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Minireview

Functional plasticity of CH domains

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Abstract With the refinement of algorithms for the identification of distinct motifs from sequence databases, especially those using secondary structure predictions, new protein modules have been determined in recent years. Calponin homology (CH) domains were identified in a variety of proteins ranging from actin cross-linking to signaling and have been proposed to function either as autonomous actin binding motifs or serve a regulatory function. Despite the overall structural conservation of the unique CH domain fold, the individual modules display a quite striking functional variability. Analysis of the actopaxin/parvin protein family suggests the existence of novel (type 4 and type 5) CH domain families which require special attention, as they appear to be a good example for how CH domains may function as scaffolds for other functional motifs of different properties.

Key words: Calponin; Calponin homology domain; Actin binding; Actopaxin; Paxillin binding; Vav; α-Actinin; Dystrophin; Fimbrin; Spectrin; Utrophin

1. Introduction

The 100 amino acid calponin (CaP) homology (CH) domain is one of about a dozen protein domains which are shared by both signaling and cytoskeletal proteins. Whilst a repeating pattern, now known to be equivalent to the VH domain, had been identified in a number of actin binding proteins [1] the definitive description of the CH module by Matti Saraste's group in 1995 [2] caused workers in the field to take a closer look at previously described actin binding domains (ABDs). The division of ABDs into two dissimilar CH domains (an N-terminal or type 1 CH domain and a C-terminal or type 2 CH domain) helped to explain differences observed in the actin binding affinities of the two separated CH domains of the α-actinin ABD, but also provided a paradox in terms of explaining the presence of a CH domain which may not be involved in actin binding.

Banuelos et al. [3], using a structural approach to shed more light on this puzzle postulated an auxiliary function for the second CH domain in an ABD. As a general rule (and yes, there is also an exception to it) one can assume that if a protein displays two CH domains in tandem they likely form an ABD. This hypothesis appears to be close to the biological truth since reports are now accumulating in which the second, but not the first, CH domain in an ABD displays additional functions, some unrelated to the 'primary' function, namely actin binding. However, a tandem array itself may not be sufficient for the formation of an actin binding interface, but appears to require the conjunction of a type 1 and a type 2 CH domain. Functional heterogeneity in CH domains is well-documented in the CH domains of the α-actinin ABD as regards their actin binding abilities. In addition, recent findings have revealed a novel function of CH domains: they also contain specific binding sites for proteins and phosphoinositides.

The CH domain is defined by a number of almost invariant core residues. These core residues are likely to be the major factors involved in stabilising the three-dimensional (3D) structure. The conservation of these residues in CH domains throughout species points towards an evolutionarily conserved structure. Yet, functional diversity is the prevailing theme in CH domains. This is best reflected by the single (type 3) CH domains which can be found in a large and diverse family of proteins, which contains both cytoskeletal and signaling proteins. The actin binding ability of type 3 CH domains in isolation is still questionable. However, a number of other functions have been postulated for this module. Here we will discuss the current ideas concerning the structural, functional and evolutionary aspects of CH domains. We will describe the structural parameters, which define a CH domain, propose a nomenclature for the distinction of CH domains and analyse a few of the recent developments relevant to the biological function(s) of CH domains.

2. Type 1 and type 2 CH domains in ABDs

The type 1 and type 2 CH domains together form the actin binding region of a large number of F-actin interacting proteins, involved in a variety of cytoskeleton and cytoskeleton–membrane linkages. This ranges from actin binding/bundling proteins, like α-actinin, spectrin and filamin, cytolinkers connecting F-actin to other filament networks, like spectrin, plec-

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Abbreviations: CaP, calponin; CH domain, calponin homology domain; ABD, actin binding domain; FAK, focal adhesion kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate

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tin and dystonin, and proteins connecting the actin cytoskeleton to various membrane-associated proteins including cell adhesion receptors (e.g. dystrophin, utrophin). In all cases the canonical type 1/2 CH domain-containing ABD is an efficient F-actin binding domain with an affinity for F-actin in the low μM range with no other significant effect on actin dynamics and to all intents and purposes functionally equal. However, several of these proteins have the ability to dimerise. When this happens, these proteins can now crosslink actin filaments (e.g. α-actinin, spectrin and filamin). Beyond the ability to bind to F-actin, a multitude of further functions is conferred by one or more of the many other protein–protein interaction modules present in these often large and complex proteins (Fig. 1).

2.1. CH1 versus CH2

Whilst the ABDs as a whole are functionally equivalent, in as much as they interact with F-actin with ~5–50 μM affinity, the CH domains they comprise are functionally distinct [4]. Type 1 CH domains have the intrinsic ability to interact with F-actin whilst type 2 CH domains do not. In all cases so far examined (α-actinin, β-spectrin, dystrophin, utrophin), the type 1 CH domain alone has an affinity for F-actin in the region of 10-fold lower than the complete actin binding region comprising both CH domains, whilst the type 2 CH domain does not bind actin at all [5–7]. Clearly, however, despite the type 2 CH domain having no intrinsic actin binding activity, it contributes substantially to the interaction of the complete actin binding domain, perhaps by acting as a locator or low affinity docking site on the actin filament. Such a role has also been proposed for the type 3 CH domain of CaP, which also lacks intrinsic actin binding activity (see below). Despite this functional difference, where structures are known for the canonical type 1/2 CH domains, there is little to choose between them on a structural basis. The functional difference therefore must come down to discrete sequence elements exposed on the potential actin binding surfaces of these molecules. A considerable effort has been put into identifying such sequences and work from a number of laboratories (see [8] and references therein) has identified three regions within the ABDs as im-

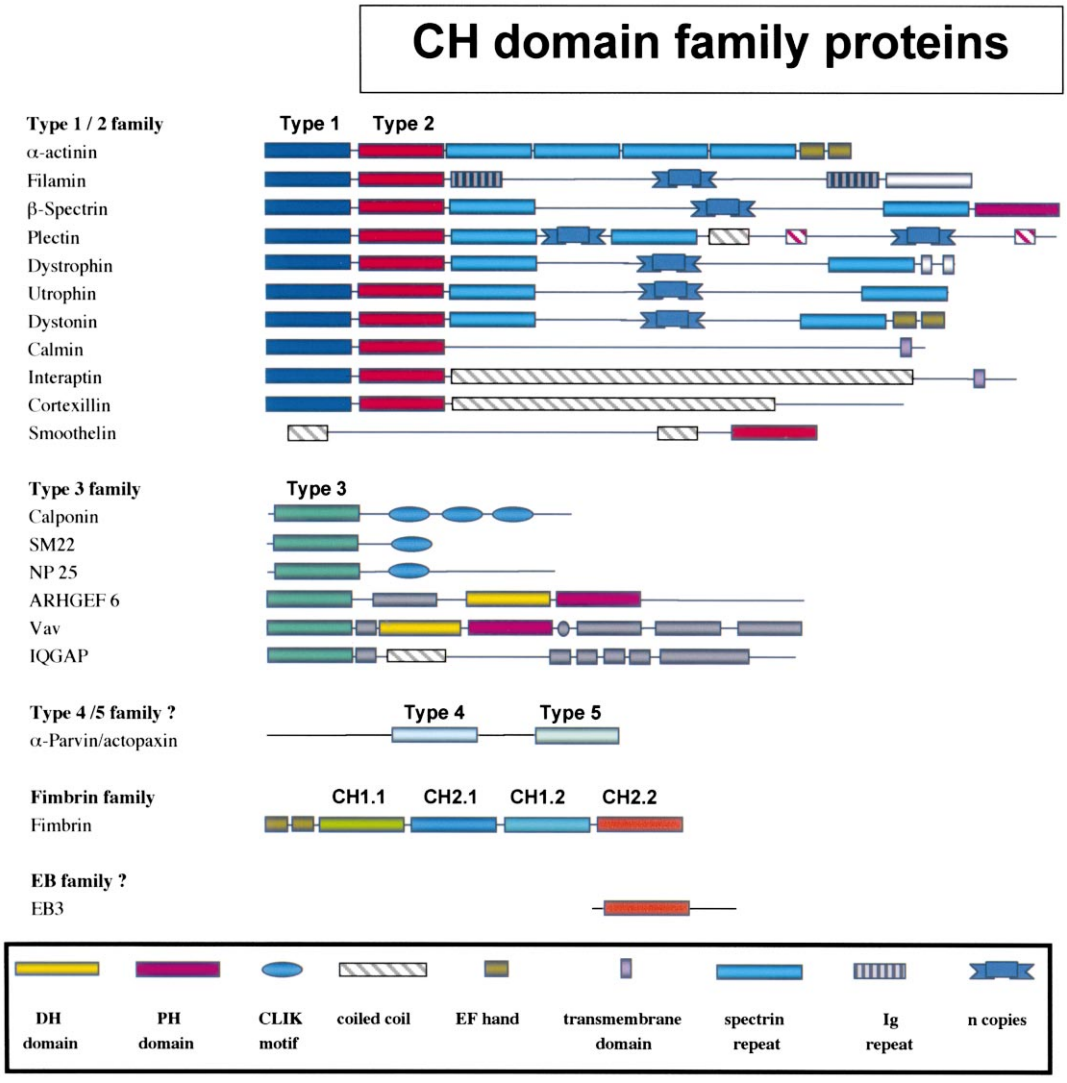

Fig. 1. The superfamily of CH domain-containing molecules. All entries are human, with the exception of cortexillin and interaptin which are unique for *Dictyostelium discoideum*. Light blue ribbons symbolise multiple (*n*) copies of identical domains. The Simple Modular Architecture Research Tool (SMART) which is available at http://smart.embl-heidelberg.de is an easy-to-use, rapid and reliable source for identifying CH domain proteins and for obtaining total domain organisations of proteins.

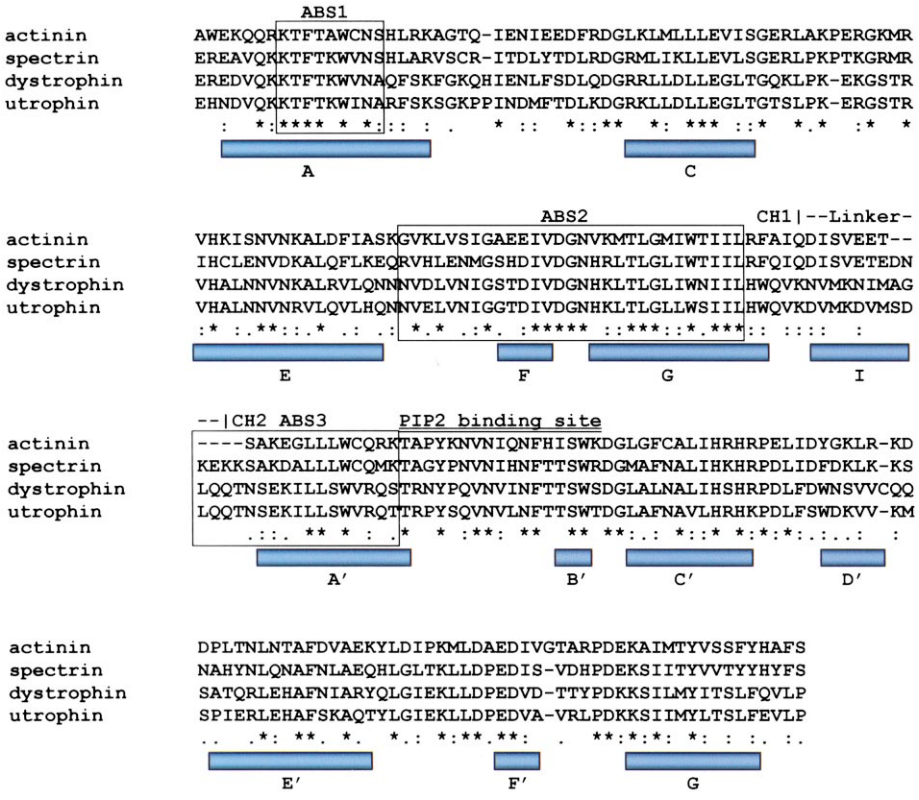

Fig. 2. Sequence alignment and secondary structure cartoon of α-actinin, β-spectrin, utrophin and dystrophin with annotation of ABS1–3 and the PIP_2 binding site.

portant for actin binding. These have been termed actin binding sites (ABS) 1–3 from N to C terminus and correspond to: helix A in the type 1 CH domain; the loop and helices F and G in the type 1 CH domain; helix A in type 2 CH domain (Fig. 2). As the stoichiometry of actin binding by these ABDs is 1:1 rather than 3:1 it was predicted that these three ABSs would come together in the tertiary structure to form a single actin binding interface on the surface of the ABD [6]. Whilst this did not quite turn out to be true (see below) the three ABSs do all appear to participate in F-actin binding, at least in the case of utrophin [9].

2.2. Structural conservation versus functional plasticity

The architecture of the CH domain is dominated by four α-helices (A, C, E and G) (Fig. 3), comprising 11–18 residues and connected by long loops. Three short and less regular helices (B, D and F) are minor secondary structure elements. The structure can be described in terms of three layers, the core being composed of two parallel α-helices (C and G), which are sandwiched on one side by helix E, and on the other side by the N-terminal helix A. This scaffolding is markedly different from the known 3D structures of actin severing, capping or regulating proteins such as gelsolin, villin, severin, profilin and destrin, which are characterised by a central β-sheet sandwiched between one or more α-helices. The 3D structure of the CH domain can be analysed with respect to the conservation of key residues and its predicted actin binding activity. A very conserved region of the molecule is helix C, which builds the hydrophobic core of the protein. This segment belongs to a sequence profile that has been used for detection of CH domains in proteins [2].

The recently published crystal structures of dystrophin, fimbrin, spectrin and utrophin CH domains have shed considerable light on several aspects of CH domain function but inevitably also raised questions [7,8,10–12]. The ABDs of dystrophin and utrophin crystallised as antiparallel dimers with the two CH domains in a relatively extended or open conformation [8,12], whereas fimbrin was a more compact monomer in the crystal with the two CH domains closely associated [10]. The orientation of the CH domains in the utrophin crystal, however, was such that the type 1 and type 2 CH domains from each of the separate chains in the crystal dimer closely resembled the orientation of the 2 CH domains in the single chain in the fimbrin crystal. Utrophin and dystrophin ABDs are known to be monomeric in solution, indeed whole purified dystrophin–glycoprotein complex does not exhibit any actin bundling activity suggesting it too is monomeric for dystrophin at least [13,14], and so the utrophin and dystrophin dimers in the crystal are likely to be an artefact of crystallisation. The preservation of an interface between two domains in monomeric (fimbrin) and oligomeric forms (utrophin and dystrophin) is a phenomenon known as 3D domain swapping (reviewed in [15]). This suggests therefore that in solution, dystrophin and utrophin are likely to adopt a conformation similar to fimbrin, with the type 1 and type 2 CH domains in close apposition. This, however, does not fit with the recent cryo-electron microscopy reconstructions of utrophin with F-actin, which show utrophin interacting with F-actin in the more open conformation adopted by utrophin in the crystal [9]. This raises the exciting and as yet untested hypothesis that utrophin, and perhaps all type 1/2 CH domains, undergo a dramatic conformational change

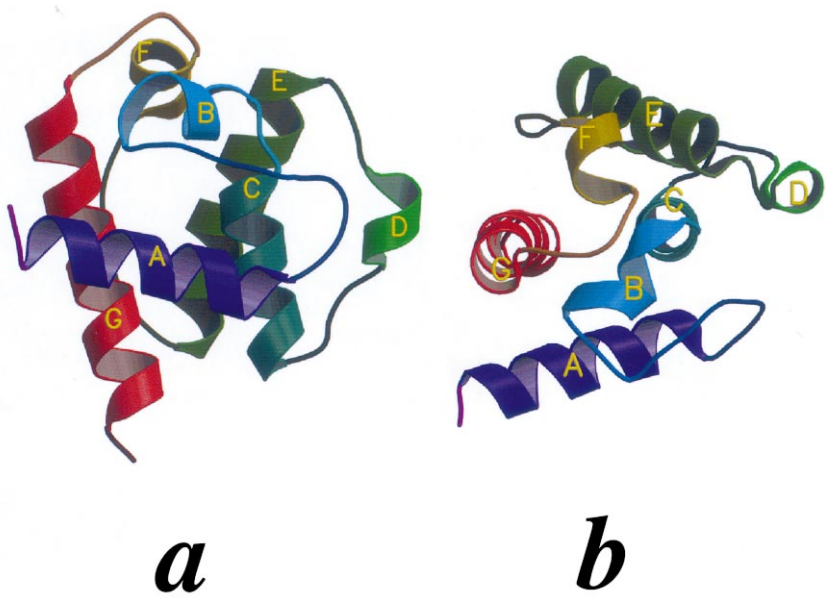

Fig. 3. Ribbon presentations of the molecular architecture of the β-spectrin type 2 CH domain as seen perpendicular (a) and parallel (b) to the two core α-helices C and G. Colour coding ranges from violet for N-terminal to red for the C-terminal residues. Ribbon diagrams were generated using programs Molscript [54], and Raster3d [55].

upon interaction with F-actin. The same cannot be said of fimbrin, however (see below).

A large number of biochemical studies using mutagenesis, cross-linking and spectroscopy have implicated three segments (defined above as ABS 1, 2 and 3) of an actin binding region in actin binding. These amino acids are mostly hydrophobic, which agrees with the current paradigm that the force driving the interaction with actin filaments is at least in part hydrophobic. The combination of available biochemical information and structural analysis suggests that the protein surface centred around the last helix of the first CH domain, and probably spanning to the N-terminus of the second CH domain is essential for actin binding. The importance of this area may explain why the affinity of the isolated type 1 CH domain for actin is one order of magnitude lower than that of the complete actin binding region. Amino acid sequence analysis of residues in ABSs (ABS 1, 2, 3) of the actin binding regions shows that the characteristic pattern of conserved residues on the molecular surface is only conserved in the type 1 and type 2 CH domains. This is of course in line with its clear biological function, binding to actin filaments. The signal is lost in type 3–5 CH domains (see below) suggesting their functional diversity and/or alternative modes of interaction with actin filaments.

2.3. Regulation

In common with many other actin binding proteins, it has been known for some time that the actin binding properties of some α-actinin isoforms and fimbrin can be regulated by calcium. In the case of α-actinin and spectrin this is achieved by virtue of the presence of EF hand regions present in these molecules rather than any direct effect of Ca^{2+} on the CH domains themselves. Several other mechanisms have evolved for the direct regulation of type 1/2 CH domain interaction with actin. α-Actinin in the muscle Z-line was shown to contain bound phosphatidylinositol (4,5)-bisphosphate (PIP_2) [16], furthermore in non-muscle cells actin-associated α-actinin contained bound PIP_2 whereas cytosolic α-actinin was free of PIP_2, suggesting that PIP_2 activated the actin bundling activity of α-actinin [17]. The PIP_2 binding site on α-actinin was delineated as a 17 amino acid region immediately C-terminal of the ABS3 [18] (see Fig. 2). Precisely how PIP_2 binding to this site promotes actin binding is not known. If α-actinin associates with F-actin in an orientation similar to utrophin [9], with which it shares over 50% sequence identity (and presumably also high structural homology) then this region of α-actinin would be expected to be in the F-actin interface and PIP_2 would be more likely to inhibit actin binding than promote it. Indeed, PIP_2 binding to both filamin and dystrophin does inhibit their interactions with F-actin [19,20] though the precise binding sites in either of these molecules are unknown and the physiological significance is uncertain. The precise mechanistic details of how PIP_2 promotes actin bundling by α-actinin also remain to be elucidated, but the difference between α-actinin and dystrophin and filamin may be due to the immediate environment of the CH domains, which in the α-actinin dimer includes the EF hands from the opposing antiparallel dimer.

The special situation in α-actinin is further underscored by the identification of DFRXXL-like motifs in the molecule, one (DFRXXL) immediately adjacent to ABS 1 in the helix B–helix C turn, the other (DFRXXL in α-actinin 2 and 3; EFKXXL in α-actinin 1 and 4) embedded in the predicted EF-hand sequences at the C-terminus of the molecule. The DFRXXL motif has been shown to constitute a novel actin binding motif which links myosin light chain kinase (MLCK) to the actin filament by occupying a novel interface along the filament [21,22]. The antiparallel dimerisation of α-actinin likely brings the C-terminal motif in close proximity of the N-terminal CH domain-based motif. Future studies will have to address the influence of this motif in the regulation of α-actinin binding to actin.

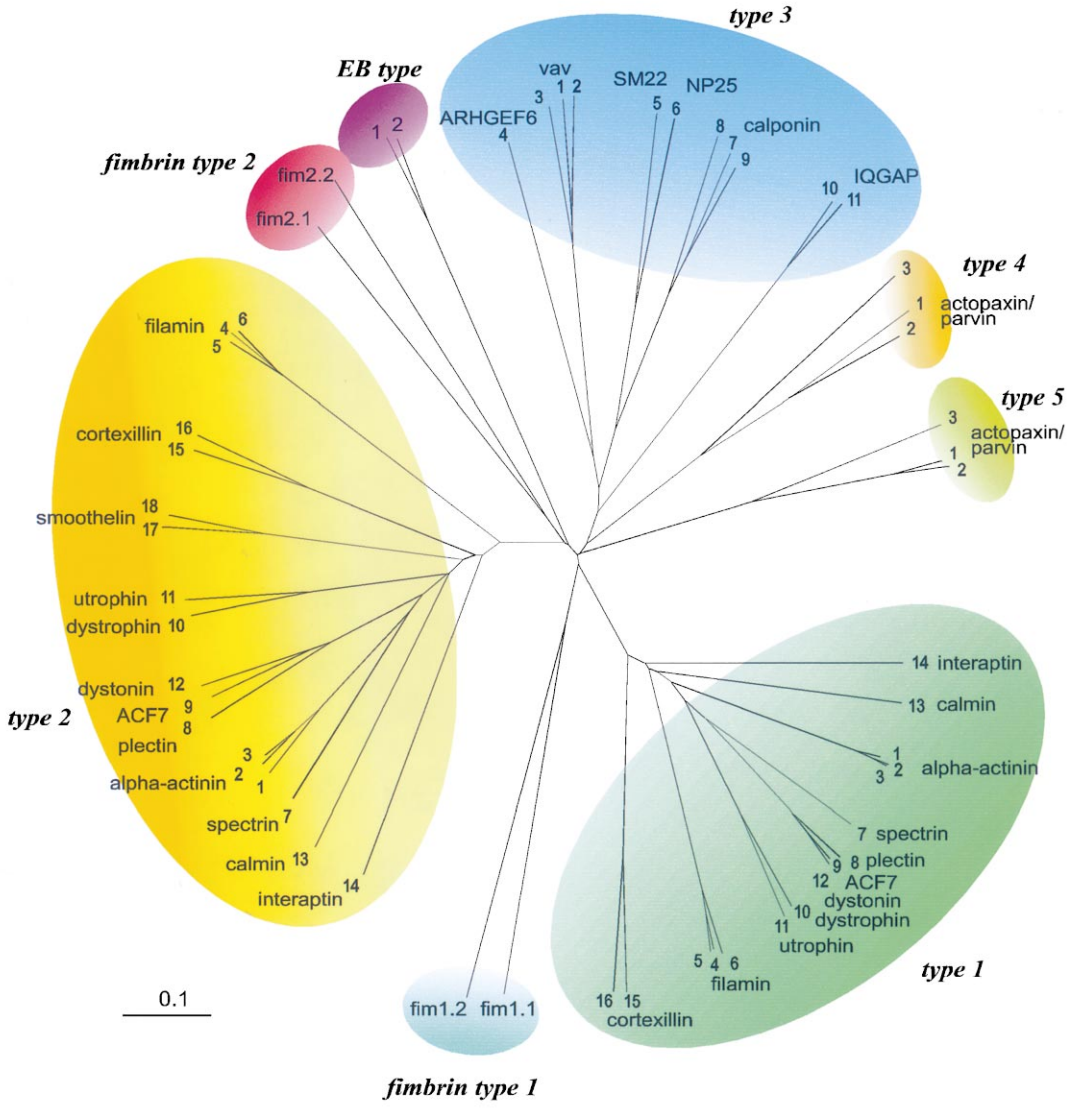

Fig. 4. Phylogenetic tree of CH domain proteins as in Fig. 1. More detailed information about core residues, subclassifications, helix–loop borders, sequence alignments and more can also be obtained at http://server1.imolbio.oeaw.ac.at/mgimona/. GenBank accession codes (all entries are human, except where otherwise noted): Type 1/type 2: (1) P12814, α-actinin 1; (2) P35609, α-actinin 2; (3) Q08043, α-actinin 3; (4) XP_048404, filamin 1; (5) XP_030806, β-filamin; (6) XP_045856, γ-filamin; (7) XP_039511, β-spectrin; (8) Q15149, plectin 1; (9) Q9UPN3, ACF7; (10) P11532, dystrophin; (11) P46939, utrophin; (12) AAC50243, dystonin isoform 1; (13) BAB59010, calmin; (14) AAC34582, interaptin (*D. discoideum*); (15) AAB62275, cortexillin I (*D. discoideum*); (16) AAB62274, cortexillin II (*D. discoideum*). Type 2 only: (17) CAA90281, smoothelin-A; (18) CAA73884, smoothelin-B. Type 3: (1) P_005419, Vav 1; (2) Q9UKW4, Vav 3; (3) XP_005638, Vav 2; (4) BAA04985, ARHGEF6; (5) JS0774, SM22; (6) Q9UI15, NP25; (7) XP_032793, CaP h1; (8) Q99439, CaP h2; (9) Q15417, acidic CaP; (10) P46940, IQGAP1; (11) genbank:NP_006624, IQGAP 2. Type 4/type 5: (1) XP_043987, α-parvin; (2) XP_043616, β-parvin; (3) XP_043623, γ-parvin. Fimbrin-type: P13797, T-plastin. EB type: (1) CAA63923, T-cell activation protein; (2) BAA82958, EB3 protein.

Calmodulin has also been shown to regulate the interaction of dystrophin, utrophin and α-actinin ABDs with F-actin through direct binding to the CH domains, though again the physiological relevance of this is not clear [23–26]. Most recently it has been shown that α-actinin is phosphorylated on tyrosine at position 12 by focal adhesion kinase (FAK) and that FAK phosphorylation of α-actinin reduces its affinity for F-actin [27]. Interestingly, however, this tyrosine phosphorylation lies N-terminal of the first CH domain in a variable region of differing length found in all type 1/2 CH domain ABDs. It has previously been noted that the amino terminal extension of the utrophin ABD was somehow important for actin binding affinity, as monoclonal antibodies raised against this region inhibited actin binding [28] and deletion of this 28 amino acid variable region in utrophin reduced actin binding affinity ~four-fold [8].

2.4. Smoothelin: single type 2 CH domains?

Smoothelin is a cytoskeletal protein specifically expressed in differentiated smooth muscle cells and has been shown to colocalise with smooth muscle α-actin. In addition to the small smoothelin isoform of 59 kDa, a larger isoform of 117 kDa and several alternatively spliced isoforms have also been described. Smoothelins harbour a single CH domain at

their C-termini, which displays the closest sequence similarity with the type 2 CH domain of α-actinin, β-spectrin and filamin. There are no reports available describing actin binding of smoothelin and the protein itself appears to form a filamentous network [29]. It is not clear at this point whether or not smoothelin can bind directly to actin, and if the type 2 CH domain is involved in any of the described functions or in the molecule's subcellular localisation.

2.5. Fimbrin

Although fimbrin does not strictly contain a type1/2 CH domain ABD it does have an ABD that contains canonical CH domains and will be discussed here for completeness. By virtue of the large inter-helical loops within the CH domains of fimbrin, the four CH domains present within the two ABDs of fimbrin fall into their own distinct classes (Fig. 4). Furthermore the large inter-CH domain linker in fimbrin also sets it aside from the classical type 1/2 CH domain ABD found in α-actinin and homologues (see also below). Nevertheless, the large amount of biochemical as well as structural information from both crystallographic and cryo-electron microscopy reconstructions has made fimbrin a useful model for CH domain F-actin interactions [10,30–32]. Unlike utrophin and dystrophin, fimbrin appears to associate with F-actin in a more compact conformation [31,32]. There doesn't appear to be any requirement for the fimbrin CH domains to adopt a more extended arrangement. This may be because the length of the fimbrin inter-CH domain linker is long enough to allow a stable interface between the two CH domains of the ABD, or due to the fundamental differences in the fimbrin CH domains per se, allowing fimbrin simply to interact with actin via a different mechanism. The combined data on the atomic modeling of human T-fimbrin from Hanein et al. [32] and the reported discrepancy in K_d values for the plant AtFim1 [33] substantiate a different mode of interfacing with the actin filament for the two ABDs in fimbrin. In addition to actin binding, Correia et al. [34] described an interaction of fimbrin with the intermediate filament protein vimentin via the first CH domain. Based on the location of this binding site on fimbrin, the molecule was implicated in the regulation of vimentin assembly, an important feature which appears to be repeated in the interaction of the CH domain of CaP with desmin (see below) and thus pointing towards a possibly conserved functional inter-relationship between CH domain proteins and intermediate filament proteins.

2.6. EB-family proteins

A single CH domain with highest sequence similarity to the second (type 2?) CH domain of the C-terminal ABD of fimbrin has been identified in the APC-binding, microtubule plus-end localised protein EB1 and its relatives EB2 and EB3 (see Fig. 1). These molecules harbour the CH module close to their respective N-termini. There is too little experimental data on the protein to hypothesise about the function of its CH domain but, considering the possibility of intermediate filament interaction of fimbrin-type CH domains and the reported localisation of EB proteins, it is tempting to speculate on an involvement of the EB CH domains in tubulin interactions. Experiments are underway testing the consequences of CH domain deletion and exchange on the APC-binding and microtubule targeting of EB1.

3. Inter-CH domain sequences

In α-actinin-like molecules the sequences separating the two CH domains are almost negligible as they span a mere six residues and may simply contribute to the helix (G) connecting the first and second CH domain. Thus, the 3D structure of a type 1/type 2 CH domain ABD may be viewed rather as a unit than as a tandem array of individual modules. Fimbrin, however, differs in this respect. Although it contains a tandem CH domain ABD and strongly binds and bundles actin, the CH domains are so unique that there is a strong likelihood for alternative functions. Moreover, the spacer sequences are longer than those found in α-actinin. Since the inter-CH domain linker sequence in the second ABD of fimbrin is almost four times longer than that of the first one, we may expect the identification of further new (regulatory?) functions for this domain in the future. Calmin, enaptin and NUANCE (and possibly also interaptin) are novel proteins with a C-terminal transmembrane domain and a classical type 1/type 2 ABD, but there is little functional data available as regards their actin binding capacity. What is noteworthy, however, is the unusually long serine-rich inter-CH domain linker, which exceeds that of fimbrin. The longest spacer sequences are the 60 residue inter-CH domain linkers of the actopaxin/parvin family. However, as discussed below, it is questionable if this stretch indeed separates the two halves of a functional ABD (separating ABS 2 and 3) or if this region is more similar to that found between the two ABDs in fimbrin.

4. Single (type 3) CH domains in cytoskeletal and signaling molecules

CaP family proteins form a separate branch in the phylogenetic tree of CH domains, together with (almost all) other proteins containing a single CH domain (Vav, IQGAP, ARHGEF6, SM22; see Figs. 1 and 4). Furthermore, the predicted or experimentally identified functions of the CH domains in this family differ markedly from the type 1 and type 2 modules and have therefore been classified as type 3 CH domains.

4.1. CaP family proteins

In contrast to the actin cross-linking and bundling proteins described above, CaP contains only a single CH domain, which shares little sequence similarity with type 1 or type 2 CH domains. CaP binds to filamentous actin in the absence of the amino terminal CH domain both in vitro and in vivo. Conversely, isolated CH domains fail to associate with F-actin structures in living cells and do not co-sediment with F-actin. The two, mapped ABSs in CaP are C-terminal of the CH domain [35] and function autonomously. So, what role for the CH domain in CaP? CaP binding to other cytoskeletal components, including tubulin and the intermediate filament protein desmin has been reported and it has been hypothesised that the binding sites on CaP reside within the CH domain. Thus, CaP has been suggested to function as a scaffolding protein for cytoskeletal structures. So far, however, overexpression experiments have failed to induce detectable bundling of intermediate filaments or microtubules in transfected cultured cells.

An alternative function for the CaP CH domain has come from the laboratory of Kathy Morgan [36,37]. This group has

shown a direct physical interaction of the CaP and α-actinin CH domains with the MAP kinase signaling molecules ERK1 and ERK 2. This puts CaP in the position to function as an adaptor molecule in signaling pathways, an attractive idea since several type 3 CH domain-containing molecules are indeed present at different positions in the Rho-signaling pathway. In this context it appears relevant that CaP has been identified as a direct target for Rho-associated kinase (ROCK), a multifunctional serine/threonine kinase involved in the regulation of actomyosin-based contractility in smooth- and non-muscle cells [38].

4.2. Vav family proteins

In the RhoGEF Vav the amino terminal type 3 CH domain appears to have adopted yet another function and may act as a regulatory domain. It has been suggested that it interacts intramolecularly with another motif to sterically block the docking site for Rho family GTPases in the DH–PH binding interface [39]. Most notably, domain-swap experiments in which the Vav CH domain has been replaced as a whole with the analogous type 3 domain from either ARHGEF6 [40] or the actin-associated proteins CaP or SM22 (Kranewitter and Gimona, unpublished) fail to 'silence' the protein back to wild-type activation levels indicating that specific residues within the Vav CH domain may determine its cellular function. Actin association also seems not to be the essential function of the Vav CH domain since its deletion causes an increase in the cytoskeletal association of Vav [41]. Fusion of Vav to the entire functional ABD of α-actinin efficiently translocates the molecule to stress fibres but this mistargeting has only marginal influence on the protein's GEF activity (Kranewitter and Gimona, unpublished). The structural data presented recently on the DH–PH catalytic unit of Vav [42] demonstrate further that the acidic region containing the critical residue Y174, C-terminal of the CH domain forms a helix which becomes inserted between the DH and PH domains and the authors of the study suggested that this intercalation causes the functional block of Vav's GEF activity. However, some open questions still remain. Why are partial N-terminal deletion mutants of Vav constitutively active despite the presence of the acidic domain (harbouring Y174) and part of the CH domain? Where does the CH domain go in this process of conformational change? Based on the above one might speculate that the CH domain regulates the positioning of the acidic helix by regulated interactions with other domains on the Vav molecule or by an intermolecular interaction with other factors present in the Vav signaling complex. A candidate for this is the nucleotide dissociation inhibitor RhoGDI. A homologue of this, LyGDI was found to interact with the N-terminal region of Vav in two hybrid analyses and in pull down assays in vitro, and partial deletion of the Vav CH domain abolished this interaction [43]. The intriguing model emerging from these data is that CH domain interactions could potentially regulate GEF activity of certain exchange factors.

4.3. αPIX/COOL/ARHGEF6

ARHGEF6 (also known as αPIX and Cool2; accession number BAA04985, coding for a 773 residue protein) has recently been identified as a dbl family GEF. Like Vav, ARHGEF6 contains an N-terminal type 3 CH domain, which is closer to the Vav-specific consensus than that of any other type 3 CH domain. Most notably, the very amino terminal A helix contains a number of residues conserved specifically in the two GEF families, and the loop connecting helix A and B is of similar length. The relation with Vav is also striking, considering the devastating effect of an amino terminal deletion within the ARHGEF 6 CH domain caused by the skipping of exon 2. This deletion has been shown to cause massive mental abnormalities in patients carrying this defect [44,45]. Yet, as discussed above, in domain swap experiments the ARHGEF6 CH domain fails to functionally substitute for the Vav 1 CH domain.

4.4. IQGAP family proteins

Considerably less information is available on the function of the amino terminal type 3 CH domain in IQGAP. The CH module of this Rho family GTPase activating molecule is believed to directly interact with actin and thereby target the molecule to the sites of actomyosin interaction, most prominently the contractile ring at the budding site in yeast [46,47]. However, this effect appears to require the dimerisation of the molecule via the more C-terminal coiled-coil domain in IQGAP. This attractive idea awaits proof at the molecular level, but the possibility of an actin binding domain being formed by the heterologous dimerisation of CH domains from two separate molecules is interesting. Such a hypothesis is supported in part by the apparent ability of CH domains from type 1/2 ABDs to participate in 3D domain swapping (see above).

5. The actopaxin/parvin family: type 4 and type 5 CH domains?

With the help of yeast two hybrid screens several groups have (almost simultaneously) described a novel family of small focal adhesion-associated proteins termed actopaxin/parvin/CH-ILKBP/Affixin/CLINT ([48–51]; C.E. Turner, personal communication). The proteins contain two unconventional CH domains in 'tandem' which do not match the consensus of the type 1/2 ABDs and are in addition separated by a 60-residue linker region. Three genetic isoforms of actopaxin/parvin (α, β and γ) have been identified [49]. CH-ILKBP is identical to the β isoform, as is affixin. CLINT, by contrast is most homologous to β-parvin, but contains a unique amino terminal extension. Sequence analysis demonstrates a higher similarity of the first CH domain of actopaxin with the type 1 CH domain of spectrin, whereas the second CH domain appears to have more in common with the type 1 CH domain of α-actinin. Hence, one of the names, parvin, was chosen to document that this molecule might be a 'tiny' α-actinin [49]. However, in agreement with the analysis presented by Olski et al. [49] both actopaxin/parvin CH domains form a separate phylogenetic branch, similar to the CH domains of fimbrin. Most remarkably, Turner et al. [48] characterised a conserved paxillin binding site (PBS) in the loop between helices A and B of actopaxin's second CH domain. Paxillin can bind to the second CH domains in the absence of the first (tentatively named type 5 and type 4 CH domains, respectively), and the second (type 5) CH domain of actopaxin (affixin; CH-ILKBP) also binds integrin-linked kinase (ILK), but does not require the PBS for this binding. The specificity of this latter interaction is further underscored by a prominent cellular localisation in both focal and fibre-like adhesions, but also

by the abolition of ILK binding (and adhesion localisation) by a single point mutation of F271D in the type 5 CH domain [51]. Assuming the general fold of a CH domain, this phenylalanine residue (which is unique to the α and β actopaxins/parvins) resides in the centre of the A helix in close proximity to the autonomous PBS. However, recent studies by Nikolopoulos and Turner ([52]; C.E. Turner, personal communication) have seriously questioned the effectiveness of this mutation for ILK binding and focal adhesion targeting of actopaxin/parvin and these authors suggested that the F271D mutation rather affects paxillin binding due to structural perturbation of the neighbouring PBS. The actin binding potential of actopaxin/parvin proposed to reside in the N-terminal type 4 CH domain still requires some additional investigation.

6. Conclusions

6.1. Structure/function

CH domains display an unusually high structural conservation but appear, by comparison, to be highly diverse in their biological functions. To fully understand the variety of functions displayed by the several CH domains more information on the atomic structure of the module is needed. The first (A) helix of the CH domain and the region bridging between helix A and helix B may contain the relevant information for functional diversity while the more C-terminal (C, F and G) helices may contribute primarily to the structural conservation. In accordance with this assumption the most prominent differences between the five CH domain families are found in the A–B loop (e.g. DFRXXL and PiP binding motifs in type 1 and type 2 CH domains in α-actinins; 5–7 residue inserts in the type 3 CH domains of GEF family proteins; functional ILK and PBSs in the type 5 CH domains of actopaxin/parvin), and even entire individual subfamilies (like α-actinin, plectin, CaP, Vav etc.) display unique conserved residues in the A helix.

Our understanding of the potential influence of the type 2 CH domains is only in its infancy. With a better understanding of how certain types of CH domains can contribute to protein function we will be able in the future to create chimera to investigate the level of independence and cooperativity between the individual CH domains in an ABD. The discovery of the CH domain has fuelled a new branch of research on ABDs, the impact of which may be only appreciated in the years to come. Certainly, we may expect new insights into the alteration, stabilisation and dynamics of actin structures (and possibly also other cytoskeletal components) by CH domains in the near future and it is conceivable that CH domain family proteins play a considerable role in the regulation of the cytoskeleton and as adaptors and regulators for signaling molecules.

6.2. Evolution

CH domains represent a conserved structural unit used in a variety of modes to display different functions on its surface. The potential involvement of CH domains in regulating the actin cytoskeleton, but potentially also of microtubules and intermediate filaments may be taken as support of the above. The general principle of polymer formation and the resulting exposure of interfaces along the surface of these polymers may be directly related to the conservation of a general structure, able to recognise these sites. Thus, the evolutionary aspect of CH domain function adds another facet to the issue. Both single type 3 and tandem type 1 and type 2 CH domain proteins have been identified from yeast to man.

The phylogenetic tree in Fig. 4, based on a structure-oriented sequence alignment of CH domains clearly reveals the five subfamilies described above. Three have already been identified before [3,53]. The inclusion of recently discovered proteins of the actopaxin/parvin family in the sequence analysis revealed two new types of CH domains. The first three branches correspond to the CH domains of the cytoskeletal actin binding proteins and to the type 3 CH domains of the CaP family, and of some signaling proteins, respectively. The branching of the CH domain tree must have functional implications. It is, however, not possible to say whether the group of single CH domains is evolutionarily closer to the type 1 or type 2 branch. It is conceivable that the type 2 CH domain of actin binding proteins lost its function and adopted an auxiliary role after an ancient duplication event. Sequence analysis of the entire actin binding region composed of two consecutive CH domains [3] shows close similarity between proteins that bind to intermediate filaments (dystonin, ACF7 and plectin), and the proteins of the spectrin family. In plectin, a domain following the actin binding region is homologous to the first structural repeat of spectrin. This suggests that proteins of the plectin family acquired their actin binding region from an ancestor similar to spectrin by a shuffling mechanism. Another interesting aspect concerns the possible duplication of the actin binding region of fimbrin. The inspection of the evolutionary tree indicates that such an event was relatively early, taking place before the divergence of human fimbrin and its yeast homologue Sac6p.

6.3. Future prospects

The tendency with which we are able to extrapolate from the current status of knowledge points towards a specific function for CH domains in almost every molecular subfamily. CH domains in Vav likely perform different functions than they do in IQGAPs. Even actin binding ABDs are likely to function differently and are certainly regulated by different molecular processes (compare α-actinin and filamin). A detailed analysis of the relative influences and contribution of the individual CH domains is required in order to understand the functions of this module. Comprehensive domain swap studies may help in the future to reveal the enigmatic relationships of the type 1/type 2 CH domains of ABDs and may help to explain how single CH domains function. Studies on the CH domain may have already come a long way since its conception in 1995, but our understanding of the functional diversity of this module is still in its infancy and there is certainly a lot further to go to maturity.

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