://www.sanger.ac.uk/Projects/C_elegans/]
This site includes a BLAST server for searching genomic, cDNA and protein sequences.
68. **Ensembl human genome server** [http://www.ensembl.org/Homo_sapiens/]
Access to the human genome sequence and gene predictions.
69. Gillett GT, Fox MF, Rowe P, Casimir CM, Povey S: **Mapping of human nonmuscle type cofilin (cfl1) to chromosome-11q13 and muscle-type cofilin (cfl2) to chromosome-14.** *Annals Human Genet* 1996, **60:**201-211.
Discovery of the chromosomal location of the two cofilin genes in humans.
70. Moon AL, Janmey PA, Louie KA, Drubin DG: **Cofilin is an essential component of the yeast cortical cytoskeleton.** *J Cell Biol* 1993, **120:**421-435.
The isolation of cofilin from *Saccharomyces cerevisiae,* characterization of its actin binding and the cloning of the yeast cofilin gene. Yeast cofilin localizes to actin patches and bundles and is an essential gene. The gene was simultaneously cloned by others [20].
71. **The *Schizosaccharomyces pombe* genome sequencing project** [http://www.sanger.ac.uk/Projects/S_pombe/]
Access to the genome sequence and other information about *S. pombe*.
72. Hatanaka H, Ogura K, Moriyama M, Ichikawa S, Yahara I, Inagaki F: **Tertiary structure of destrin and structural similarity between two actin-regulating protein families.** *Cell* 1996, **85:**1047-1055.
The first report of an ADF/cofilin structure, accomplished with NMR. The main surprise was that it had structural homology but not sequence homology to the gelsolin fold.
73. Leonard S, Gittis A, Petrulla E, Pollard T, Lattman E: **Crystal structure of the actin-binding protein actophorin from A*canthamoeba*.** *Nat Struct Biol* 1997, **4:**369-373.
The crystal structure of *Acanthamoeba* actophorin was found to be very like that of other ADF/cofilins.
74. Blanchoin L, Robinson RC, Choe S, Pollard TD: **Phosphorylation of *Acanthamoeba* actophorin (ADF/cofilin) blocks interaction with actin without a change in atomic structure.** *J Mol Biol* 2000, **295:**203-211.
The structure of phosphorylated actophorin was found to be essentially identical to the unphosphorylated form. Phosphorylation was found to remove actophorin's actin-binding capacity.
75. Federov A, Lappalainen P, Federov E, Drubin D, Almo S: **Structure determination of yeast cofilin.** *Nat Struct Biol* 1997, **4:**366-369.
The solution of the structure of yeast cofilin by crystallography to 2.3 angstroms.
76. **The ADF/Cofilin homepage** [http://www.bms.ed.ac.uk/research/smaciver/Cofilin.htm]
A continuously updated listing of information on ADF/cofilins, including a bibliography.
77. **European Bioinformatics Institute: SwissProt** [http://www.ebi.ac.uk/swissprot/]
One of the most comprehensive protein sequence databases.
78. **Protein Information Resource** [http://pir.georgetown.edu/]
A non-redundant, expertly annotated protein sequence database.
79. **Sea urchin genome project** [http://sugp.caltech.edu/]
A collection of genomic and cDNA sequence and mapping data for the sea urchin *S. purpuratus.*
80. Abe H, Obinata T, Minamide L, Bamburg J: ***Xenopus laevis* actin-depolymerizing factor/cofilin: a phosphorylation-regulated protein essential for development.** *J Cell Biol* 1996, **132:** 871-885.
The cloning of two *Xenopus* ADF/cofilins, their pH-dependent interaction with F-actin and their expression. Inhibition of their function with antibodies inhibited the progression of the cleavage furrow. ADF/cofilin phosphorylation changed dramatically with development.
81. Abe H, Endo T, Yamamoto K, Obinata T: **Sequence of cDNAs encoding actin depolymerizing factor and cofilin of embryonic chicken skeletal muscle: two functionally distinct actin-regulatory proteins exhibit high structural homology.** *Biochemistry* 1990, **29:**7420-7425.
The cloning of ADF and cofilin from chicken and their comparison to pig cofilin.
82. Edwards KA, Montague RA, Shepard S, Edgar BA, Erikson RL, Kiehart DP: **Identification of *Drosophila* cytoskeletal proteins by induction of abnormal cell shape in fission yeast.** *Proc Natl Acad Sci USA* 1994, **91:**4589-4593.
Drosophila ADF/cofilin was found to be identical to Twinstar, a *Drosophila* ADF/cofilin reported at the same time [11].
83. **The TIGR *Entamoeba histolytica* genome project** [http://www.tigr.org/tdb/e2k1/eha1/]
A collection of information on Entamoeba genome research at TIGR.