Filamins: promiscuous organizers of the cytoskeleton

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Filamins are elongated homodimeric proteins that crosslink F-actin. Each monomer chain of filamin comprises an actin-binding domain, and a rod segment consisting of six (Dictystelium filamin) up to 24 (human filamin) highly homologous repeats of ~96 amino acid residues, which adopt an immunoglobulin-like fold. Two hinges in the rod segment, together with the reversible unfolding of single repeats, might be the structural basis for the intrinsic flexibility of the actin networks generated by filamins. There are numerous filamin-binding proteins that associate, in most cases, along the repeats of the rod repeats. This rather promiscuous behaviour renders filamin a versatile scaffold between the actin network and finely tuned molecular cascades from the membrane to the cytoskeleton.

Introduction
The cytoskeleton provides the foundation for the spatial organization of cells and their movement. Despite our inclination to consider the cell ‘skeleton’ as a rigid structure, the real cytoskeleton is dynamic, undergoing continual reorganization and modification. In a non-muscle cell, the actin cytoskeleton consists of a pool of globular monomeric actin (G-actin), which can reversibly polymerize into filamentous actin (F-actin) and thus markedly alter the viscosity, elasticity or mechanical resistance of the whole cell. Actin filaments are polymerized, depolymerized, crosslinked, bundled or fragmented with the help of specific proteins that either change the polymerization kinetics or stabilize the existing network by connecting single filaments.

The best-studied F-actin crosslinking proteins are spectrin, fimbrin, α-actinin and filamin; these proteins are characterized by a conserved actin-binding domain (ABD), which is frequently followed by an extended rod-like domain. They can form dimers or tetramers, enabling them to bundle or to crosslink filaments. Whereas spectrin, fimbrin and α-actinin are thought to form primarily parallel actin bundles, filamins can crosslink actin filaments to form orthogonal networks to bundles depending on their concentration [1]. In filamins, the rod segment consists of highly homologous repeats of ~96 amino acids that are characterized structurally by the presence of an immunoglobulin-like fold, in which seven β strands are arranged in an antiparallel way [2,3]. By contrast, the spectrin and α-actinin rod segments consist of α-helical regions [4].

In this review, we focus on recent progress in structural research on filamins and seek to link their physiological functions with this new information. Although filamins are found in many organisms, the best studied are the Dictyostelium discoideum filamin (ddFLN) and mammalian filamin. These two prototypical filamins have an ABD comprising two tandem calponin homology domains and a rod region built by six (in ddFLN) or 24 (in human filamin) repeats [2,5] (Figure 1). The last repeat of the rod is responsible for dimerization. In addition, human filamin has two unique long hinges positioned between repeats 15–16 (27 residues) and 23–24 (35 residues) that are postulated to be flexible [6]. The human filamin family consists of three members, filamins A, B and C, that share 70% sequence homology, except for the less homologous hinges. Recent studies show that filamins not only are mechanical linkers for actin filaments but also act as interaction partners for many proteins of great functional diversity ranging from signal transduction to transcription [7]. Furthermore, recent genetic studies have demonstrated the significance of filamins in several diseases affecting brain [7–9], bone and the cardiovascular system [10,11].

Mechanism of filamin dimerization
As mentioned, dimerization of filamin is mediated by the last rod repeat. Three structures of fragments of the rod region including the dimerization region have been solved (Figure 1b): two from ddFLN, which show an identical mode of dimerization; and one from human filamin [12–14]. In ddFLN the structure of the last repeat differs from the other repeats of the rod, whereas in human filamin the repeat responsible for dimerization is similar to the other repeats.

In ddFLN, repeat 6 lacks 12 residues at the N terminus and, up to the middle of the second β strand, shares no sequence homology with the other repeats. It also contains an additional strand at the C terminus of the repeat. Repeats 6 and 6′ in the two monomers form an antiparallel dimer by β-sheet extension. The most extensive interactions are between the first strand and the penultimate strand of repeats 6 and 6′. The dimer
interface is large, comprising ~4500Å (of which 69% is the primary dimerization contribution of the repeat and 22% is the contact surface of a buried linker between repeats 5 and 6). Repeats 6 and 6' form a large β-sandwich of six strands on one side and eight on the other (Figures 1, 2b). The N-terminal regions of both structural units embrace neighbouring repeats and are also deeply buried between their strand-interconnecting loops. Such organization of the linker brings the fifth repeat closer to the sixth repeat of the second molecule than to the sixth repeat of the same chain. This arrangement provides a high degree of rigidity between repeats 5 and 6.

The structures of the dimerization repeats of ddFLN and human filamin are similar: the root mean square deviation for the core parts is only 1.2 Å (Figure 2). This similarity refers particularly to the global arrangement of

Figure 1. Structural properties of filamins. (a) Architecture of human filamin and Dictyostelium filamin (ddFLN). Human filamin comprises an ABD and a rod region built by 24 repeats. The rod is divided by two 'hinge' linkers located between repeats 15 and 16, and repeats 23 and 24. Each repeat has an immunoglobulin-like fold. The last repeat is the homodimerization domain. ddFLN has a similar ABD and a rod of six repeats. (b) Structures of fragments of the ddFLN rod segment (repeats 4-6), human filamin dimerizing repeat 24 (the monomers that build the homodimer are shown in different colours) and human filamin repeat 17 (FLN17). The structure of repeat 17 (red) was solved in complex with the receptor GPIbX (blue), highlighting a common binding motif of filamin-associated proteins. (c) Ribbon plot of an ABD from α-actinin [19]. The ABD is composed of two calponin homology domains, CH1 and CH2. On the basis of sequence similarity, filamin is expected to have a similar domain at its N terminus.
dimerization; for example, the repeats in human filamin are arranged with an exact two-fold symmetry as they are in ddFLN. Furthermore, dimers of human filamin also form through the extension of a β sheet of each monomer to form an elongated β sandwich. However, the strands responsible for direct interaction differ in human filamin, and the internal organization of the strands also varies from that in ddFLN. Differences affering dimerization are found at the C and N termini of the repeats: the dimerizing strands of the repeat seem to be the third (C) and fourth (D) β strands in human filamin, and the first (A) and sixth (F) β strand in ddFLN. The lengths of the interacting strands are shorter in human filamin than in ddFLN. In addition, there is no buried linker at the N terminus of the repeat in human filamin. A long hinge region of 35 residues precedes repeat 24 in human filamin. The structure of this region is unknown and shows the greatest sequence divergence. The main function of this region is probably mechanical, enabling a high degree of flexibility and structural adaptability [14]. One study has indicated that this region might have a regulatory function for dimerization [15]. The interface of dimerization is much smaller in human filamin than in ddFLN: the buried surface area is 1109 Å², taking up 19% of the molecular surface. The crystallographic model is supported by mutagenesis studies, which exclude the possibility of artefacts and prove that the dimerization mechanism of the wild-type vertebrate filamin is altered and presumably weaker than that of ddFLN [14].

Overall, the comparison between ddFLN and human filamin reveals that the dimerization interfaces in filamins have changed during evolution, although the same mechanism of a double-sided β-sheet extension of immunoglobulin-like repeats has remained. The rigid and strong interaction in filamins of lower organisms has been replaced by more sophisticated, weaker, and more flexible dimerization interfaces in higher organisms. Future studies on longer constructs of filamins are expected to provide information on the spatial organization of their repeats and their rigidity.

**Inter-repeat organization**

The mechanical properties of the whole rod region are essential for both the F-actin crosslinking capabilities of filamin and the characteristics of the F-actin networks that they create. Except for the two hinge regions in human filamin, the linkers between the repeats are also short in vertebrate filamins: they comprise only a few residues and are rich in proline. Additional salt bridges have been identified between neighbouring repeats.

The two structures of the ddFLN multirepeat constructs show that the repeats of the rod remain in the same spatial position regardless of crystal packing and crystallization conditions. The structures maintain an exact two-fold symmetry. The root mean square deviation between the structures is only 1.3 Å; thus, the whole rod seems to be a long, extended, spring-like molecule with an inter-repeat tilt angle of ~115°. Such conformations support the proposition that in ddFLN the whole rod is an extended structure of limited flexibility. Of course, some flexibility must be provided to facilitate crosslinking of actin filaments and adaptation of the F-actin network generated. This particular feature might be realized by a long linker between the ABD and the rod [13] (Figure 3). It seems that the primary function of ddFLN in *Dictyostelium* is to reduce the degree of freedom of the bound actin filaments while facilitating the organization of an orthogonal and highly flexible filamentous network.

There are no structural data on the inter-repeat organization of vertebrate filamins; however, the rod segment of human filamin, which is almost five times longer than that of ddFLN, is likely to be organized in the same way. Remarkable sequence similarities support such a model; in addition, the two long hinge regions in human filamin have a larger amino acid diversity and their length would enable the whole rod to be more flexible and to crosslink actin filaments in many orientations. The hinges might also present potential proteolytic cleavage sites. A naturally occurring proteolytic fragment of human filamin (FLNa; repeats 16–24), generated by cleavage at the protease-cleavage site between repeats 15 and 16, translocates to the nucleus and acts as a nuclear transcription modulator [16]. However, a region before repeat 24, which is considered to be flexible [17], has been reported to regulate dimerization [15]. In addition to its mechanical function,
The actin-binding domain
Members of the α-actinin superfamily of actin crosslinking proteins share the same mechanism of interaction with F-actin: filamins, α-actinin, spectrin, nesprins, plectin, dystrophin and fimbrin use a similar motif of two calponin homology domains to construct their ABDs [18]. A typical ABD has ~250 residues and shares 20–60% of sequence identity with other ABDs in the family. Structures of the ABD of α-actinin show that the two subdomains form a fully α-helical globular domain [19] (Figure 1b). The subdomains seem to have either a closed or open conformation, depending on the contact area between them. These conformations present various possible subdomain organizations [20], and it is not known which one is present in the ABDs of filamins.

The interface between F-actin and α-actinin has been studied extensively with peptides and antibodies of known specificities. The primary binding site for F-actin in the ABD of α-actinin is located between residues 121 and 147. The interaction is mostly hydrophobic, but the susceptibility of the binding to ionic strength suggests that hydrophilic interactions are also involved. The amino acids of actin that participate in binding are located between residues 112–125 and 360–372 [21].

A recent report suggests that binding of the ABD of FLNs to actin is regulated by calmodulin, an abundant Ca^{2+}-binding protein. Binding is facilitated by Ca^{2+} and calmodulin together: in other words, neither Ca^{2+} ions nor calmodulin alone show regulatory properties. The calmodulin-binding region in the ABD has been mapped to a region between residues 50 and 96 in the first calponin homology domain. An interaction between calmodulin and the whole ABD is not observed, however, which might suggest that calmodulin uses a cryptic binding site in the ABD that is uncovered only after the ABD binds to the actin filament [22].

Unfolding of the ddFLN rod
Detailed studies using atomic force microscopy have been recently carried out to obtain more information on the mechanical properties of the rod repeats in ddFLN. These experiments indicate that individual repeats unfold before the dimer is broken. To break the dimer, a force of ~200 pN is necessary. Notably, the fourth repeat shows a pattern of unfolding that differs from that of all other repeats. It unfolds the most easily and seems to be the only repeat that has a stable folding intermediate. In the first stage of unfolding, ~40 residues are stripped from the molecule and the remaining 60 stay folded. The former 40 residues correspond to the first two β-strands [23]. The intermediate is a stable structure that can fold on itself. The two-stage folding of the fourth repeat is also the fastest process of folding observed for the rod region [24].

The biological significance of this feature is potentially interesting. Easy unfolding and fast refolding of repeat 4 would enable the whole rod to nearly double its length and then come back to its native state. It is also possible that the folding speed of an elongated molecule would be increased by the presence of the intermediate. The free-energy barriers between the unfolded state and the intermediate, and, in the next step, between the

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Figure 3. Model of the whole ddFLN molecule as a homodimer. The model is built on the basis of known parts of the structure. Actin-binding domains are green; rod regions are red and blue. The extended molecule can bind actin filaments over a distance of ~400 Å [13]. Reproduced, with permission, from Ref. [13].

the rod region of vertebrate filamins has been shown to be an important scaffold for binding many proteins involved in a broad range of cellular processes [6,7].
intermediate and a fully folded protein, are easier to break than the barrier for direct transition to the folded state. Another interesting feature of this unfolding mechanism is linked to proteins that can bind to the rod region in the region of repeat 4. Mechanical stress might cause unfolding of the repeat together with dissociation of the binding partner or association of a new one to the intermediate. The bound or released interacting molecules might be part of a signalling pathway that could function as a cellular ‘sensor’ of mechanical forces present in the cytoskeleton.

Experiments investigating vertebrate filamin mechanics have been done on the whole filamin molecule and have shown that different repeats unfold under different force [25,26]. To unfold some of the strongest repeats requires a force more than three times greater than that needed to unfold the weakest ones. It has been also shown that repeats can refold spontaneously without stress. The authors of these studies [25,26] postulated that eventual unfolding of the filamin rod during cytoskeletal stresses is a method to protect the whole architecture from being mechanically separated. Formation of a new complex between filamin and its putative binding partner would be much slower and less likely to occur than a folding cycle. Under these circumstances, the spatial organization of cytoskeletal structures might be preserved under mechanical stress.

This hypothesis requires a thorough analysis of the binding affinities between the ABD of filamin and the actin filament. On the one hand, if the on- and off-rates are high, the rod repeats would never unfold. On the other hand, our knowledge about the binding characteristics at the filament – that is, the putative associated proteins in vivo that loosen or strengthen the interaction between filamin

### Table 1. Interaction partners of filamin

<table>
<thead>
<tr>
<th>Binding partner</th>
<th>Function of binding partner</th>
<th>Function of filamin in complex with partner</th>
<th>Binding site on filamin</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicystostelium filamin</td>
<td>FIP</td>
<td>Membrane to actin link, signal transduction</td>
<td>Repeats 2–4</td>
<td>[58]</td>
</tr>
<tr>
<td>Vertebrate filamins</td>
<td>FILIP</td>
<td>Downregulated by FILIP</td>
<td>Unknown</td>
<td>[33]</td>
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<tr>
<td></td>
<td>TRAF1, TRAF2</td>
<td>Anchoring and receptor internalization and recycling</td>
<td>Repeats 15–19</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>CaR extracellular Ca2+ receptor</td>
<td>Receptor to actin anchoring</td>
<td>Repeats 14–16</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>Furin</td>
<td>Sorting, compartmentalization and stabilization</td>
<td>Unknown</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td>FAP52</td>
<td>Nuclear scaffold (?)</td>
<td>Repeats 13–16, aa 571–866</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>FOXC1</td>
<td></td>
<td>aa 867–1154</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aa 1779–2284</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GP Ibα</td>
<td>Platelet adhesion receptor</td>
<td>Transport of GP Ibα from ER to cell surface</td>
<td>[37,51]</td>
</tr>
<tr>
<td></td>
<td>SHIP-2</td>
<td>Receptor to actin anchoring</td>
<td>Unknown</td>
<td>[60]</td>
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<tr>
<td></td>
<td>HCN1</td>
<td>Receptor to actin anchoring</td>
<td>Repeat 24</td>
<td>[38]</td>
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<tr>
<td></td>
<td>D2/D3 dopamine receptors</td>
<td>Receptor to actin anchoring</td>
<td>Repeat 19</td>
<td>[39]</td>
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<tr>
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<td>Glutamate receptor type 7</td>
<td>Receptor to actin anchoring</td>
<td>Repeat 21–22</td>
<td>[40]</td>
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<td></td>
<td>Calcitonin receptor</td>
<td>Anchoring and receptor internalization and recycling</td>
<td>Repeat 20–22</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Androgen receptor</td>
<td>Downregulates AR in nucleus</td>
<td>Repeats 16–24 after cleavage</td>
<td>[16,57]</td>
</tr>
<tr>
<td></td>
<td>SEK-1</td>
<td>Tumour necrosis factor-α activation</td>
<td>Repeats 21–23</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>BRCA-2</td>
<td>Promotes recovery from G2 arrest after DNA damage</td>
<td>Repeat 21–24 in nucleus</td>
<td>[55,56]</td>
</tr>
<tr>
<td></td>
<td>RaIA</td>
<td>Cytoskeleton regulation, filopodia formation</td>
<td>Repeat 24</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>Kir2.1</td>
<td>Receptor to actin anchoring</td>
<td>Repeats 23–24</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Smad</td>
<td>Anchoring and phosphorylation promotion</td>
<td>Repeats 20–23</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>Caveolin-1</td>
<td>Anchoring caveolae to cytoskeleton</td>
<td>Repeats 22–24</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Protein kinase Cz</td>
<td>Signal transduction</td>
<td>Scaffold for signalling pathway</td>
<td>Repeat 1–3; hinge 2 to repeat 24</td>
</tr>
<tr>
<td></td>
<td>Integrin</td>
<td>ECM receptor</td>
<td>Receptor to actin anchoring</td>
<td>Repeat 19–24</td>
</tr>
<tr>
<td></td>
<td>Pak1</td>
<td>Ruffle formation</td>
<td>Receptor to actin anchoring</td>
<td>Repeat 23</td>
</tr>
<tr>
<td></td>
<td>Migfilin</td>
<td>Cell adhesion structure to cytoskeleton binding</td>
<td>Repeat 21</td>
<td>[49,50]</td>
</tr>
<tr>
<td></td>
<td>PEBP2/CBF</td>
<td>Transcription factor</td>
<td>Retains PEBP2 in cytoplasm inhibiting its nuclear activity</td>
<td>Repeat 23–24</td>
</tr>
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*Abbreviations: aa, amino acid residues; AR, androgen receptor; CaR, Ca2+ -sensing receptor; ECM, extracellular matrix; ER, endoplasmic reticulum; TGF, transforming growth factor.

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and F-actin – is currently limited. We cannot exclude the idea that binding proteins divide filaments into different and distinct populations that either adapt dynamically to a permanently changing network of actin filaments or are well anchored in distinct subcellular regions and sense mechanical stress by repeated folding cycles.

Geometry and mechanics of F-actin crosslinking
Filamin is a potent gelation factor in an actin network; in fact, only one molecule of filamin per filament of actin is needed to induce gelation [27]. Actin gels that are crosslinked by filamin show a significant increase in elasticity and a nearly constant viscosity. Because these properties are similar to those of actin polymerized at high concentrations, it has been postulated that filamin functions as an ‘actin-saving’ protein; in other words, without filamin much more actin would have to be used to build such actin networks. Other actin crosslinking proteins, such as α-actinin, do not develop these properties [28].

Vertebrate filamins have been extensively studied by electron microscopy. Human filamin seems to have a V-shaped organization and is fundamentally more flexible, probably because of its two long hinges [29], than ddFLN. It seems that actin networks built by filamin have mostly a perpendicular organization of actin filaments and that filamin acts as a bracket, holding actin filaments perpendicular to each other. The T-, X- or L-shaped junctions created by human filamin have been shown to have myosin arrows pointing towards the branch points of actin filaments [29,30].

The intensive discussion in the past about the roles of the Arp2/3 complex and filamin during the generation of branched F-actin networks has cooled down considerably with the acknowledgement that the different structures of Arp2/3 and filamin consequently lead to different architectures of the filamentous meshwork. Arp2/3-induced nucleation along an existing actin filament forms strictly oriented daughter filaments, which start polymerization from the pointed ends, are capped very early and easily break at the branching point. By contrast, filamins are frequently found at the intersections of existing filaments, where they facilitate a flexible three-dimensional, orthogonal, fairly resistant meshwork that continuously adapts to morphological changes that are typical of slow migration [31].

The presence of the first hinge region between repeats 15 and 16 has been recently reported to be essential for maintaining the viscoelastic properties of actin networks [32]. The nonlinearity of a stress strain produced at large forces, which is characteristic of living cells, can be reproduced in the in vitro actin networks only when the crosslinking filamin possesses this region. When recombinant filamin lacking this hinge is used in such experiments, the networks show linear stiffening and break at much lower stresses [32].

Filamins are not picky about binding partners
In recent publications, filamins have revealed many new roles that are equally as important as their primary function of crosslinking F-actin. Vertebrate filamins have been found to interact with > 20 proteins; many of these interactions are unexpected (Table 1). Thus, filamins have an important role in signalling towards changes in cytoskeletal rearrangements [33,34] and have a scaffolding function. This latter function has a broad range of applications: first, it anchors membrane receptors to the actin cytoskeleton, thereby facilitating precise localization and transport of the receptors [35–43] and other proteins [44]; second, it acts as a colocalization factor for signalling pathways [45]; and third, it serves as a mechanical element in caveolae and membrane ruffle formation [46,47].

It has been proved that the expression level of filamin is essential for functioning of the Ca^{2+}-sensing receptor (CaR) signalling pathway because the SEK-1 protein downstream of the receptor is also a binding partner of filamin [48]. Thus, filamin facilitates the proper colocalization of signalling pathway proteins. Filamins also have an important role in interactions between cells and the extracellular matrix; there, together with extracellular matrix receptors, they provide an important interface between the cytoskeleton and the exoskeleton [43,49–52].

In addition, a few unusual functions of filamin have been identified. It seems that this protein might hold transcription factors in the cytoplasmic compartment, thereby preventing them from being active in the nucleus [53]. Surprisingly, filamin has been recently reported as a nuclear protein that binds to transcription factors. It has been postulated that an elongated filamin molecule might function as a kind of a nuclear ‘cytoskeleton’ for colocalization of nuclear functional complexes [54–56] participating in the nucleocytoplasmic transport [57]. The cleaved C-terminal part of human filamin, representing repeats 16–24, has been also found in the nucleus, where it downregulates the androgen receptor [16].

Two important structures of single rod repeats of human filamin A bound to cytoplasmic fragments of its

Figure 4. The interaction between human filamin repeat 21 and the cytoplasmic tail of integrin β7. The filamin repeat is shown as a surface representation; the bound peptide is shown in stick representation with carbon atoms coloured yellow, nitrogen atoms blue, and oxygen atoms red. Residues Pro776 to Pro788 of integrin form a β strand that expands the filament-in-sheet comprising strands C, F and G.
interacting receptors have been recently published: namely, repeat 17 of FLNa bound to a peptide of the platelet adhesion glycoprotein GPIbα [51], and repeat 21 bound to the cytoplasmic tail of integrin β7 [52]. In both of these structures, filamin uses the same structural region of the repeat domain for binding – that is, the face of the molecule that comprises β-strands C and D. Interestingly, this interface is also used in repeat 24 for dimerization. The peptides assume a fully extended conformation: their β strands are hydrogen-bonded to strand C and have several hydrophobic contacts to residues of both strands C and D of filamin (Figure 4). Although the sequence similarity is low between GPIbα and the β7 integrin cytoplasmic tail, the filamin-binding site in both proteins is flanked by proline residues. On the basis of these structural similarities, the C–D interface of filamin repeats has been proposed to be a general ligand-binding area, where specificity is determined by the different hydrogen-bonding capabilities and hydrophobic interactions of the binding proteins (Figure 4). Kiema et al. [52] have also reported that filamin cross-competes with talin for integrin. The fact that filamin has competitors for binding its ligands is important for understanding why filamin has so many interaction partners and how these interactions are regulated.

Remarkably, most of the filamin-interacting proteins bind to filamin between repeats 16 and 24. The mapped binding regions of different binding partners overlap but are seldom identical. This observation has led to the hypothesis that the primary function of repeats 16–24 is to bind to large proteins such as receptors, whereas that of repeats 1–15 is to bind to a few smaller proteins that participate in signalling processes. We can thus assume that the ABD is an anchor, repeats 1–15 form a chain, and the region of repeats 16–24 is a capstan. In light of the recently identified functions of filamins, it is clear that the primary function (F-actin crosslinking) of filamins has been complemented during evolution by additional tasks (Figure 5).
So far, only one binding partner other than actin has been identified for ddFLN. The filamin-interacting protein (FIP), a elongated molecule of 224 kDa, is important for the developmental cycle, formation of multicellular aggregates and phototoxic behaviour [58]. Its binding region includes rod repeat 4, which might support the hypothesis that unfolding of repeat 4 is important for ligand binding.

Concluding remarks
Filamins present a wonderful example of multifunctional proteins. Their primary and pralvs functions of crosslinking actin filaaments have been supplemented during evolution by various additional tasks. Filamin and actin cooperate in the creation of exceptional mechanical and dynamical properties of the cytoskeleton. Filamin also acts as a scaffold for membrane-receptor-associated signalling proteins. The medical relevance of filamins, especially in the field of developmental malfunctions, is indisputable [7].

At the current stage of our knowledge of filamin, it seems necessary to introduce a more holistic approach to this protein. Our information on the structural properties of filamins, and vertebrate filamins in particular, is still too limited to link many of their various biochemical functions to structure. The most logical way forward for structural investigation would seem to be to study filamin complexes with other proteins and to search for a ‘filamin-binding motif or domain’ in its interaction partners. The challenge is to study the structure of filamin in complex with its target proteins and to explain the discrepancy between its structural and mechanical properties. Much can be done in this field; however, other properties of filamins, such as regulation of actin binding and inter-domain orientation, must be also studied.

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