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Review

A new twist to coiled coil

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ABSTRACT

Spectrin repeats have been largely considered as passive linkers or spacers with little functional role other than to convey flexibility to a protein. Whilst this is undoubtedly part of their function, it is by no means all. Whilst the overt structure of all spectrin repeats is a simple triple-helical coiled coil, the linkages between repeats and the surface properties of repeats vary widely. Spectrin repeats in different proteins can act as dimerisation interfaces, platforms for the recruitment of signalling molecules, and as a site for the interaction with cytoskeletal elements and even direct association with membrane lipids. In the case of dystrophin several of these functions overlap in the space of a few repeats.
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1. Introduction

The presence of multiple coiled coil modules, and in particular spectrin repeats is a common feature of cytoskeletal linking proteins, or cytolinkers. Cytolinker was a term originally coined to describe plectin [1] but has now been adopted to describe a broader group of proteins including plakins, nesprins and spectrin family proteins, all of which contain multiple spectrin repeats. The presence of spectrin repeats in these cytolinkers can mediate self association and permits flexible and perhaps extensible linkages so the proteins can connect between different cytoskeletal filament systems, or between cytoskeletal systems and cellular membranes. However in recent years evidence has emerged of a role for the spectrin repeat as a binding interface in its own right, making direct protein–protein interactions and in some cases protein-lipid interactions.

2. Overview of the family of spectrin repeat containing proteins

More than 97% of known spectrin repeat containing proteins are found in metazoans with over two thirds of those in chordates [2]. Despite scattered examples of spectrin repeats in all other kingdoms, this would tend to suggest that the spectrin repeat arose with the evolution of the animal kingdom. With the exception of one or two outliers, as mentioned above, most proteins are considered as cytolinkers and can be broadly grouped into 2 or 3 families depending on ones perspective. The eponymous family from which the repeat derives its name includes the proteins α-actinin, spectrins themselves and dystrophin and utrophin. These proteins share a variable number of spectrin-like repeats, from 4 in α-actinin to 24 in dystrophin, and depending on the protein also have an amino-terminal actin binding domain comprising tandem CH domains and carboxy-terminal calcium binding EF hands. In addition different family members have acquired additional domains specific to their cellular functions, including PH, SH3, WW and ZnF (Fig. 1A). Full listings and domain compositions can be found in several online databases for example SMART, PFAM and Domain Club (http://smart.embl-heidelberg.de/, http://pfam.

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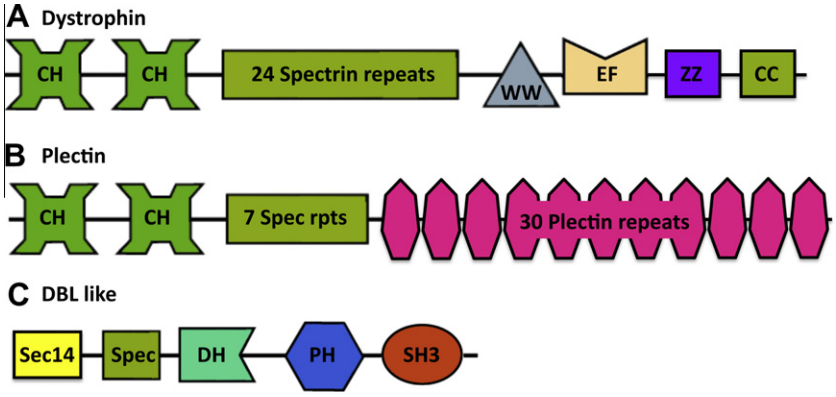

Fig. 1. Schematic representation of selected members of spectrin repeat containing proteins from the three main families. (A) Dystrophin; (B) Plectin and (C) DBL-like. Modules are coded according to the style and nomenclature of Pawson and Nash [53] with the addition of the Sec14 domain [54] (yellow box) and ZZ domain [55] (purple box) and plectin repeats (magenta heptagons). Definitive publications to the modules shown above are: CC [56] CH [57] DH [58] EF [59] PH [60,61] Plec [62] SH3 [63,64] Spec [7] WW [65].

sanger.ac.uk/, http://pawsonlab.mshri.on.ca/DomainClub/domainClub.php). For the purposes of this review we will discuss in more detail new functions and properties of the spectrin repeats of the spectrin family member dystrophin.

The plakin family of proteins is characterised by the presence of plectin repeats and spectrin repeats often interspersed with other sequences with a propensity to form dimeric coiled coils. Other modules found in plakins include tandem CH domains, EF hands, SH3 and KASH again dependent on cellular function (Fig. 1B). Plakin family proteins function to interconnect different cytoskeletal filament networks with each other and directly to membranes or membrane associated structures. Depending on their domain composition they variously connect intermediate filaments via their plectin repeats (e.g. desmoplakin, plectin and some nesprin isoforms), actin filaments via their CH domains (plectin, MACF1 and some nesprin isoforms) and microtubules (plectin, MACF1). In turn they associate with other membrane anchored proteins in the plasma membrane at adhesion sites such as costameres, desmosomes and hemidesmosomes. The plakins also have roles in organelle positioning including mitochondria and Golgi, and in the maintenance of nuclear membrane connectivity to the cytoskeleton as well as the structure of the nuclear lamina. Readers are referred to more authoritative reviews for details of these functions [3,4].

In addition to multiple repeated copies of spectrin repeats in the cytolinker proteins mentioned above, spectrin repeats also occur sparsely in some Rho family guanine nucleotide exchange factors (RhoGEFs) including the RhoGEF DBL, its big sister MCF2L and more distant relatives such as trio and kalirin. With the exception of DBL itself, these all contain an amino terminal SEC14 domain, followed by one or more spectrin repeats followed by one or more copies of the DH and PH domains characteristic of GEFs also with SH3 and S/T kinase domains (Fig. 1C). Loss of the spectrin repeat in kalirin alters is effects on actin based structures such as dendritic spines [5]. In a similar manner deletion of the spectrin repeat from Dbl contributes to its oncogenic potential by removing binding sites for HSc70 and a ubiquitin ligase that serve to maintain low steady state levels of the protein [6]. Thus the functions of the spectrin repeats in kalirin and Dbl appear to control GEF function and/or targeting of the GEF activity.

3. Spectrin family repeat structure

The core elements of the spectrin repeat are a triple-helical coiled-coil bundle, with the 3 helices forming the domain gently curving and wrapping around each other in a left-handed supercoil (Fig. 2). The archetypal spectrin repeat structure obtained from the direct protein sequencing of spectrin in the early nineteen eighties revealed a repeating 106 amino acid sequence with conserved periodic hydrophobic and charged residues [7]. Predicted sequences for dystrophin and α-actinin obtained slightly later by DNA sequencing also revealed similarities to the repeating regions of spectrin [8–10] but with slightly different average repeat lengths of 122 and 109 residues for α-actinin and dystrophin, respectively. An evolutionary relationship has also been proposed for this protein family from a likely α-actinin ancestor and subsequent diversification to spectrins and then dystrophin/utrophin [11–13]. The repeats of α-actinin and spectrin are known to form dimers, indeed, the regular repeat length and conserved surface charge particularly in the e and g positions in the heptad lends itself to dimerisation. By convention the amino acids in helices are lettered from a to g to represent the 7 residues per two turns of the helix, i.e. the heptad. Whilst hydrophobic residues at the a and d positions in the heptad, a hallmark of a triple-helical coiled coil, are conserved in dystrophin and its autosomal homologue utrophin, they both lack the conservation of repeat length and charged residues at the e and g positions in the heptad to form stable dimers [14–16] (Fig. 2).

The first structures of single spectrin repeats as predicted [17] revealed tight triple α-helical coiled-coils [18,19]. However these single repeat structures did not reveal the true spectrin coiled coil structure due to either the long helix folding back on itself or the repeat dimerising. It was only later when multiple repeats were solved that the continuous relationship between the helices in the repeat junction was elucidated [20–22] (Fig. 2). Furthermore the crystal structures of a repeat pair from α-spectrin in multiple crystal forms revealed the potential flexibility of spectrin repeats [21] whereas structures of the four spectrin repeats from α-actinin yielded a rather rigid dimerised structure [20,22]. As noted above, dystrophin repeats are more variable in length and have more frequent insertions in the helices [14] (Fig. 2). In addition, and in contrast to spectrin molecules, four predicted hinges separating the rod region into three sub-regions were speculated to confer additional flexibility to the molecule (Fig. 3) [9]. The alpha-helical nature of the dystrophin spectrin-like repeats was confirmed, however additional residues were required to extend the helices into the adjoining helices in order to produce a stable fold [23–25]. These studies suggested that the dystrophin repeats may fold in an overlapping or nested manner with the structural integrity of each repeat being reliant in part on its neighbours [16,26,27]. This

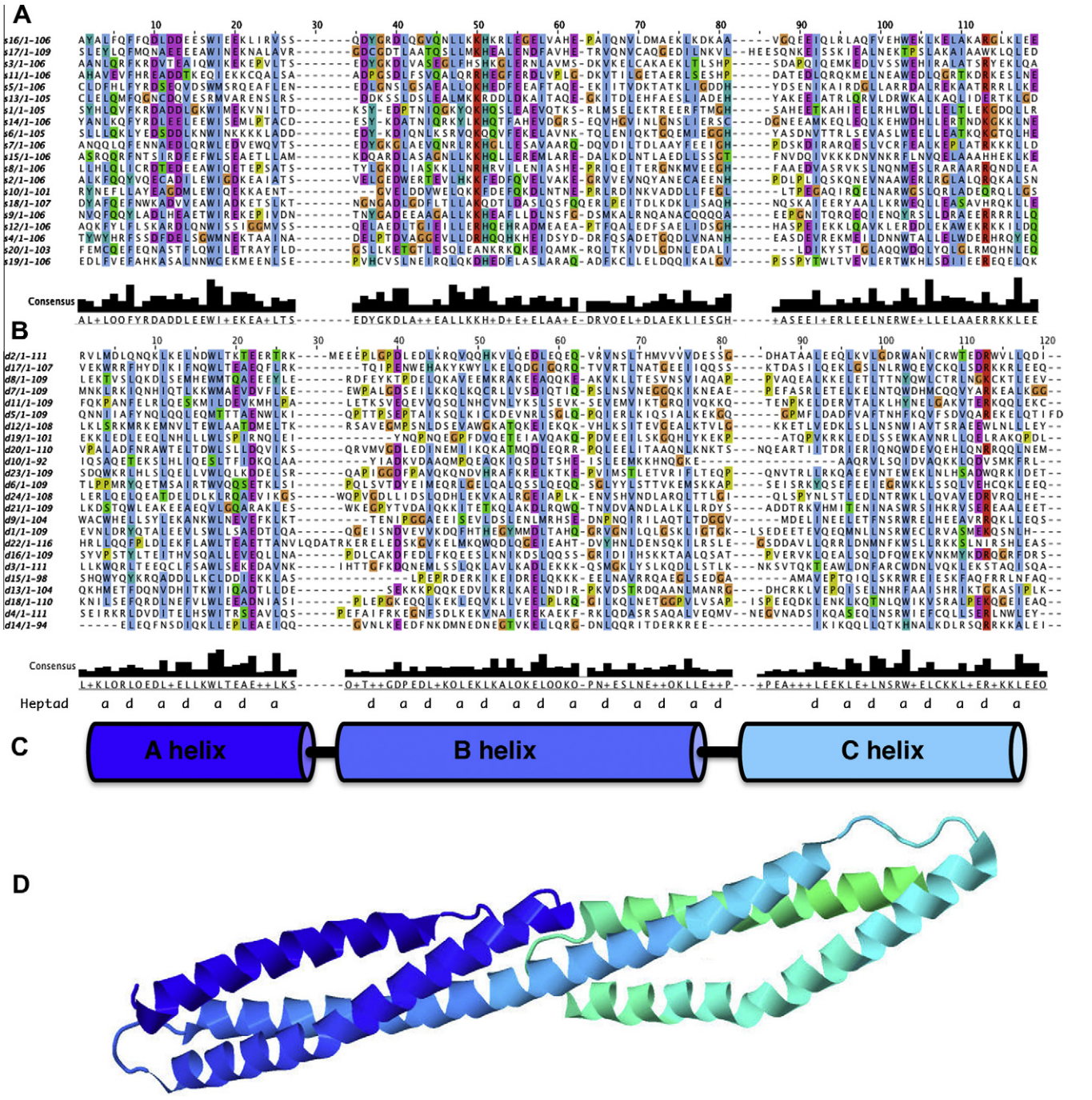

Fig. 2. Clustal X derived sequence alignments and consensus sequence with confidence histogram in black, of the repeats from α-spectrin (A) and dystrophin (B). The original sequence alignments from [14] were 'realigned' using Clustal X with the BLOSUM weight matrix 'gap creation' and 'gap extension' penalties set to maximum to preserve the repeat boundaries of the original alignment. Conservation of the hydrophobic residues in the a and d positions of the heptad in blue is clear for spectrin and dystrophin, however compared to dystrophin, the central spectrin B helix has a consistent length and has fewer proline residues (yellow) at the boundaries. Furthermore there is a much higher degree of conservation in other positions in the heptad in α-spectrin repeats as indicated by the magenta (acidic), red (basic) and green (polar) with glycines in orange. (C) Is a cylinder representation of the helices in a single repeat that by convention are named A, B and C and coded by colour to match the actual structure of an α-spectrin repeat pair [66] shown in D. As can be seen in (D) the C helix in the N-terminal repeat (pale blue) is continuous with the A helix in the C-terminal repeat (sky blue).

remains an unresolved problem, whilst we have some biochemical understanding of the folding properties of the dystrophin repeats, see for example [28] and reviewed in [29], we are still no closer to a true structure of any of them. Nonetheless considerable insight has been gained recently into dystrophin spectrin-repeat structure and function from comprehensive molecular modelling studies. By systematically analysing all successive pairwise dystrophin repeats along the length of the dystrophin it was found that the inter-helical linkers were for the majority helical, but for some containing proline residues, these regions were modelled as small loops and interpreted as likely points of additional flexibility [30]. The same points are also observed between the equivalent repeats in utrophin but not in any spectrins [30]. Furthermore, analysis of the surface properties of the repeat pairs revealed considerable differences in hydrophobic and electrostatics surfaces [30], a degree of variability which is in contrast to the uniformity of

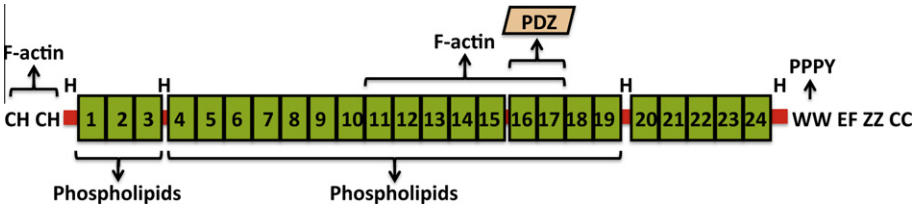

Fig. 3. Schematic overview of spectrin repeat interactions in dystrophin. In keeping with established nomenclature [67] 4 hinge regions (H) are shown in red, however a fifth extended region between repeats 15 and 16 which should probably be considered a hinge [68] is also depicted in red. Precise molecular details of which single repeats interact with F-actin, phospholipids and the PDZ domain of nNOS are not known, and are therefore bracketed by repeat number. As can be seen repeats 16 and 17 have overlapping functions as discussed in the text. The key interactions of the tandem CH domains with F-actin [69] and the WW domain with the PPPY of β-dystroglycan [70] are also represented.

structure and the probable uniformity of surface properties of spectrin repeats (Fig. 2A). In turn it is this variability that is likely to confer unique properties to the dystrophin repeats that will be discussed in more detail below.

4. Spectrin-repeat interactions in dystrophin

4.1. Actin binding

Many members of the spectrin-repeat containing protein family interact with the actin cytoskeleton. In most cases direct interaction with F-actin is mediated by a pair of amino-terminal CH domains, a well characterised actin binding module found in a large number of F-actin binding proteins [31]. However in addition to the CH domain mediated actin binding activity, the Ervasti lab also characterised a second major actin binding region in dystrophin situated in repeats 11–17 [32,33] (Fig. 3). Whilst the affinities of either the CH domain actin binding site and the dystrophin-repeat actin binding site are individually relatively low, when present together in the same molecule they act synergistically to provide a high affinity interaction with F-actin [33,34]. Despite the high degree of sequence homology over the whole length of dystrophin and utrophin, utrophin does not share the same actin binding functions in the central repeats [35]. Repeats 11–17 of dystrophin are relatively basic in nature which facilitates an electrostatic interaction with F-actin, whereas the equivalent repeats in utrophin are acidic. However, the addition of up to ten canonical repeats to the utrophin CH domains stabilised the interaction between utrophin and actin increasing the affinity by up to 20-fold [36]. The addition of up to two repeats to the β-spectrin actin binding domain had a similar effect [37], but in both the case of β-spectrin and utrophin the repeats have no intrinsic actin binding properties alone and absolutely require the CH domains. Despite both dystrophin and utrophin being able to interact along the length of actin filaments through at least half of their molecular length, the binding was not competitive, with both proteins able to interact with F-actin simultaneously [34]. One consequence of the binding of dystrophin or utrophin laterally on actin filaments is to greatly increase the torsional flexibility of actin [38], a property that could be of great functional significance for the protection of the sarcolemma during the deformation induced by contraction and relaxation.

4.2. Lipid Binding

Mapping of the lipid binding properties of dystrophin repeats revealed that repeats 1–19 can bind to anionic lipids whereas repeats 20–24 do not [39,40]. This has led to the suggestion that dystrophin may in fact associate directly with the membrane and for part of its repeat region lies along the membrane, reviewed in [29]. Furthermore biophysical studies of the lipid binding repeats 1–3 and the non-lipid-binding repeats 20–24 revealed that the interaction of repeats 1–3 with membrane lipids was largely electrostatic with no modification of the helical secondary structure of the protein [41]. This is in contrast to the interaction of repeat 14 of β-spectrin with lipids, which undergoes significant unfolding in order to interact with phospholipids [42]. Of the dystrophin repeats that do interact with lipids, repeats 11–15 are unique in that they are able to bind to both anionic and zwitterionic lipids [40]. Interestingly repeats 11–17 have also been demonstrated to interact with F-actin [33] (see above). A more detailed biophysical analysis of repeats 11–15 revealed that this region is able to create very strong protein networks at the interface of both anionic and zwitterionic lipid membranes [43]. This confirmed the amphiphilic nature of these repeats and their propensity to spread onto the membrane surface dependent on the surface pressure and the lipid packing. Given that the interaction of dystrophin is dependent on the surface pressure of the lipid monolayer, these properties are likely to have important biological consequences as they could relate to the association of dystrophin with the sarcolemma during changes in surface pressure due to the muscle contraction-relaxation cycle.

The ability of repeats 11–15 to bind to actin and to lipid membranes suggests a possible role for these associations in stabilising the membrane. Neither dystrophin repeats 11–15 not F-actin alone has any significant effect on the viscoelasticity of the lipid membrane, however when actin and dystrophin repeats 11–15 are added together there is a highly significant increase in membrane stiffness that is dependent on dystrophin repeat concentration and actin polymerisation, as well as lipid type and surface pressure [44]. Taken together these findings present a new paradigm for the functional role of dystrophin in protecting the membrane from contraction-associated damage by forming a more continuous bridge between sarcolemma and underlying cytoskeletal elements such as actin, a role mediated by the dystrophin repeats.

4.3. nNOS

Neuronal nitric oxide synthase (nNOS) is a sarcolemma associated enzyme important for skeletal muscle vasomodulation [45]. nNOS is lost from the sarcolemma due to the loss of dystrophin and associated proteins in Duchenne muscular dystrophy [46]. Evidence from Becker muscular dystrophy patients who lack only part of the dystrophin gene, and from the *mdx* mouse, a model of Duchenne muscular dystrophy, highlighted a requirement for the repeat region of dystrophin [47–49]. However experiments in *mdx* mice also highlight a requirement for syntrophin, one of the dystrophin associated proteins [48], though syntrophin alone is not sufficient to restore nNOS to the sarcolemma [49]. More detailed analysis of a potential ternary complex between nNOS, dystrophin repeats and syntrophin reveals a direct interaction between the PDZ domain of nNOS an repeats 16 and 17 of dystrophin [50].

5. Biomedical significance

A significant proportion of DMD mutations arise in the repeat region of dystrophin, many as a consequence of deletions of one or several exons and nonsense mutations. Recent advances in molecular medicine now make it theoretically possible to correct these mutation using exon-skipping strategies, see [51] for a recent comprehensive review. However for these strategies to be successful, one not only requires a knowledge of genomic structure and protein structure but also protein function. The importance of maintaining the phasing of the repeats in dystrophin has been realised for some time [14], however it is only more recently that the importance of which dystrophin repeats are required for a functional rescue of dystrophic muscle has become apparent, reviewed in [29]. As an aide to basic and clinical scientists alike we have developed an eDystrophin database devoted to the analysis and human DMD mutations and prediction of resulting protein structure with particular emphasis on the repeat region. The eDystrophin database is available: http://edystrophin.genouest.org/ [52]. As is apparent from the analysis described above, the overlapping functions of actin binding, phospholipid binding and nNOS binding makes the restoration of dystrophin repeats 11–17 critical to the success of any therapy.

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