Structural Portrait of Filamin Interaction Mechanisms

Kristina Djinovic-Carugo[1,2,*] and Oliviero Carugo[1,3,*]

[1]*Department for Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria;* [2]*Department of Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia;* [3]*Department of General Chemistry, University of Pavia, Pavia, Italy*

Abstract: We review the most recent findings on human filamin structure, with particular emphasis on the relationships between structure, function, and interaction. Filamin is a cytoskeletal actin-binding protein and it is therefore crucial in providing cells with the necessary mechanical and dynamical properties. Filamentous actin cross-linking by filamin is regulated by a number of other proteins and the molecular mechanisms of this complex interaction network can be understood by highlighting the structural features of isolated filamin moieties and of their complexes with several partners. Here we describe first the structure-function relationships of the isolated filamin, its flexibility, and its dimerization mechanism. Secondly, we illustrate the structural mechanism with which filamin can recognize its partners, both the actin filaments and the regulatory proteins.

Keywords: Filamin, actin, actin-binding proteins, cystoskeleton; Z-disk.

1. FILAMIN AND CELLULAR ORGANIZATION

The cytoskeleton of eukaryotic cells is made of filamentous systems composed of polymers of actin, tubulin or intermediate filament proteins. Their filamentous nature provides the cell with networks of interactions that are very specific and at the same time highly dynamics. Cytoskeletal structures are involved in a number of important functions: maintenance of cell's internal scaffold, provision of mechanical stability, locomotion, intracellular transport of organelles, as well as chromosome separation in mitosis and meiosis.

In migrating cells the dynamic assembly/disassembly of actin network drives cell motility, while in a muscle a stable acto-myosin system composes the contractile apparatus (for review see [1, 2]. Actin-binding proteins, in particular their size, molecular architecture and flexibility, dictate the formation of actin bundles or networks.

Among the best characterized actin binding proteins are α-actinin, spectrin, and filamin. They are composed of an N-terminal actin binding domain (ABD) made of two consecutive calponin homology domains (CH), followed by a rod made of a variable number of different domains. They can form dimers (filamin, α-actinin) or tetramers (spectrin) allowing them to bundle or to crosslink filaments. The rod domain of spectrin and α-actinin is composed of spectrin-like repeats adopting three-helix bundles, while in filamin the rod domain is formed by immunoglubulin-like domains (Ig-like domains).

*Address correspondence to these authors at the Department of Structural and Computational Biology, Max F. Perutz Laboratories, Vienna University, Campus Vienna Biocenter 5, 1030 Vienna, Austria; Tel: +43 1 4277 52208; Fax: +43 1 4277 9522; E-mail: oliviero.carugo@univie.ac.at and Department of Structural and Computational Biology Max F. Perutz Laboratories, Vienna University, Campus Vienna Biocenter 5, 1030 Vienna, Austria;
Tel: +43 1 4277 52203; Fax: +43 1 4277 9522;
E-mail: kristina.djinovic@univie.ac.at

Two prototypical filamins have been studied very well: *Dictyostelium discoideum* filamin (ddFLN) and mammalian filamin. ddFLN has only six Ig-like domains while there are 24 in human filamin. In both molecules the very C-terminal domain is responsible for dimerisation, which in case of ddFLN results in a formation of an anti-parallel dimer, while the human filamin assembles in a V-shaped structure [3-8]. Furthermore, two flexible hinge regions have been identified between Ig-like domains 15 and 16 and 23 and 24, dissecting the molecule into rod 1 and rod 2 segments, respectively Fig. (**1**).

To date, three human filamin gene paralogues (*FLNA*, *FLNB* and *FLNC*) have been identified. Contrary to the rather ubiquitous distribution of filamins A and B, the C isoform is expressed mainly in the sarcomeric Z-disk region of striated muscular tissue [9].

Filamin A is the most prominent actin filaments cross-linking protein in non-muscle cells. It acts as a mechanical linker and confers elastic properties on actin networks subjected to prestress [10]. Expression of filamin A is essential for mammalian development [11-13]. Deletions or point mutations in filamin A lead to various congenital anomalies [14-16]. Cultured cells impaired of filamin A expression, on the other hand, have unstable surfaces, poor locomotion, and reduced mechanical resistance [17, 18]. Apart from binding to actin filaments, filamins A and B bind to a number of other partners, a current directory enumerates more than 20 interacting proteins (reviewed in [19]). Filamin acts as a scaffold protein which renders it an important player in a complex system in which signalling centred around filamin organises actin architectures that in turn regulates signalling [7] Filamin facilitates precise localization and transport of receptors by anchoring membrane imbedded receptors to actin cytoskeleton through interaction with tumor necrosis factor receptor associated factor, calcium-sensing receptor, glycoprotein Ibα, pacemaker channel HCN1, dopamine D2

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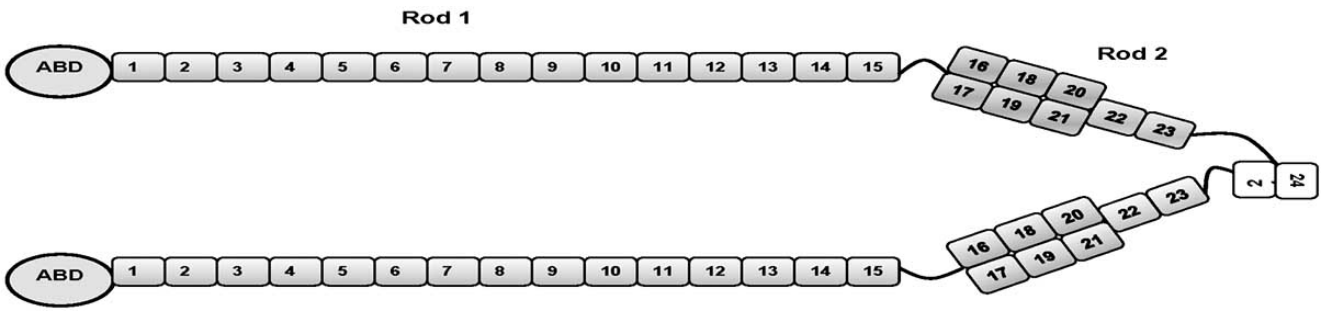

Fig. (1). Schematic view of human filamin (after [46]). The N-terminal actin binding region (ABD) that contains two CH domains is followed by a 24 Ig-like domains, the C-terminal of which is responsible for the dimerization. The pairs of Ig-like domains 16-17, 18-19, and 20-21 are shown as a compact region since they interpenetrate into each other.

and D3 receptors, glutamate receptor type 7, calcitonin receptor, potassium channel, Kir2.1 and integrin β7, to name a few [20-28]. It acts as a colocalization factor for signalling pathways by being a ligand and *in vivo* substrate for protein kinase Cα [29]. Furthermore it has a mechanical role in caveolae and membrane ruffle formation [30, 31]. Together with extracellular matrix receptors, filamins provide an important link between the cytoskeleton and the exoskeleton *via* interactions with migfilin, glycoprotein Iβ and integrins [27, 32-35].

Filamin C is largely restricted to skeletal muscle [36, 37]. It hosts a unique insertion of 81 amino acid residues at repeat 20, which is not present in the other filamins and is thought to be responsible for its recruitment into the myofibrillar Z-disks. The C-terminal portion of the rod 2 portion of the molecule is responsible for a number of specific protein-protein interactions, e.g. with myotilin [38] and FATZ/ calsarcin/myozenin [39], XIRP2, Ky-protein, myopodin, synaptopodin, tritopodin and FILIP [40, 41]. The fraction of filamin C that is localized at the sarcolemma binds to sarcoglycans at costameres [9] and to Xin at myotendinous junctions [40] and acts as scaffold for transmembrane receptors, signaling and adapter proteins. A recent study based on analysis of filamin C deficient mice indicated an important role for filamin C in muscle development and maintenance of muscle structural integrity [42]. Furthermore, mutations on the filamin C encoding gene have been implicated in myofibrillar myopathies leading to abnormal accumulation of an array of proteins at ectopic sites as well as accumulation of degraded myofibrillar proteins forming large aggregates and manifesting itself clinically in limb muscle weakness [43-45].

Interestingly, many filamin-interacting partners bind to the rod 2 segment encompassing Ig-like domains 16 - 24. The mapped binding regions of different binding partners overlap but are seldom identical [19].

2. STRUCTURAL SNAPSHOTS AND GLOBAL PICTURES

2.1 Experimental Data and Flexibility

Owing to its large dimension, full length filamin is not amenable for crystallization. Lower resolution structural information of full-length protein and of its segments has been generated by EM and SAXS studies [6, 7, 46]. Even if high resolution structures of full length isoforms were available, they would likely bring limited biological information. It is in fact reasonable to suppose that this long and modular protein is flexible, especially in the rod1 region where a series of globular domains follow each other and are separated by short and flexible linear linkers, and it is also reasonable to suppose that this flexibility is functionally important, allowing the molecule to adapt to different stereochemical arrangements.

However, several structural analyses on filamin domains or larger fragments were carried out (Table **1**). In the Protein Data Bank the structural information is available for each Ig-like domain and for the N-terminal actin binding domain [47], with the exception of the first eight Ig-like domains. Although most of the structural data are on the isoform B of filamin, four of the Ig-like domains were characterized structurally in all the three isoforms (R16, R17, R23, and R24). Interestingly, the data are quite recent – no more than five years old – and about half of them were produced by structural genomics consortia. Only one third of the structures were determined by X-ray crystallographic methods, while the rest was determined by NMR spectroscopy.

The structures covering the rod 1 portion of filamin are limited to single domains. Consequently they do not provide much information on the molecular architecture and give little basis for functional analysis. However, given that several of these structures have overlapping polypeptide sequences, it is possible to attempt to model *in silico* a larger section of filamin, by connecting in a sort of "lego" approach the individual domains or domain pairs. Moreover, since most of these structures were determined in solution by NMR spectroscopy and were deposited into the Protein Data Bank as ensembles of alternative models, this modeling strategy results in a series of alternative models.

After removing eventual artificial tags, the last five residues of a domain were superposed to the first five residues of the next domain (these five residues were the same in the filamin sequence). Given that a single domain is represented by twenty NMR determined structural models, this process produces four hundred models of the protein fragment

Table 1. List of the PDB Files that Contain Some Fragments of Human Filamin. Fragments of Filamin A are Shown in Italic; Those of Filamin B in Plain Text; and Those of Filamin C in Bold

PDB id	Fragment	Isoform	Technique	Resolution	Reference	Deposition Year
2di9	R9	B	NMR	-	(i)	2006
2dia	R10	B	NMR	-	(i)	2006
2dib	R11	B	NMR	-	(i)	2006
2dic	R12	B	NMR	-	(i)	2006
2dj4	R13	B	NMR	-	(i)	2006
2d7m	R14	C	NMR	-	(ii)	2005
2e9j	R14	C	NMR	-	(iii)	2007
2dmb	R15	B	NMR	-	(iv)	2006
2d7n	R16	C	NMR	-	(ii)	2005
2ee9	R16	B	NMR	-	(iii)	2007
2aav	*R17*	*A*	*NMR*	*-*	*(v)*	*2005*
2bp3	*R17 (a)*	*A*	*X-ray*	*2.32*	*(v)*	*2005*
2eea	R17	B	NMR	-	(iii)	2007
2dmc	R18	B	NMR	-	(i)	2006
2di8	R19	B	NMR	-	(vi)	2006
2e9i	R20	B	NMR	-	(iii)	2007
2dlg	R20	B	NMR	-	(i)	2006
2brq	*R21 (b)*	*A*	*X-ray*	*2.10*	*(vii)*	*2005*
2jf1	*R21 (c)*	*A*	*X-ray*	*2.20*	*(viii)*	*2007*
2w0p	*R21 (d)*	*A*	*X-ray*	*1.90*	*(ix)*	*2008*
2ee6	R21	B	NMR	-	(iii)	2007
2eeb	R22	B	NMR	-	(iii)	2007
2eec	R23	B	NMR	-	(iii)	2007
2eed	R24	B	NMR	-	(iii)	2007
2d7o	R17	C	NMR	-	(ii)	2006
2d7p	R22	C	NMR	-	(ii)	2005
2d7q	R23	C	NMR	-	(ii)	2005

(Table 1) contd….

PDB id	Fragment	Isoform	Technique	Resolution	Reference	Deposition Year
1v05	R24	C	X-ray	1.43	(x)	2004
2nqc	R23	C	X-ray	2.05	(xi)	2006
2j3s	*R19-R21*	*A*	*X-ray*	*2.50*	*(xii)*	*2006*
2k7p	*R16-17*	*A*	*NMR*	-	*(xiii)*	*2009*
2k7q	*R18-19*	*A*	*NMR*	-	*(xiii)*	*2009*
2wa5	CH1-CH2	B	X-ray	1.90	(xiv)	2009
2wa6	CH1-CH2 (e)	B	X-ray	1.95	(xiv)	2009
2wa7	CH1-CH2 (f)	B	X-ray	1.85	(xiv)	2009
2k3t	*R23*	*A*	*NMR*	-	*(xv)*	*2009*
3cnk	*R24*	*A*	*X-ray*	*1.65*	*(xvi)*	*2009*
3fer	CH1-CH2	B	X-ray	2.40	(xvii)	2009
2k9u	R21 (d)	C	NMR	-	(xviii)	2008
2d7o	R17	C	NMR	-	(ii)	2006
2aav	*R17*	*A*	*NMR*	-	*(v)*	

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(ii) Tomizawa, T.,Kigawa, T.,Koshiba, S.,Inoue, M.,Yokoyama, S. (2005). Authors of the PDB file (RIKEN Structural Genomics/Proteomics Initiative - RSGI)
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(xviii) Ithychanda, S.N., Qin, J. (2008) Authors of the PDB file
(a) complexed with the GPIB alpha cytoplasmic domain
(b) complexed with the integrin beta7 cytoplasmic tail peptide
(c) complexed with the integrin beta2 cytoplasmic tail peptide
(d) complexed with MIG FILIN peptide
(e) W148R mutant
(f) M202V mutant

formed by the two consecutive domains. Such a procedure was then extended to a third domain and following domains. At each step, models with severe inter-atomic clashes were removed and the remaining ones grouped into clusters, by using a hierarchical cluster analysis algorithm, adopting the nearest neighbour clustering criterion and the root-mean-square-distance between equivalent Cα atoms as a measure of proximity. By retaining only one representative model per cluster, the number of models to be considered was drastically lowered.

We in this way built 9 alternative models of a fragment of filamin containing the 705 residues encompassing Ig-like domains R9, R10, R11, R12, R13, R14, and R15 see Fig. (**2**). These structures are very different from each other. The distances between the N- and the C-termini vary from 130 Å, when the molecule assumes a bent shape (like a letter C), to 260 Å when the Ig-like domains are arranged in a linear manner. This clearly suggests that filamin is likely to be very flexible and can assume many different shapes. It is important to note that this modeling exercise is quite different from that reported by Kesner *et al.* [48], where the reciprocal orientation of pairs of Ig-like domains was kept fixed, according to that observed between two domains of *Dictyostelium discoideum* filamin [3]. However, given the structural plasticity and shape variability of filamin Ig-like domains 8-15 as observed by Nakamura *et al.* [7] it is reasonable to suppose that both modelling exercises capture only a part of the reality, which is the plastic filamin structure in the cytosol together with its binding partners.

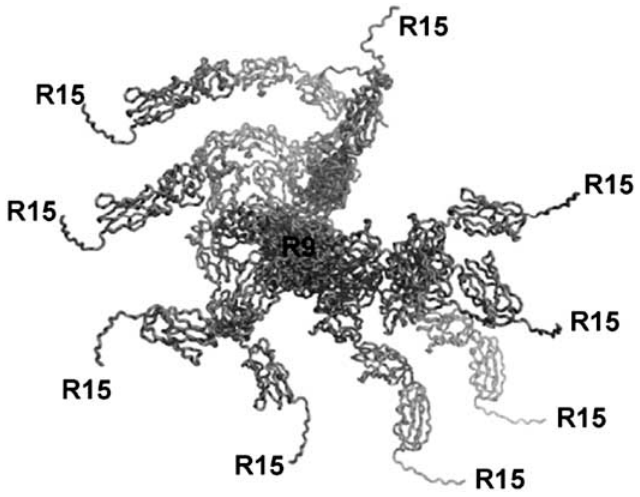

Fig. (2). Computational models of the filamin fragment containing the Ig-like domains R9-R15. The domain R9 is equally oriented in all the nine models. R9 and R15 are labeled.

Despite this modeling exercise, the results of which might be highly questionable, a body of experimental data exists on the inter-domain structure and dynamics. The most interesting results can be summarized into three points. First, the filamin is a V shaped dimer, formed through antiparallel dimerisation of Ig-like domains 24 [5-7, 47]. Second, information has been generated on the relative dynamics of the adjacent Ig-like domains (see section 2.3). Third, some insight has been reached on the structural features and the functional mechanisms of the recognition of filamin binding partners (see section 2.4). All these points will be discussed in the following chapters.

2.2. Dimerization Mechanism

Given that filamin subunit has only one actin binding site, it must dimerize (or oligomerize) in order to become a bidentate (or multidentate) ligand, able to cross-link actin filaments. Also other actin binding proteins are dimeric, like for example alpha-actinin, since their subunits contain only one actin binding domain. The dimerization of filamin is due to the C-terminal Ig-like domain 24. The dimerization mode is conserved between isoforms B and C, since it is possible to form *in vitro* a dimer between a domain 24 of filamin B and a domain 24 of filamin C (the isoform A is however unable to form these non-native hetero dimers) [49] though the *in vitro* dissociation constant, measured with analytical ultracentrifugation is in the microM range [5]. It has moreover been hypothesized that dimerization might be regulated in order to influence the molecular properties like in the case of platelet activation where the linkers between the Ig-like domains 15 and 16 and 23 and 24 are proteolyzed by calpain [49-51].

The dimerization mechanism seems to be the same in all the three isoforms of filamin. It implies a two-fold rotation axis, nearly perpendicular to the β sheets of each domain, close to the β-sheet edges formed by the strands C and D Fig. (**3**). As a consequence of a twofold axis rotation, two strands C and two strands D face each other in an antiparallel way. However, while the interaction of a strand C of one subunit with the strand C of the other subunit is due to hydrophobic interactions and to hydrogen bonds mediated by water molecules, the two strands D interact also *via* hydrogen bonds between backbone atoms, like in a typical interac-

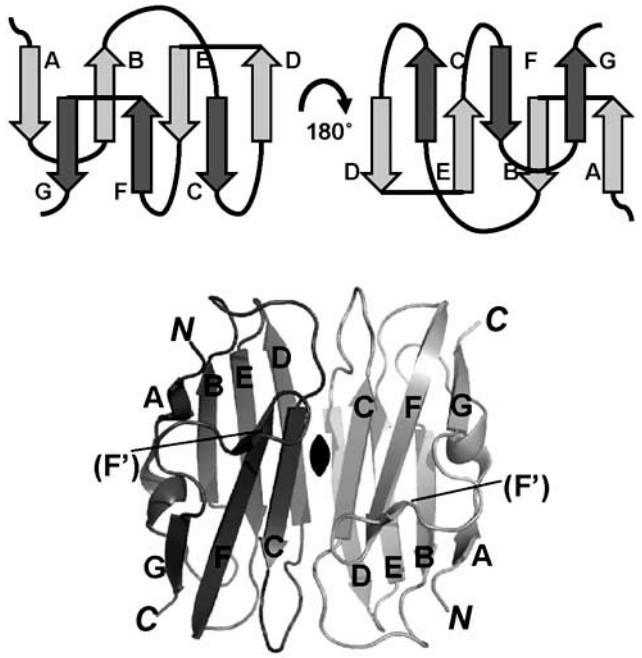

Fig. (3). Schematic view of the dimer formed by the C-terminal Ig-like domain of vertebrate filamin A. A two-fold axis in the middle of the intermolecular interface is perpendicular to the plot.

tion between two adjacent, anti-parallel β-strands belonging to the same β-sheet. It can thus be hypothesized that the nature and role of the interface between the two β-strands C is different from the role of the interface between the pairs of D β-strands.

Interestingly, this dimerization mechanism, which seems on the basis of amino acid sequence comparisons to be common to all vertebrates, is different in the gelation factor from *Dictyostelium discoideum* [3]. The difference can be attributed to the different topology of the Ig-like domains that are responsible for the formation of the homo-dimeric assembly [8].

2.3. C-Terminal Rod 2 Domains

Recent structural determinations of tandem Ig-like domain pairs R16-R17, R18-R19, R19-R20-R21 [46, 52] allow to examine the mutual influence of adjacent Ig-like domains. Surprisingly, in crystal structure of the triplet of Ig-like domains R19-R20-R21 from filamin A [52] it was observed that these domains interact with each other through an extensive interaction pattern. Namely, the first β-strand of domain 20 is, in the reality, part of domain 21 see Fig. (**4**). As a consequence, the three domains are quite different from each other. Domain 19 adopts the usual Ig-like fold, with two sandwiched, anti-parallel β-sheets, one containing three and the other constituted by four β-strands. Domain 20 looses one β-strand, despite its topology is still unclear since a fraction of its residues were not observed experimentally. On the contrary, domain 21 has an extra β-strand, given by domain 20 and is for the rest, similar to domain 19. Another surprising observation is that, although the three domains form a nearly cylindrical structure, the domains 19 and 20 are at the two ends of the cylinder, while domain 21 is in the middle. Moreover, while the β-strands of domains 19 and 21 are nearly parallel to the cylinder, those of domain 20 are nearly normal to the cylinder axis see Fig. (**4**).

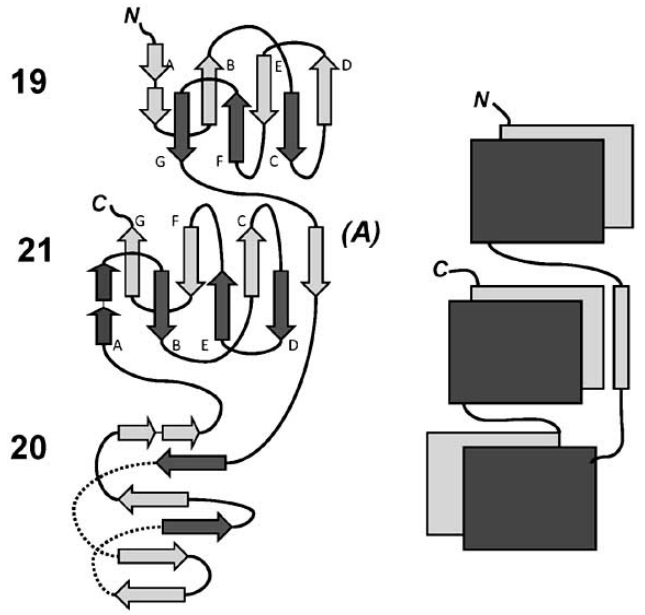

Fig. (4). Schematic view of the filamin A fragment containing the Ig-like domains 19, 20, and 21.

These results are so surprising and unexpected that one might suspect that they are unfortunate experimental artifact. There is however a series of supporting data that reinforce their validity. In particular, the first β-strand of domain 20, which extends one of the two β-sheets of domain 21, occupies the binding site of integrin suggesting that this conjugation between domains 20 and 21 might be a regulatory mechanism controlling the association of filamin with its ligands [52]. It was furthermore suggested that the autoinhibition of ligand binding to the Ig-like domains by the preceding, even-numbered domain, may be a general feature of filamin A regulation [52].

On the contrary the small angle X-rays scattering (SAXS) analysis of the construct containing Ig-like domains 23 and 24 of filamin C did not show any reciprocal influence or interaction of the two domains that are connected by a flexible hinge region [6]. Similarly, SAXS data on tandem Ig-like domains 12-13 and 22-23 of filamin A also suggested that the domains do not interact tightly with each other and form elongated structures [46]. On the contrary, both SAXS and NMR structures show that the tandem Ig-like domains 18 and 19 of filamin A adopt a shape closely similar to that observed in the construct containing domains 20 and 21 [46] where the first β-strand of domain 18 is actually found in domain 19, where it extends a pre-existing β-sheet by flanking the β-strand C of domain 19 in an anti-parallel way see Fig. (**5**). Consequently, the two domains assume two different folds. Another type of association was observed in the filamin A fragment that includes the Ig-like domains 16 and 17 [46]. Here the first β-strand of domain 16 is observed to be conformationally disordered and it is not inserted into the following domain see Fig. (**5**). The inter-domain association is due to hydrophobic interactions, partially made possible by the unfolding of the first β-strand of domain 16, which exposes to the solvent part of its hydrophobic. Interestingly, the interaction between domain 17 and GPIb-α [34] is not influenced by domain 16 as the domain-domain interaction takes place at the opposite side of GPIb-α binding site.

2.4. N-Terminal Rod 1 Domains

No structures of the single domains from R1 to R8 have been determined as well as no structures groups or pairs of domains of this region of the filamin are so far available. In the absence of experimental data, only simulations and modeling as reported here are possible. Fig. (**6**) shows the computed isoelectric points of all the 26 domains of the three isoforms of human filamin (2 CH domain and 24 Ig-like domains). Surprisingly, with the partial exception of filamin C, there is an alternation of high and low pI values, which is especially pronounced at the N-terminus: the pI of the first CH domain of filamin A is close to 10, while the pI of the second CH domain is close to 4; pI of the first Ig-like domain is close to 10, the one of the second Ig-like domain is close to 5, and so on. The oscillations between pI values above and below 7 are much more pronounced than the expected error on the computed pIs, implying that there is an alternation of positively and negatively charged domains at physiological pH, a phenomenon that is extremely uncommon in multi-domain proteins [53].This trend does not involve only the N-terminal Ig-like domains but also the two CH domains.

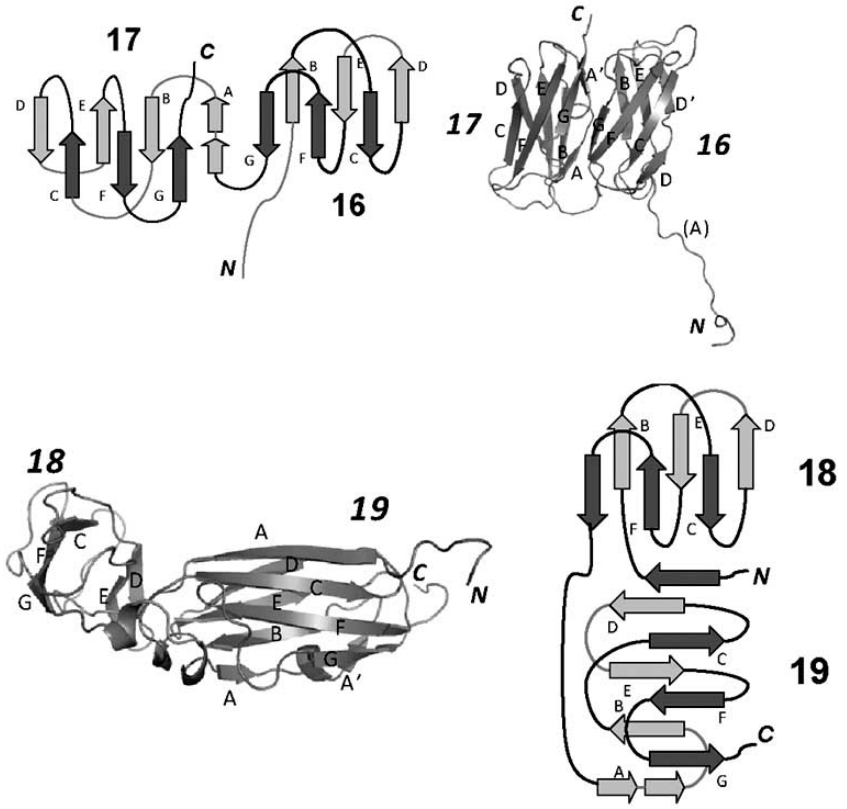

Fig. (5). Schematic view of the structures of the pairs of domains 16-17 and 18-19 of filamin A.

As a consequence of this molecular property, the N-terminal segment of filamin (up to the Ig-like domain number 5 or 6) tends to be rather rigid and extended, with all the domains following each other along a straight line [7]. A similar, though less pronounced trend of pI alternations is also observed at the C terminus, which on the other had adopts more compact structure due to inter-domain interactions. This rigidity might be functionally important, since a linear rod at the N-terminal moiety of filamin might ensure formation of actin networks and gel-like structures with certain mesh characteristics.

3. PARTNERS AND DEFAILLANCES

3.1. Actin Binding Domain

Human filamins bind to actin filaments through a canonical actin binding domain (ABD) composed of two calponin homology domains in a row [54]. ABDs are found in a number of actin filaments binding, bundling and cross linking proteins such as the spectrin superfamily of proteins (α-actinin, spectrin, dystrophin, utrophin), in filamin, plectin, calmin and cortexillin as well as in fimbrin and parvins. Furthermore, single CH domains can be found in different proteins such as calponin, Vav, IQGAP, SM22 and EB1, and are known to have other functions than binding to actin filaments [54]. A series of mutational and deletion studies identified three actin filament binding sites (ABS1-3): the N-terminal αA helix of CH1 (ABS1), the C-terminal αG helix of CH1 (ABS2) and the interdomain linker flanked by the N-terminal segment of CH2 domain comprising helix αA (ABS3) [55-59]. The large body of structural information on ABDs from diverse actin binding proteins revealed the two CH domains in a closed conformation [60-66], in which the ABS1 is largely buried between the two CH domains, suggesting the need for some interdomain structural rearrangements to render ABD fully capable of binding to actin filaments. In ABD structures from utrophin and dystrophin the closed conformation is brought about by the domain swapping between two molecules [62, 63].

The structural analysis of ABDs from filamin A [47] and filamin B (Kuzin *et al*. authors of PDB entry 3fer) showed CH domains arranged in the expected closed conformation Fig. (**7**). Several genetic diseases causing abnormalities in brain, bone and cardiovascular system development have been linked to mutations in the genes coding for filamin A and B. Some of the identified mutations causing otopalatodigital syndrome (OPD) spectrum disorders or periventricular nodular heterotopia (PVNH) have been mapped in the ABD of filamin A [67, 68]. Structural analysis of the PVNH mutations suggests that two mutations (A128V and S149P) that map in a proximity of ABS2 and disrupt the helix of ABS2, respectively, might have a direct effect binding to actin filaments, while E82V and M102V might have an only an indirect influence on binding to actin filaments [47]. The structural analysis of OPD mutations, which spread over the CH domain, revealed some that map to the ABS3 (Q170P, L172F) or face ABS1 (E245R) and might have effect on binding to actin filaments Fig. (**7**), while many other OPD mutations might have local effects at distal part of CH2 or in

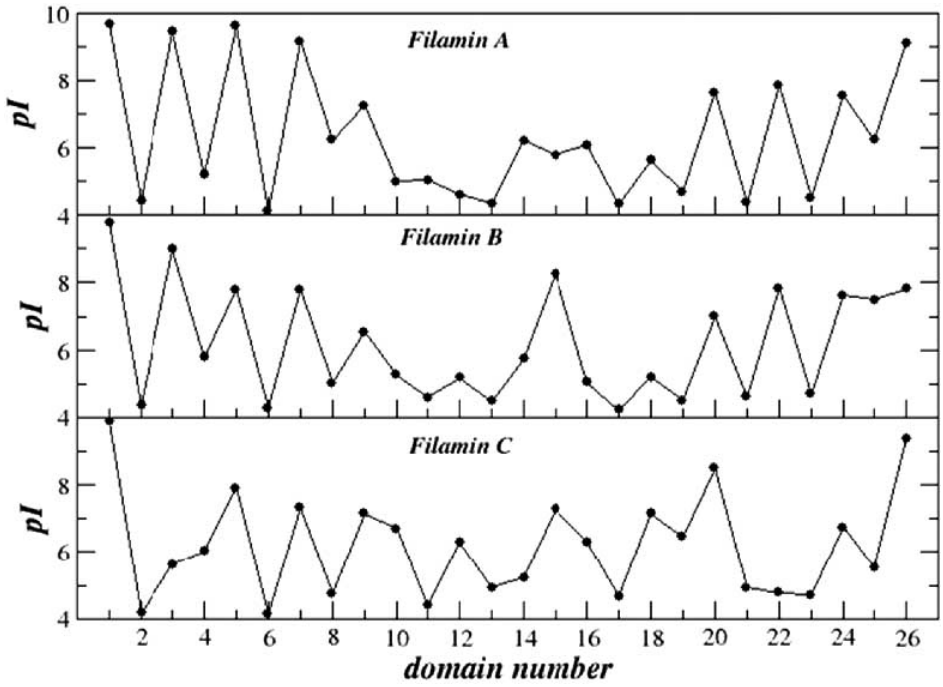

Fig. (6). Isoelectricpoints (pI) of the domains of the three isoformsof filamin.

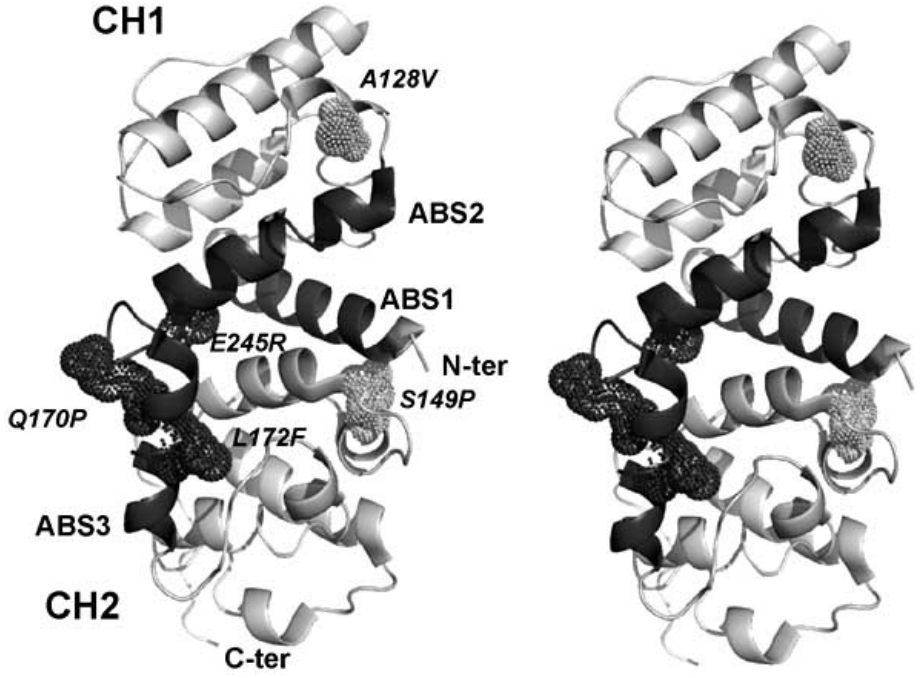

Fig. (7). Ribbon presentation of ABD of filamin A. ABS1-3 are highlighted in dark grey. PVNH mutations that might have effect on actin binding (A128V, A149P) are indicated in light grey, OPD mutation with potential local effect on actin binding (Q170P, L172F, E245R) are highlighted in black.

the interface between the CH domains, and largely retain their capacity to bind to actin filaments [68].

3.2. Promiscuity and Fidelity

Human filamins are known to participate in a number of intermolecular interactions. Besides to crosslink actin filaments, they interact with cellular membranes and with signaling pathways. Tens of filamin-binding proteins have been discovered [19, 69-71]. Most of the interactions involve the rod 2 region (Ig-like domains 16-24) while few of them occur in the rod 1 moiety (domains 1-15).

Some structural insight on the molecular mechanisms of these interactions has been unraveled recently. Ylanne and co-workers determined the three dimensional structures of

the complexes between the Ig-like domain 21 of filamin A and polypeptide fragments of migflin [72], β7 and β2 integrin [35, 73], as well as the Ig-like domain 17 of filamin A in complex with a peptide moiety of platelet adhesion glycoprotein GPIbα [34]. Schematic views of these structures are depicted in Fig. (**8**).

Some rather surprising trends appear from the structural and functional analyses of these complexes. First, the peptide fragment of the filamin partners adopts always a β-strand backbone conformation. Second, this β-strand flanks in an antiparallel orientation the β-strand C of the filamin Ig-like domains 17 and 21, respectively. Consequently, a four stranded antiparallel β-sheet is formed with the β-strands C, F, and G of the filamin Ig-like domain and the additional strand of the filamin ligand. Apart from the aforementioned hydrogen bonding network, the partner peptides interact with Ig-like domains through several hydrophobic interactions with residues of strands C and D. It is the combination of polar and hydrophobic contacts that renders this interaction specific. Interestingly, the interaction of integrin peptides is very similar to the GPIbα despite low sequence similarity: the peptides in all cases on one hand adopt a beta-strand conformation to interact with strand C and on the other conserved but not identical hydrophobic residues point toward strand D.

In the crystal structure of the filamin-migfilin complex, the migfilin moiety is in crystal even able to bridge two Ig-like domains, with the formation of a seven-stranded β-sheet, though the stoichiometry of the complex is 1:1 in solution [72] see Fig. (**8**)

Curiously, this structural mechanism of complex formation with binding partners is very similar to the filamin dimerization mechanism that involves the Ig-like domain 24 see Fig. (**3**). It also extremely similar to the interactions between the Ig-like domains 18-19 and 20-21, where the first β-strand of the first domain is antiparallel to strand C of the next domain see Fig. (**4**) and Fig. (**5**). Given the structural similarity between the four complexes with peptides of ligand and between some pairs of filamin Ig-like domains, Ylanne and coworkers hypothesized that the C and D β-strands of the filamin domain are a sort of "filamin-binding motif of filamin-binding partners" [34]. On the basis of these observations, it is possible to predict the binding mode of other filamin partners, for example the dopamine D2 and D3 receptors that bind the domain 19 of filamin A.

However, more subtle is the question of the specificity: why migfilin and integrin moieties form a complex with the Ig-like domain 21 of filamin A and the polypeptide fragment of the glycoprotein Ib interacts preferably with the domain 17? Of course, the sequences of these pairs of partners are

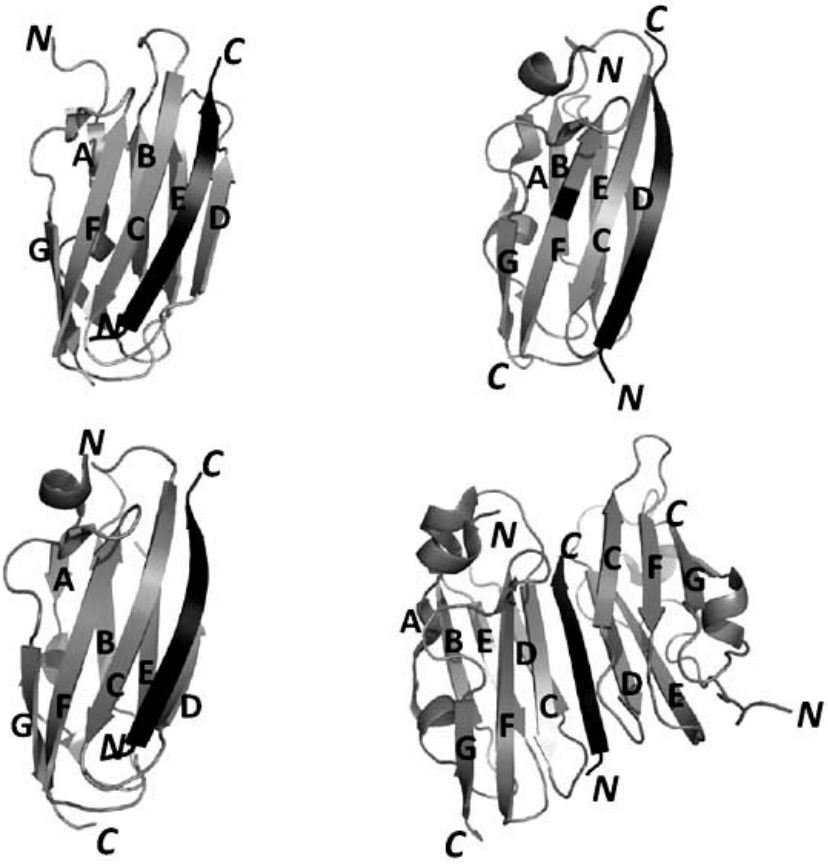

Fig. (8). Schematic view of the structures of the complexes of filamin. Top left: complex between domain 17 of filamin B and the platelet adhesion glycoprotein GPIbα (PDB file 2bp3). Top right: complex between domain 21 of filamin A and the integrin β7 cytoplasmic tail peptide (2brq). Bottom left: complex between the domain 21 of filamin A and the integrin beta2 cytoplasmic tail peptide (2jf1). Bottom right: complex between the domain 21 of filamin A and the migfilin peptide (2w0p). While filamin is shown in grey, its ligand is shown in black.

not identical. However, their interaction strategy is the same. This question is made even more complicated, since, the specificity seems to be relatively modest at least *in vitro*. The N-terminal segment of migfilin, which is conformationally disordered *in vitro*, interacts not only with the Ig-like domain 21 but also with domains 19 and 22, tough with minor affinity [72]. Analogously, the cytoplasmic tail of integrins and the peptide fragment of the glycoprotein Ibα show affinity in the micromolar range for the Ig-like domain 17 of filamin A [34].

Two major scenarios, compatible with each other, were proposed. On the one hand, the side-chain-side-chain interactions play an important role. Therefore, the extension of the β-sheet of the strands C-F-G to the β-strand of the ligand does not depend only on the formation of the required backbone-backbone hydrogen bonds but also on specific interactions between the side-chains. These interactions are not confined to the Ig-like domain strand C and to the β-strand of the filamin-binding protein but involve also the β-strand D of the Ig-like domain, with the consequence that the entire surface patch formed by the β-strands C and D of the Ig-like domain can be considered essential for binding and specificity. This is the case, for example, of the peptide fragment of the glycoprotein Ibα, where the side chains of two residues (Phe 568 and Trp 570) extensively interact with the CD face of the immunoglobulin sandwich [74].

On the other hand, beside specific side-chain-side-chain interactions, phosphorylations may also play a role in regulating the formation of complexes between filamin and other molecules. A particularly well characterized example is the interaction between filamin A and the β2 integrin cytoplasmic tail. The unphosphorylated integrin binds filamin and integrin phosphorylation (at the Thr 758) inhibits this interaction and promotes the complex formation between integrin and tallin [73].

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