Minireview

The spectrin repeat: a structural platform for cytoskeletal protein assemblies

Kristina Djinovic-Carugo[a,*], Mathias Gautel[b,*], Jari Ylänne[c], Paul Young[d]

[a] *Structural Biology Laboratory, Sincrotrone Trieste in Area Science Park, S.S. 14 Km 163,5, 34012 Trieste, Italy*
[b] *Max-Planck-Institut für Molekulare Physiologie, Abteilung Physikalische Biochemie, Postfach 500 247, 44202 Dortmund, Germany*
[c] *Department of Biochemistry, University of Oulu, Linnanmaan, 90570 Oulu, Finland*
[d] *Department of Neurobiology, Bryan Research Building, Duke University Medical Centre, Research Drive, Durham, NC 27710, USA*

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Abstract Spectrin repeats are three-helix bundle structures which occur in a large number of diverse proteins, either as single copies or in tandem arrangements of multiple repeats. They can serve structural purposes, by coordination of cytoskeletal interactions with high spatial precision, as well as a 'switchboard' for interactions with multiple proteins with a more regulatory role. We describe the structure of the α-actinin spectrin repeats as a prototypical example, their assembly in a defined antiparallel dimer, and the interactions of spectrin repeats with multiple other proteins. The α-actinin rod domain shares several features common to other spectrin repeats. (1) The rod domain forms a rigid connection between two actin-binding domains positioned at the two ends of the α-actinin dimer. The exact distance and rigidity are important, for example, for organizing the muscle Z-line and maintaining its architecture during muscle contraction. (2) The spectrin repeats of α-actinin have evolved to make tight antiparallel homodimer contacts. (3) The spectrin repeats are important interaction sites for multiple structural and signalling proteins. The interactions of spectrin repeats are, however, diverse and defy any simple classification of their preferred interaction sites, which is possible for other domains (e.g. src-homology domains 3 or 2). Nevertheless, the binding properties of the repeats perform important roles in the biology of the proteins where they are found, and lead to the assembly of complex, multiprotein structures involved both in cytoskeletal architecture as well as in forming large signal transduction complexes.

Key words: Cytoskeleton; Spectrin repeat; Actin filament; α-Actinin; Spectrin

1. Introduction

Cytoskeletal structures are both highly dynamic as well as highly stable cellular scaffolds, maintaining and modelling cell shape, providing routes for intracellular traffic, and organizing networks for inter- and intracellular communication. The cytoskeleton consists of a number of filamentous systems, composed of polymers of actin, tubulin or intermediate filament proteins. These form the filaments of actin stress fibers, microtubules or intermediate filaments. Their filamentous state provides the cell with internal scaffolds which maintain cell shape and provide routes for intracellular traffic. The dynamic exchange of subunits, and the linking of the different systems by multi-specific cross-linking proteins, allows the rapid remodelling in response to altered mechanical needs.

An important family of cytoskeletal proteins are those cross-linking actin filaments, or linking actin filaments to the cell membrane. These proteins include the actin cross-linking protein α-actinin, the actin-bundling protein filamin, or the membrane-associated actin-binding proteins spectrin, dystrophin and utrophin.

A common structural element of all of these proteins is evidently an actin-binding domain, arranged in specific spatial relationship to other functional motifs by protein domains with a predominantly architectural function. Filamin is assembled predominantly from immunoglobulin (Ig)-like domains, and the spectrin/dystrophin/α-actinin family from spectrin-like repeats. In the following, the structural functions of this motif, as well as its increasingly recognized function as a protein interaction module, will be discussed.

In this article we will shortly review the proteins that contain spectrin repeats, discuss the structural and functional aspects of the domain and use the spectrin repeats of α-actinin as an example to illustrate some functional features.

2. Spectrin repeats in diverse proteins

The spectrin repeat is a domain composed of three α-helices (Fig. 1). A number of aromatic residues in the hydrophobic core of the domain are typically conserved. Structurally, the spectrin repeat can be distinguished from other three-helix domains via its characteristic length, its left-handed twist and localization of the termini to the distal ends of the domain. The spectrin repeat seems to be specific to the evolution of the animal kingdom.

Spectrin repeats are best known from the spectrin superfamily of proteins (spectrin, α-actinin, dystrophin and utrophin), in which they are typically found together with actin-binding calponin-homology (CH) domains, EF-hand type motifs, calcium-binding motifs and various signalling domains.

*Corresponding authors.
E-mail addresses: djinovic@elettra.trieste.it (K. Djinovic-Carugo), mathias.gautel@mpi-dortmund.mpg.de (M. Gautel).

Abbreviations: PH, pleckstrin homology; SH3, src-homology domain 3; GEF, GDP/GTP exchange factor; Ig, immunoglobulin

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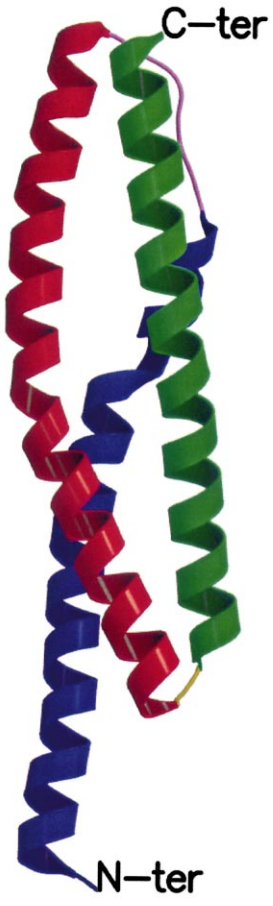

Fig. 1. Ribbon presentation of the second spectrin repeat of α-actinin. Helices 1, 2, and 3 are depicted in blue, red and green, respectively. Ribbon diagrams were generated using programs Molscript [41], and Raster3d [42].

Typically, there are several (from 4 to over 20) consecutive spectrin repeats in these proteins. The genome projects have revealed some novel members of this family, including new heavy isoforms of spectrin. Additionally, several transcripts or putative proteins have been found containing spectrin repeats without the other hallmark domains of the spectrin superfamily. Some of these sequences contain multiple spectrin repeats with signalling domains such as Rho-GEF (GDP/GTP exchange factor), PH (pleckstrin homology), src-homology domain 3 (SH3), WW, Sec14, GAS2 and protein kinase domains, as well as with structural motifs like ZZ zinc-finger, Ig, fibronectin-3, and plectin domains. In addition, there are a couple of protein sequences with only one or few predicted spectrin repeats together with signalling domains. For instance, the DPN protein contains a potential Sec14-homology domain, one or two spectrin repeats, and Rho-GEF, PH and SH3 domains. The dbl proto-oncogene contains a single spectrin repeat together with a Rho-GEF domain. This pattern is expanded by numerous further spectrin repeats in the Rho-GEFs trio and kalirin. (for an updated overview of spectrin repeat-containing proteins visit, e.g., the SMART site at EMBL, http://smart.embl-heidelberg.de).

3. What is the function of a spectrin repeat?

Traditionally, spectrin repeats have been viewed as modules that are used to build long, extended molecules. This fits well with the observation that they normally occur in multiple copies and seem to separate other functional parts of the proteins. For instance, in the actin cross-linking proteins of the spectrin superfamily, they control the specific distance between functional domains at the N- and C-termini [1]. It is the length in this spacer that determines whether the protein would bundle or cross-link actin filaments. However, this seems not to be the only function of spectrin repeats. Dbl, as mentioned above, contains only a single spectrin repeat together with non-modular sequences and the Rho-GEF domain, both of which are domains which are not involved directly in cytoskeletal (architectural) functions. Recently, the ability of spectrin repeats to serve as a docking surface for cytoskeletal and signal transduction proteins has been widely accepted. In addition, certain spectrin repeats seem to have specialized in making dimers, determining in this way the functional molecular architecture of the overall multimeric protein. Furthermore, the mechanical properties of spectrin repeats are also interesting. They are often found in structures that are exposed to great mechanical stress, such as the cell cortex, the muscle sarcomere, and stress fibers. Single-molecule measurements have shown that spectrin repeats have elastic properties that might be important for the deformability of the cell cortex [2]. Further elastic contributions may arise from structural flexibility between adjacent spectrin repeats [3], and from interactions between separate molecules in the macromolecular assemblies.

4. Structural principle of the spectrin repeat

The fold of the spectrin repeat is determined by three α-helices in a coiled-coil assembly (Fig. 1). Antiparallel coiled coils have also been referred to as bundles [4]; here the coiled-

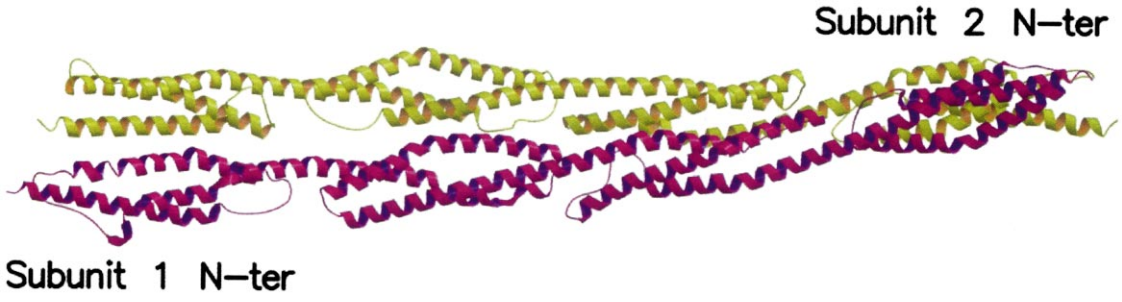

Fig. 2. Ribbon presentation of the α-actinin rod. The two antiparallel subunits of the α-actinin rod are depicted in yellow and magenta, respectively. Note the antiparallel arrangement of the interacting pairs of spectrin repeats and the 90° twist in the quaternary structure. Ribbon diagrams were generated using programs Molscript [41], and Raster3d [42].

Table 1
Interactions mediated by spectrin-like repeats

Spectrin repeat-containing protein	Interacting protein	Domain mediating the interaction	Methods	Reference
Spectrin				
β-Spectrin (rep 15–17)	Munc13	Doc2 interacting domain	Y2H, in vitro bind	[37]
β-Spectrin (rep 15)	ankyrin	spectrin-binding domain	in vitro bind	[38]
β-Spectrin (rep 1)	F-actin	F-actin	actin Co-Sed	[13]
Dystrophin				
Dystrophin (rep 11–14)	F-actin	F-actin	actin Co-Sed	[12,14]
α-Actinin				
α-Actinin 2 (rep 2–4)	ALP	PDZ	Y2H, in vitro bind, Co-IP	[27]
α-Actinin 1,4 (rep 1–4)	CLP36	PDZ	Co-IP	[31]
α-Actinin 1 (rep 2–3)	α-catenin	residues 325–394	Y2H, Co-IP	[39]
α-Actinin 1,2 (rep 3)	PKN	N-terminal region	Y2H, Co-IP	[35]
α-Actinin 2 (rep 3–4)	myotilin	N-terminal 215 residues	Y2H, in vitro bind	[26]
α-Actinin 1,2 (rep 2–3)	titin	Zq-region	Y2H, in vitro bind	[28]
α-Actinin 2 (rep 2–4)	NMDAR	NR1, NR2B cytoplasmic tails	Y2H,	[23]
α-Actinin 1 (rep 1–4)	ICAM2	cytoplasmic domain	peptide-binding assays	[19]
α-Actinin 2 (rep 3–4)	FATZ	carboxy terminal region	Y2H	[25]
Kalirin				
Kalirin (rep 4–6)	PAM	cytoplasmic domain	Y2H, in vitro bind	[40]

Homo- and heterodimeric interactions of α-actinin and spectrin are not included. Only interactions which have been unambiguously mapped to the spectrin repeat regions of the stated proteins are shown. Abbreviations: rep (repeats); NMDAR (*N*-methyl-D-aspartate receptor); PAM (peptidylglycine α-amidating monooxygenase); Y2H (yeast two-hybrid assay); in vitro bind (in vitro-binding assays); Co-IP (Co-immunoprecipitation); actin Co-Sed (actin co-sedimentation assays).

coil term will be used following Brown et al., 1993. The helices (1, 2 and 3) forming the domain are not straight but curve gently and wrap around each other in a left-handed supercoil. Comparison of available structures of spectrin repeats (R16 [5], R17–R18 [3], R2–R3 from α-actinin [6], R1–R2–R3–R4 from α-actinin [7]) reveals that the repeats are structurally very similar. The major differences – as expected – reside in the connecting loops, which differ in length and conformation. The heptad periodicity has been suggested to promote stable folding of a single spectrin repeat [8]. The periodicity can be observed in the primary sequence, and is clearly reflected in the three-dimensional structure. As is customary, the amino acids forming each heptad repeat are termed *a* to *g*, with residues *a* and *d* constituting the hydrophobic core of the coiled coil. Apart from the hydrophobic packing in the core, electrostatic interactions between charged side chains of variable residues mainly at *g* and *e* positions also contribute to the stabilization of the repeat fold.

The striking feature that emerged from crystal structures of constructs composed of two or four consecutive spectrin repeats (R17–R18 [3], R2–R3 from α-actinin [6]), R1–R2–R3–R4 from α-actinin [7] is the clearly α-helical region between the repeating units (Fig. 2). Originally, the linker region was predicted to be non-helical [9], however, subsequent analyses have predicted a helical linker region [8,10]. In the structures, there is no obvious break, discontinuity or change in the secondary structure to delineate the linker region. This region can be defined from the end of the linker of one repeat to the beginning of the heptad periodicity of the subsequent repeat. The linearity of the linker together with the topology of the triple-helix coiled coil adopted by a single repeat leads to the formation of elongated, non-globular structures. A feature common to structures of multiple spectrin repeats in a row is a twist between the relative orientations of the repeats. In the α-actinin rod, the twist leads to a structure in which the terminal faces of the rod are related to each other by an angle of 90° (Fig. 2), resulting in important functional implications for cross-linking of actin filaments by α-actinin in different cytoskeletal scenarios.

5. The α-actinin rod structure as a paradigm of spectrin repeat-based cytoskeletal proteins

The domain arrangement of α-actinin reveals four spectrin repeats between actin-binding domains composed of two CH domains at the N-terminus of the protein, and a calmodulin-homology domain with EF-hand type motifs at the C-terminus of the protein. α-actinin forms an antiparallel homodimer, in which the rod domain composed of spectrin repeats separates the head domains. In electron microscopy images, the rod domain is rather rigid, and there seems to be a flexible hinge region between the rod and the head.

α-actinin is thought to be an ancient member of the spectrin superfamily [5,36], from which the larger actin cross-linking proteins are believed to have evolved by multiple duplication events of the spectrin repeats. The N-terminus of α-actinin, containing the actin-binding CH domains and spectrin repeats 1 and 2, is highly homologous to the N-terminus of β-spectrin. The C-terminal repeats 3 and 4 and the EF-hands of α-actinin are again homologous to the C-terminus of α-spectrin. The major difference between the α-actinin type of spectrin repeats with most others is that their inter-repeat linker sequences are longer, while most of the repeats in spectrin have a short linker sequence, which probably leads to partial stacking of the consequent repeats. The linear organization of α-actinin type spectrin repeats together with specific surface properties of individual subunits allows them to form dimers. In the dimer, the spectrin repeats 1 and 2 form a long interaction surface with the repeats 4 and 3. This seems to be the case also with the homologous repeats of spectrin. Dystrophin and utrophin do not have α-actinin-type spectrin repeats and they were not reported not to form dimers.

Clearly, the specific feature of the spectrin repeats in α-actinin is dimer formation. The crystal structures of the rod domain of the human muscle α-actinin isoform [6,7] revealed that the dimer interface spans the whole length of the curved rod domain. The curved interface is probably important for stabilizing the rod structure. An emerging general structural principle of high affinity protein interaction sites is indeed a

curved interface. This interface also causes the total α-actinin rod domain to be axially twisted by roughly 90° from one end to the other. The twisted structure may make the rod mechanically more stable. Another consequence of the twist is that the actin-binding domains are also rotated by 90° in relation to each other.

The crystal structure of the α-actinin rod domain also allowed analysis of the surface features and the prediction of possible protein–protein interaction sites. In general, the rod surface is rather smooth and acidic. One long surface is predominantly acidic. When the conservation of surface residues is plotted on the model, the acidic surface emerges as the most conserved area [7]. This is suggestive of a conserved interaction surface. A literature survey revealed that the cytoplasmic domains of transmembrane receptors that have been reported to interact with the α-actinin rod are predominantly basic (see also Table 1). This led us to suggest that the acidic surface of the rod domain would be an interaction site for these cytoplasmic domains [7]. The symmetry properties of the rod domain predict that there would be two symmetry-related interaction sites. These hypotheses are currently being tested.

6. Interactions of spectrin repeats

As we have outlined above, the spectrin repeat module seems to have initially arisen as a self-association motif in an ancestral α-actinin type molecule [11]. Since then, spectrin repeats in the various descendant molecules have evolved the ability to interact with many other proteins. Table 1 lists some proteins, which have been shown to bind to spectrin repeats. It should be noted that for many proteins reported to bind spectrin superfamily members, particularly those that were characterized biochemically, the exact binding sites have not been mapped, thus the list in Table 1 is obviously incomplete. The power of the yeast two-hybrid system in identifying and mapping interactions is evident from the number of spectrin repeat-associated proteins discovered using this method in the last 5 years. The proteins listed in Table 1 are diverse, and their association with spectrin repeats does not seem to be mediated by a common motif or domain. Indeed, many of the interactions are mediated by sequences that do not contain any identifiable domains. However, many of the interactions can be grouped on a functional basis.

6.1. Structural interactions

Spectrin superfamily members function as flexible scaffolding molecules in the cell cortex where they link the actin cytoskeleton to the plasma membrane. This role is especially important in cells that are under extreme mechanical strain such as muscle cells or erythrocytes. Many of the repeat interactions therefore serve as links to either the cytoskeleton or the cell membrane, often providing multiple attachments to these structures. In both spectrin and dystrophin, the repeats have been shown to contribute to actin binding [12,13]. A cluster of basic repeats in the middle of the dystrophin rod region can bind actin and may align dystrophin molecules partly along the actin filaments [12,14]. In spectrin, repeat 1 has been shown to increase the affinity of the adjacent actin-binding domain for actin, and has led to a model where each spectrin molecule may contact two or more actin subunits [13]. Thus, the actin-binding properties of dystrophin and spectrin may be modified by binding of the repeats to actin filaments.

The repeats can provide either direct or indirect links to transmembrane proteins. In erythrocytes, the spectrin–actin network is attached to the membrane in large part by the interaction of repeat 15 of β-spectrin with ankyrin, which in turn binds the cytoplasmic domain of the anion exchanger ([15] and references therein). Direct linkage of spectrin repeat proteins to the cytoplasmic domains of transmembrane proteins is probably more common, and α-actinin, for example, can interact with the cytoplasmic domains of integrins [16,17], ICAMs [18,19], L-selectin [20], Ep-CAM [21], ADAM12 [22] and NMDA receptor subunits [23]. For all of these interactions, the binding site in the cytoplasmic domains of these proteins has been mapped to relatively short basic peptides. The crystal structure of the α-actinin rod reveals a conserved acidic surface which has been postulated as a potential binding site for these peptides [24]. Precise mapping of the binding sites for these proteins on α-actinin, as well as future structural studies will be required to confirm if this is indeed the case.

6.2. Dynamic and regulatory interactions

A distinct group of proteins includes the proteins FATZ (filamin/ABP-L, α-actinin and telethonin binding protein of the Z-disc), ALP (actinin-associated LIM protein), myotilin, and titin which interact with the spectrin repeat region of skeletal muscle α-actinin, and which have been localized to the sarcomeric Z-disk of striated muscle [25–28]. Titin can also interact, via its alternatively spliced Z-repeats, with the calmodulin-like C-terminal domain of α-actinin [29,30]. Together these titin interactions seem to be involved in regulating the number of α-actinin cross-links and thereby the thickness of the Z-disc in different muscle types. The zinc-finger protein ALP interacts with the spectrin repeats of α-actinin via a PDZ domain, similarly to CLP36 [27,31]. Initially, ALP was regarded as a candidate gene in facioscapulohumeral muscular dystrophy, but has since been excluded [32]. It seems to play a dynamic role in control of muscle growth. FATZ and myotilin are unrelated but share the property of also binding to a muscle-specific isoform of filamin [25,26,33]. Additionally, FATZ was reported to interact with telethonin, a protein involved in myofibrilogenesis and which binds the N-terminus of titin in the Z-disk [25]. The importance of these interactions is highlighted by the identification of mutations in myotilin, which cause limb girdle muscular dystrophy 1A [34]. Lastly, an example of a protein that may play a mainly regulatory role in cytoskeletal rearrangement involving α-actinin is the rho-kinase type protein kinase N (PKN), which interacts with both R3 of the rod and the C-terminal calmodulin-like domain [35]. Some of these proteins, like myotilin or PKN, are not constitutive components of the Z-disk. Myotilin appears only at later stages of muscle differentiation and after initial myofibril formation. These observations suggest a dynamic exchange of α-actinin ligands during the life and differentiation of a cell.

Clearly, very complex networks of protein interactions centered on α-actinin exist. The spectrin repeats serve as multivalent binding sites for these interactions. They include structural proteins, cell membrane receptors, zinc-finger proteins, protein kinases, and scaffolding/adaptor proteins. Identifying when, and where, all these interactions occur during develop-

ment, as well as how they are regulated, will be a big future challenge.

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