Chapter 12
Dystrophin and Spectrin, Two Highly Dissimilar Sisters of the Same Family

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Abstract Dystrophin and Spectrin are two proteins essential for the organization of the cytoskeleton and for the stabilization of membrane cells. The comparison of these two sister proteins, and with the dystrophin homologue utrophin, enables us to emphasise that, despite a similar topology with common subdomains and a common structural basis of a three-helix coiled-coil, they show a large range of dissimilarities in terms of genetics, cell expression and higher level structural organisation. Interactions with cellular partners, including proteins and membrane phospholipids, also show both strikingly similar and very different behaviours. The differences between dystrophin and spectrin are also illustrated by the large variety of pathological anomalies emerging from the dysfunction or the absence of these proteins,

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D.A.D. Parry, J.M. Squire (eds.), *Fibrous Proteins: Structures and Mechanisms*, Subcellular Biochemistry 82, DOI 10.1007/978-3-319-49674-0_12

showing that they are keystones in their function of providing a scaffold that sustains cell structure.

Keywords Dystrophin • Spectrin • Scaffolding protein • Coiled-coil fold • Membrane binding • Genetic disease

12.1 Introduction

Dystrophin is a unique protein coded by a single gene, which is expressed in differentiated smooth and striated muscular and nervous cells. Spectrins, on the other hand, are a large family of several proteins, coded by several genes, leading to a high number of isoforms that are ubiquitously expressed in numerous cell types. Dystrophin and its homologue utrophin are each coded by a single gene, *DMD* and *UTRN* (Table 12.1) respectively. These two genes are associated with the expression of unique proteins (Koenig et al. 1987; Tinsley et al. 1992). However, in the case of dystrophin, several promoters allow the tissue-specific expression of shorter isoforms of dystrophin compared to the full length protein. Utrophin is only known as the full length transcript (Helliwell et al. 1992; Tinsley et al. 1992). Unlike the two single genes for dystrophin and utrophin, spectrins are coded by numerous genes. In mammals, two genes, *SPTA1* and *SPTAN1,* code the two major α isoforms of spectrin, αI and αII, respectively. αI is characteristic of erythroid cells (Sahr et al. 1990). In contrast to *SPTA1*, alternative processing of the transcript of the *SPTAN1* gene gives rise to at least four isoforms of αII spectrin (Cianci et al. 1999). Moreover, four genes (*SPTB, SPTBN1, SPTBN2, SPTBN4*) code for conventional β spectrins (β-erythrocytic and βI, II and IV, respectively; Winkelmann et al. 1990; Stankewich et al. 1998; Berghs et al. 2000) and one gene (*SPTBN5*) codes for the large βV spectrin (Stabach and Morrow 2000). Variations in mRNA splicing in the case of isoforms βI, βII and βIV generate longer or shorter C-terminal regions (Berghs et al. 2000; Hayes et al. 2000; Table 12.1). The expression of the isoforms is tightly regulated in a tissue- and time-dependent manner, which is a consequence of the

Table 12.1 Genes coding dystrophin, utrophin and spectrins in *Homo sapiens*

Gene	Chromosome	Protein
DMD	X	Dystrophin
UTRN	6	Utrophin
SPTA1	1	Spectrin α erythrocytic (αI)
SPTAN1	9	Spectrin α non-erythrocytic (αII)
SPTB	14	Spectrin β erythrocytic
SPTBN1	2	Spectrin β non-erythrocytic (βI)
SPTBN2	11	Spectrin β non-erythrocytic 2 (βII)
SPTBN4	19	Spectrin β non-erythrocytic 4 (βIV)
SPTBN5	15	Spectrin β non-erythrocytic 5 (βV)

structural complexity of mammalian cells. Various intracellular locations (e.g. plasma membrane, Golgi apparatus, endoplasmic reticulum, nucleus) of spectrins reflect the variety of their functions. Only three spectrin isoforms are present in *D. melanogaster* and *C. elegans*, where the α subunit is encoded by a gene resembling mammalian *SPTAN1* and the β subunits correspond to mammalian βII and βV (Dubreuil and Grushko 1998).

Dystrophin, utrophin and the spectrins share the unique fold of the so-called "spectrin repeat", while they also have specific additional domains. Due to their similar spectrin repeats, they have been attributed to the same vast "spectrin" family of proteins (Parry et al. 1992). The spectrin family also includes α-actinin (see Ribiero Ede et al. 2014) which we will not discuss further in this Chapter. However, the general topology of dystrophin, utrophin and the spectrins is different, with dystrophin and utrophin existing as monomers and spectrins as homodimers or higher homo-oligomers of αβ-heterodimers. Their genes are all subject to mutations, but mutation types are very specific for each gene, with the vast majority being deletions of exons for dystrophin and point mutations for spectrins. The effect of out-of-frame mutations in the *DMD* gene is, in most cases, to cause the total absence of dystrophin in the musculature, which is highly deleterious and leads to Duchenne muscular dystrophy (DMD), with patients having an expected life span of less than 30 years. The effects of in-frame mutations are highly specific for the *DMD* gene and can lead to a shortened dystrophin, which partially maintains the dystrophin function and leads in most cases to Becker muscular dystrophy (BMD), with an expected life span that is very variable. In-frame point mutations of spectrins primarily affect the tetramerization processes.

Until now, the functions of dystrophin, utrophin and the spectrin proteins have not been well defined at the molecular level. They are largely thought to be scaffolding proteins interacting with numerous partners, some of them well documented, but there are probably many unknown partners. Their scaffolding function means that they are an important part of the structure of the cell cytoskeleton. However, understanding their function at a molecular level is only in its infancy, partly due to the large size of these proteins: from 71 to 427 kDa for the longest isoform of dystrophin and ranging between 246 and 430 kDa for spectrins.

12.2 Dystrophin, Utrophin and Spectrin Structural Domains

Dystrophin is a monomeric protein comprising 3685 residues in the longest isoform, which is present in muscle and heart cells (Fig. 12.1: Koenig et al. 1987, 1988). Several promotors allow for the tissue-specific expression of isoforms of various lengths. There are three full length isoforms. Dp427m is expressed in striated muscles, where it appears at the internal face of the sarcolemma. Dp427c is found in heart and glial cells (Koenig et al. 1989) and in brain and retina (Nudel et al. 1989; Chelly et al. 1990). Dp427p occurs in Purkinje cells and in skeletal

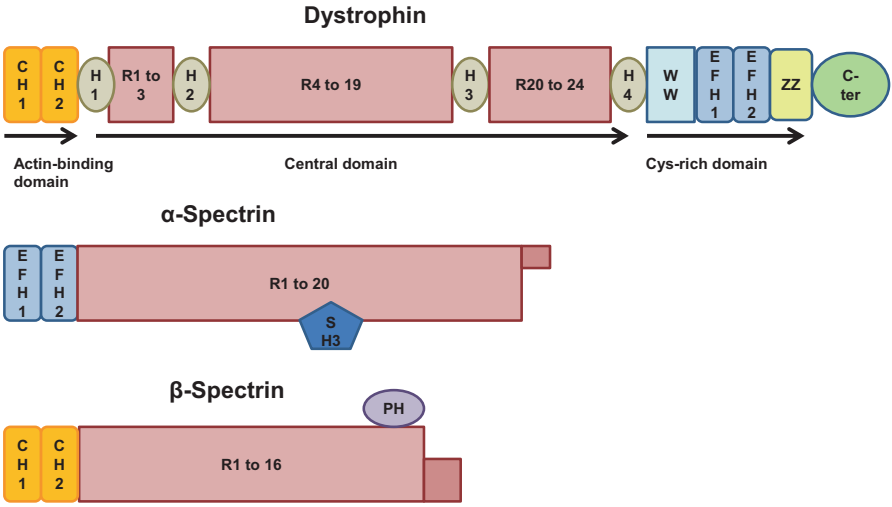

Fig. 12.1 Dystrophin and spectrin structural domains. CH1 and CH2, calponin homology domains, constituting the actin-binding domain ABD1; H1–H4, unstructured domains called hinges 1–4; R1–24, spectrin repeats, constituting the central domain; WW, Tryptophan-rich domain, EFH1 and EFH2, EF hand domains 1 and 2 and ZZ, ZZ domain, constituting the Cys-rich domain; C-ter, C-terminal domain, SH3, SH3 domain, PH, PH domain

muscle (Chelly et al. 1990). The shorter isoform, Dp160, is expressed in retina (D'Souza et al. 1995), Dp140 in brain, retina and kidney (Lidov et al. 1995), Dp116 in Schwann cells (Byers et al. 1993) and Dp71 in all tissues except skeletal muscles (Hugnot et al. 1992).

Dystrophin comprises four main domains (Le Rumeur et al. 2010). At the N-terminal end there is a first actin binding domain (ABD1) consisting of two calponin homology (CH) domains. It is followed by a very long central domain made of 24 spectrin repeats of about 100–110 residues each. They are interrupted by hinges, one between repeat 3 and 4 and one between repeats 19 and 20. A Cys-rich domain comprises WW, EFH1 and EFH and ZZ domains. The fourth domain is the C-terminal domain (Fig. 12.1). Only a few of these domains have been crystallized and have had their structures solved (Table 12.2). These are the first actin-binding domain ABD1, repeat 1 (R1) and the WW and EFH subdomains in complex with a peptide from β-dystroglycan, an essential partner of dystrophin (see below). Depending on the location of the promotor, the shorter isoforms do not maintain the ABD1 subdomain and do not present all the repeats. As an example, the shortest isoform, Dp71, consists only of the C-terminal end starting at the EFH1 domain.

Utrophin has a similar domain structure to Dystrophin, the only difference being that there are two repeats less than in dystrophin (Winder et al. 1995; Le Rumeur

Table 12.2 Three-dimensional structures of dystrophin, utrophin and spectrin subdomains

Protein	Domain	PDB ID
Dystrophin (H. sapiens)	N-terminal actin binding domain	1DXX
Dystrophin (H. sapiens)	Repeat 1	3UUN
Dystrophin (H. sapiens)	WW, EFH1, EFH2 sub-domains	1EG3, 1EG4
Utrophin (H. sapiens)	N-terminal actin-binding domain	1QAG, 1BHD
Utrophin (R. norvegicus)	Repeat 1	3UUL
Spectrin α (D. melanogaster)	Repeat 14	2SPC
Spectrin α (G. gallus)	Repeats 15, 16, 17	1U4Q
Spectrin α (G. gallus)	Repeats 15, 16	1U5P
Spectrin α (G. gallus)	Repeat 16	1AJ3
Spectrin α (G. gallus)	Repeats 16, 17	1CUN
Spectrin α (G. gallus)	SH3 domain	1SHG, 1 U06
Spectrin α (G. gallus)	Tetramerization site	3F31
Spectrin α (H. sapiens)	Tetramerization site	1OWA
Spectrin αβ (H. sapiens)	Tetramerization sites	3LBX
Spectrin β (H. sapiens)	Repeats 8, 9	1S35
Spectrin β (H. sapiens)	Repeats 13, 14, 15	3KBT
Spectrin β (H. sapiens)	Repeats 14, 15	3F57, 3EDU
Spectrin β (H. sapiens)	Repeats 14, 15, 16	3EDV
Spectrin β (H. sapiens)	CH2 domain	1AA2, 1BKR
Spectrin β (H. sapiens)	PH domain	1WJM
Spectrin β (*M. musculus*)	PH domain	1MPH

et al. 2010). Apart from the ABD1 domain, which is also found in many other proteins, the other domains show high specificity in terms of their structure and interactions (see below).

Spectrin is a large, mostly heterotetrameric protein with a characteristic modular structure of helical spectrin repeats (Fig. 12.1). While there are 20 full-length repeating units and one incomplete repeat in α spectrins, only 16 (or 29 in case of heavy isoforms) repeats, along with one incomplete repeat, occur in β spectrins. The incomplete segments together provide one full spectrin repeating unit after tetramer formation (Kotula et al. 1993; Ipsaro et al. 2010). However, spectrin is much more than just an assembly of spectrin repeats (Machnicka et al. 2014). The 280-kDa α-spectrin also contains EF hands (calmodulin-like domains) at its N-terminus, an SH3 domain within repeat 9, and a short insert (named CCC) within repeat 10 of αII, which includes sites for calmodulin binding and caspase/calpain cleavage (Czogalla and Sikorski 2005). The variety of β spectrins is reflected not only in a broad molecular weight range (246–430 kDa), but also by the presence or absence of C-terminal plecstrin homology (PH) domains. Moreover, an actin binding domain, which consists of tandem calponin homology (CH) domains, is located at the N-terminus of each of the β spectrins.

12.2.1 Common Features of Dystrophin and Spectrin

12.2.1.1 Classical ABD Domains at the N-terminal End of Dystrophin, Utrophin and β-spectrin

Dystrophin, utrophin and β-spectrins are F-actin interacting proteins through the presence of actin-binding-domains either constituted by two CH domains (respectively CH1 and CH2) situated at the N-terminal end of the mentioned proteins (ABD1 in the case of dystrophin) or constituted of spectrin repeats (ABD2) in dystrophin only.

The entire ABD1 domains of dystrophin and utrophin have been crystallized as open conformations stabilized as head-to-tail homodimers (Keep et al. 1999; Norwood et al. 2000). The relative orientation between the CH1 and CH2 subdomains is different in the dystrophin and utrophin structures and it has been proposed that this is associated with the modulation in the sequence of the linker region bridging the CH subdomains. This linker region is folded into an α-helix which could be bent (Fig. 12.2a), and its amino-acid composition (Fig. 12.2b) influences the thermodynamic stability of the whole subdomain, probably through the conformational equilibrium between the open and the closed states of the ABD in solution (Bandi et al. 2015). The solution conformation adopted by the ABD or its conformation after binding to F-actin is still controversial and may vary between dystrophin and utrophin (Sutherland-Smith et al. 2003; Lin et al. 2011; Broderick et al. 2012; Singh and Mallela 2012). Three actin binding sites (ABS) have been distinguished by experimental mapping on the dystrophin ABD1. This mapping indicated that three independent ABS regions are involved in the contact with F-actin. These are from Lys18 to Ala27 (ABS1), from Val89 to Leu116 (ABS2) and from Leu131 to Ser147 (ABS3). These sites mainly involve the CH1 subdomain (residue 9–121) and the N-terminal end of CH2 (Henderson et al. 2010).

The actin binding domain of β spectrin also consists in two CH subdomains, CH1 (residues 51–156) and CH2 (171–282). The CH2 domain was initially supposed to have a key role in the interactions with actin and this is the reason why only the structure of the CH2 domain was determined at high resolution (1.1 Å; PDB ID: 1BKR), but it appears that isolated CH2 domains bind rather weakly to actin as well (Banuelos et al. 1998). Like the corresponding subdomain in dystrophin and utrophin, CH2 is composed of four parallel helices interconnected by longer loops together with three additional helices resulting in a tight and globular structure. The most conservative elements across the various CH domains are the residues forming helices A, C and G, which play a major role in actin binding. Further on in this Chapter, we discuss a phospholipid-sensitive mechanism of actin binding by this domain.

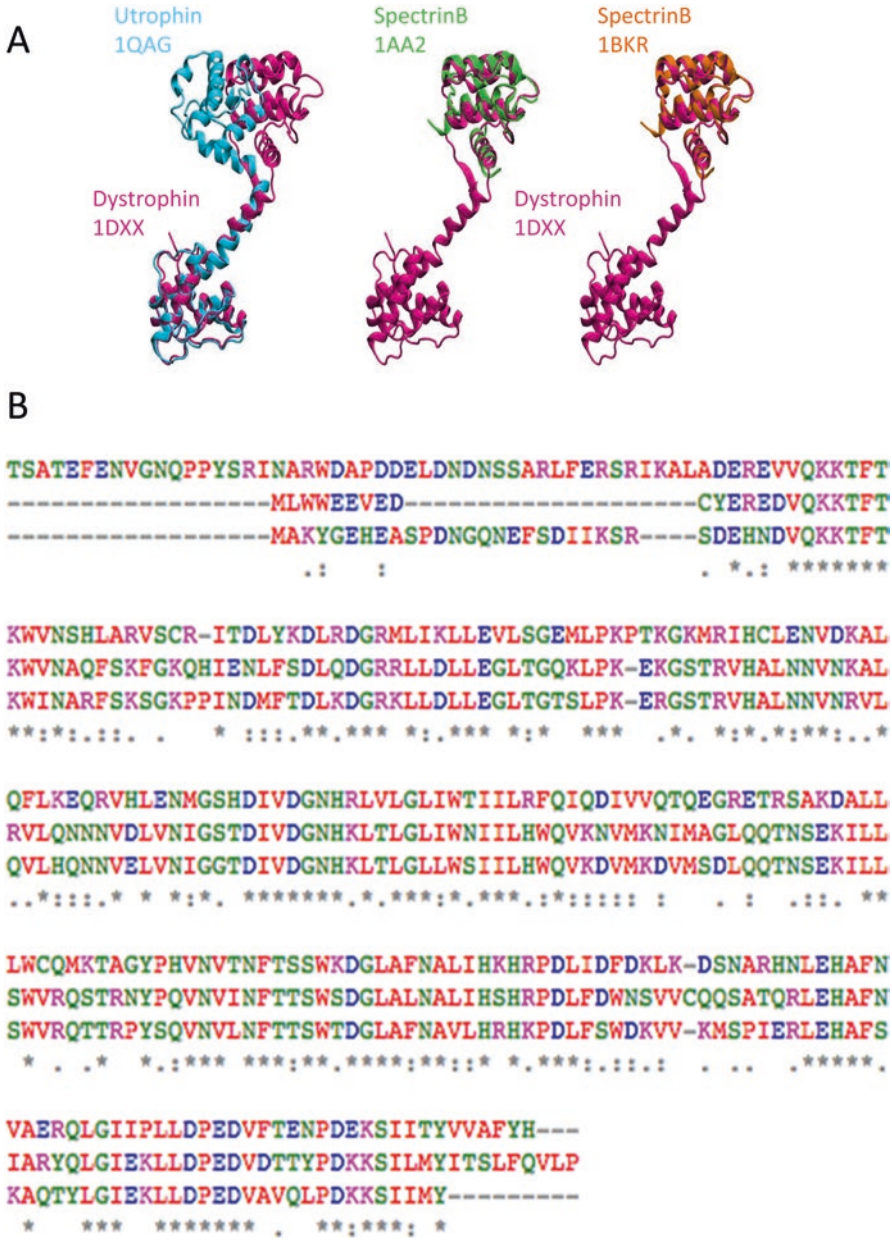

Fig. 12.2 Experimental structures of the actin-binding domain composed of two calponin homology (CH) domains obtained for human dystrophin and utrophin and the two CH2 structures of the β spectrin available in the Protein Data Bank (PDB). (**a**) Backbone representation of the ABD domain emphasizing that the two CH subdomains are mainly composed of alpha-helices. Tridimensional superimposition allows us to establish the very high degree of structural conservation of this domain. (**b**) Primary sequence alignment (spectrin B, dystrophin, utrophin) shows the highest modulations to be located at both terminal ends and at the linker region bridging the two CH subdomains

12.2.1.2 Central Domains are Composed of Repeats with Similar Folds in Triple Helical Coiled-Coils

The common feature of these three proteins is the presence of numerous so-called spectrin repeats made of about 110 residues each and folded in triple helical coiled-coils. These coiled-coils are rather unusual since they are not formed by dimerization as in, for example, myosin or tropomyosin. In this case three successive helices (A, B and C) interrupted by two loops fold back on themselves to produce a three strand coiled coil. The interaction between the three alpha-helices is due to the presence of the classical heptad pattern of residues along the sequence (Winder et al. 1995). The heptad is defined as seven residues labelled "a" to "g" where the residues in position "a" and "d" are preferentially hydrophobic while the other are mostly hydrophilic. This pattern allows the folding in coiled-coils with all the "a" and "d" residues constituting the core of the molecule (Fig. 12.3a).

Spectrin repeats are all rather similar in length being constituted by roughly 106 residues each, but there are variations between 99 and 114 amino acid residues. Unlike spectrins, the related dystrophin repeats are largely heterogeneous in length because of variations in length of their alpha-helices. As an example, repeat 14 composed of 94 amino acids (aa) only has a very short A helix (22 residues) and repeat 10 (92 aa) has a very short C helix (25 residues).

Although the sequence homology between the spectrin repeats does not go beyond 30 % (Leluk et al. 2001), and dystrophin and utrophin share a homology of less than 20 % (Nicolas et al. 2014), all spectrin repeats, as well as dystrophin and utrophin repeats, share a common fold roughly 50 Å in length and 20 Å in diameter. This was first described for the α spectrin of *D. melanogaster* (PDB ID: 2SPC; Yan et al. 1993; Pascual et al. 1997; Muthu et al. 2012; Fig. 12.3b). In a typical spectrin left-handed supercoil segment, helix B is kinked and is slightly longer than the other two helices in the segment (PDB IDs: 1CUN, 1U5P, 1U4Q; Grum et al. 1999; Kusunoki et al. 2004b). However, the positioning of loops interconnecting the helices of individual repeats may vary to some extent (Grum et al. 1999; Kusunoki et al. 2004a). It seems that conservative tryptophan residues are critical for stabilization of the triple-helical structure, although the core of some of the spectrin repeats may deviate from this pattern, as was described for R9 of human β spectrin, where water-mediated hydrogen bonding forms the core (PDB ID: 1S35; Kusunoki et al. 2004a). The tiny dissimilarities among spectrin repeats result in differences in their stabilities. Individual spectrin repeats also have different folding characteristics, as shown for repeats 15–17 of α spectrin (Kwa et al. 2014). Remarkably, helix C and helix A of the next repeat exhibit an uninterrupted helical structure (Fig. 12.3c), thus enabling cooperative propagation of forced unfolding throughout the whole molecule (Kusunoki et al. 2004b; Law et al. 2003). These features, together with a broad array of possible inter-repeat interactions and potential rearrangement of helices within a single repeat, may be keys to understanding the remarkable flexibility of spectrins (Grum et al. 1999; Mirijanian et al. 2007). It is worth underlining that upon full extension the spectrin tetramer is 180–200 nm long, but its physiological rest length is only 55–60 nm. Most recently, a model has been proposed where

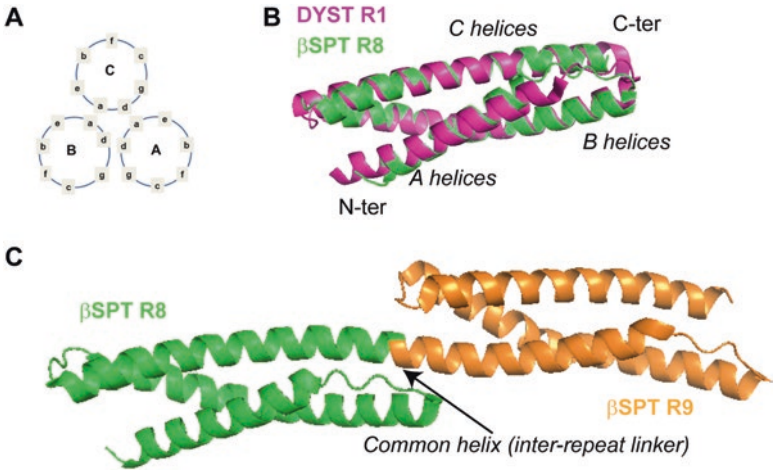

Fig. 12.3 The heptad pattern of the residues in the sequence allows the 3D folding in a triple helical coiled-coil for spectrin and dystrophin repeats. (**a**) Representation as a helix wheel of the coiled-coil structure: the "a" and "d" residues are situated in the core of the molecule while the other residues are accessible to the solvent and for interactions with partners. A, B and C are the three helices of the triple coiled-coil. (**b**) The crystal structures of repeat 8 of β-spectrin (1S35) and repeat 1 of dystrophin (3UUN) are superimposed. The rms deviation between the two structures is 1.333 Å. (**c**) The crystal structure of the R8–9 tandem repeat from β-spectrin showing the common helix constituting the inter-repeat linker joining the repeats

spectrin is a hollow cylinder and the pitch of each spectrin repeat increases during extension (Brown et al. 2015). Inter-repeat linkers do not all appear as helical in dystrophin or utrophin (Legrand et al. 2011; Nicolas et al. 2014).

12.2.1.3 EF Domains at the C-Terminal End of Dystrophin, Utrophin and α-spectrin

The structures of the EF hands of dystrophin have been determined along with the WW domain in complex with a Pro-rich motif of β-dystroglycan (Huang et al. 2000), establishing that the β-dystroglycan interaction with dystrophin is achieved through both WW and EFH1 subdomains. The recognition mechanism has been revealed to be highly similar to what is observed for Pro-rich sequences binding to SH3 domains. Very little is known about utrophin EFH subdomains. The EFH1 and EFH2 of utrophin share respectively 65 % and 77 % of identity with the corresponding subdomains in dystrophin, probably indicating a high degree of structure similarity. The C-terminus of α spectrins also exhibits structural similarities to calmodulin. The two EF-hands (EFH) present in αII spectrins bind cooperatively two calcium ions with rather low affinities in the 50–100 μM range, unlike the analogous structures in αI that do not bind Ca^{2+} (Buevich et al. 2004). It seems that EF-hands stabilize interactions of the CH2 domain of the spectrin dimer with actin

by binding the linker that comes between the actin binding domain and the first β-spectrin repeat (Korsgren and Lux 2010). Moreover, EF-hands can bind some proteins of the membrane skeleton, such as protein 4.2, in a calcium- and calmodulin-dependent manner (Korsgren et al. 2010).

12.2.2 Dystrophin and Spectrin Structural Dissimilarities

Dystrophin is a monomeric protein which has been found to be difficult to crystallize even as small fragments. The only experimentally defined structure was obtained for repeat 1 (R1) constrained to be a dimer induced by an S-S bridge between two repeats (Muthu et al. 2012). This structure was found to be similar to spectrin repeats, post-validating the reliability of homology models obtained previously based on spectrin structures (Legrand et al. 2011). However, this structure does not show how several repeats are chained together in the full length molecule, especially whether or not the linker region is helical across consecutive repeats, and it does not define their relative positioning. Because of this, in a recent study, we obtained structural models of tandem or multi-repeat fragments in solution using small angle X-ray scattering (SAXS) and molecular modelling. This work showed that the multi-repeat fragments present well-defined kinks at some inter-repeat linkers that give a tortuous and complex topology to the whole dystrophin filament (Delalande et al. to be published; Fig. 12.4).

These kinks are situated at specific linkers between successive repeats and are not uniformly distributed all along the central domain of dystrophin. Kinks between spectrin repeats are reminiscent of the angle found at the spectrin ankyrin-binding site, one of the determining features of spectrin-ankyrin interactions (see below). Similarly, the angle observed between repeats 16 and 17 shows that region to be one of the key places for the interaction of dystrophin with neuronal nitric oxide synthase (nNOS; see below; Molza et al. 2015).

Utrophin has not yet been studied with such tools, but it is likely that kinks will be present in utrophin as in dystrophin. These kinks would be placed at different sites than in dystrophin since, unlike dystrophin, repeats 10–15 of utrophin do not bind actin (Amann et al. 1999).

In contrast to dystrophin, the functional units of spectrin are tetramers or higher oligomers (Nans et al. 2011). A broad range of oligomeric states of spectrin in erythrocytes reflects the high degree of elasticity and flexibility of the red blood cell membrane. The α/β heterodimer is stabilized mostly by interactions between two C-terminal and two N-terminal repeats of the α and β subunits, respectively (Begg et al. 2000). The next level of organization is achieved by head to head dimer interactions. The key role in spectrin tetramerization is played by the incomplete repeats of both subunits. The roughly 30 residue long N-terminus of α spectrin forms a single helix and the roughly 70 C-terminal residues of β spectrin fold into two helices. These two structural motifs interact to reconstruct a full triple helical structure where hydrophobic interactions are the major players (PDB ID:

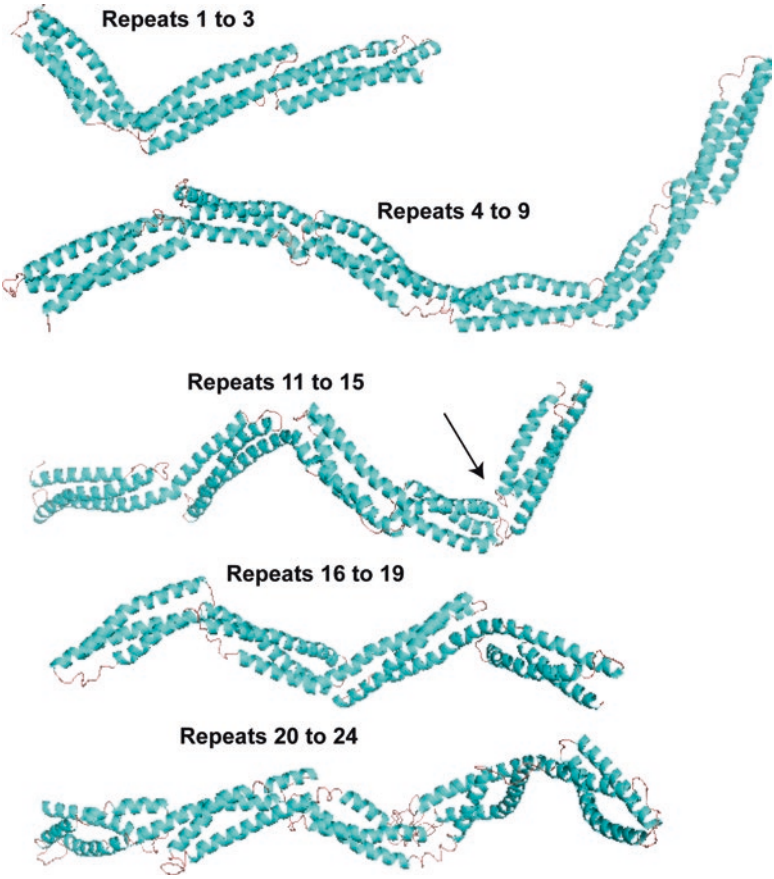

Fig. 12.4 Structural models of multirepeat fragments of dystrophin obtained by small angle X-ray scattering and molecular modeling. Five of the eight fragments studied are shown here. The N-terminal ends are to the left. They all show kinks placed at inter-repeat linkers with angles as high as 90° between repeats 14 and 15 in the R11–15 fragment (*arrow*)

3LBX; Ipsaro et al. 2010). The tetramerization regions of αI and αII (PDB IDs: 1OWA and 3F31, respectively) are not equivalent; the linker following the first helix is either unstructured or helical, respectively (Mehboob et al. 2010). However, after binding β spectrin, the conformation of the linker is helical in both cases. This feature may contribute to the differences in flexibility between various isoforms of spectrin. Although the general model presumes spectrin tetramers or higher oligomers as prerequisite elements of the membrane skeleton, tetramerization of nonerythroid spectrin appears to be unnecessary for normal development of *D. melanogaster* (Khanna et al. 2015).

In all α spectrins, repeat 9 is interrupted by an SH3 domain (Robertsson et al. 2005). The core of this domain consists of antiparallel β strands that form two β-sheets perpendicular to each other (PDB ID: 1SHG; Musacchio et al. 1992).

Recently, more and more data point out the role of the SH3 domain of spectrin in numerous processes connected to cell signalling and repair of DNA interstrand cross-links (for review see Machnicka et al. 2014).

Some isoforms of β spectrin have an extended C-terminus where a PH domain can be found (Macias et al. 1994; Zhang et al. 1995). Seven antiparallel β strands that form a barrel and a subsequent conservative α-helix are the core of such structural motifs, which are known to occur in numerous eukaryotic proteins involved in signal transduction, organization of cytoskeleton and many other cellular processes. Although little is known about the physiological role of the PH domain of spectrin, the positively charged lipid-binding surface seems to be sufficient to bind the inositide head group of PIP_2 (Hyvonen et al. 1995). Complexation to inositol-1,4,5-triphosphate seems to induce ordering of the binding loops when compared to the structure of the free domain in solution (PDB ID: 1MPH; Nilges et al. 1997). On the other hand, it appears that the presence of the PH domain is necessary for membrane targeting of spectrin in midgut copper cells of *D. melanogaster*, but the mechanism is not related to PIP_2- binding activity (Das et al. 2008).

12.3 The Scaffolding Function of Dystrophin and Spectrins: Binding to Phospholipids and Organizing Protein-Protein Assemblies

The first impression when looking at the structures of spectrins and dystrophin is that their repeating units are just modules that build proteins of elongated shape and are predominantly responsible for the observed structural and mechanical properties. In that sense, dystrophin has long been considered as a rod-shape protein linking the cytoskeletal element actin to the extracellular matrix (Koenig et al. 1988; Ervasti and Campbell 1991). However, more and more data show that such modules are also binding sites for a number of cytoskeletal and signal transduction proteins, as well as for phospholipid membranes (Machnicka et al. 2014; Djinovic-Carugo et al. 2002; Boguslawska et al. 2014b; Le Rumeur et al. 2003; Legardinier et al. 2009; Ervasti and Campbell 1993a; Lai et al. 2009; Prins et al. 2009).

12.3.1 Dystrophin and Spectrin: Two Coiled-Coil Filaments Interacting with Membrane Lipids

Phospholipid binding properties of dystrophin are essential for its main biological function to maintain the cohesion of the sarcolemma during compression and re-extension of the membranes of muscle cells. It is well established that a major part of the dystrophin central domain binds to membrane lipids, but with a heterogeneous interaction pattern all along the filament length (Fig. 12.5). The cartography

of the dystrophin central domain emphasizes that, despite a common structural pattern, different repeats show highly different molecular surface properties which define sub-domains involved in specific interactions with various partners (Legrand et al. 2011; Nicolas et al. 2014). For example, repeats R1–R3 strongly interact with anionic lipids, but repeats R20–R24 do not show any similar binding properties (Legardinier et al. 2008). This C-terminal end non-binding feature seems to be a significant difference between dystrophin and spectrin binding properties. Anionic lipids also bind strongly with the subdomain comprising repeats R4–R19 of dystrophin (Legardinier et al. 2009). Another localized binding feature has also been revealed for some repeats of the central domain. For instance, since it binds very weakly to large unilamellar vesicles (LUVs) compared to small unilamellar vesicles (SUVs), except when the LUVs contain PE and/or cholesterol, the binding properties of dystrophin repeat R2 were revealed to be highly sensitive to lipid packing (Le Rumeur et al. 2007). In addition, cholesterol has been shown to increase the insertion of several repeats of dystrophin into the membrane (Ameziane-Le Hir et al. 2014).

For some parts of the central domain, circular dichroism analysis indicated a lower degree of inter-helix interaction in the coiled-coil filament upon binding with phospholipids. This is notably the case in three-repeat fragments like R4–6, R6–9, R12–14 or R17–19. However, coiled-coils should open only partly upon lipid binding, since circular dichroism measurement modulations are not comparable to what can be observed in TFE. Such dynamic behaviour was not observed for longer fragments including R11–15 and R14–17. Among the specific binding profiles encountered along the central domain, the R11–15 fragment seems to be the only subdomain to bind to PC/PE lipids (Legardinier et al. 2009; Sarkis et al. 2011).

Both erythroid and non-erythroid spectrins possess an ability to interact with phospholipids, which has been proven for natural and model membranes (for review see Boguslawska et al. 2014a). Although spectrin repeats exhibit a general lipid binding ability, high affinity sites have recently been recognized (Fig. 12.5). These include PS recognizing repeats 8–10 of the α subunit, and repeats R2–4 and R12–14 of β spectrin (An et al. 2004a, b), as well as a binding site for PE-rich membranes within R14 of β spectrin which partly overlaps with the ankyrin binding domain (described below). Ankyrin-sensitive binding of PE-rich lipids was identified within the N-terminal part of the ankyrin binding domain of both β erythrocytic and β non-erythrocytic spectrin (Hryniewicz-Jankowska et al. 2004; Bok et al. 2007). Further structural studies showed that lipid binding activity is confined to helix C of R14, the highly amphipathic character of which correlates with its mixed 3_{10}/α-helical conformation (Czogalla et al. 2007). Remarkably, interaction with PE/PC membranes or detergents provokes partial opening of the coiled-coil structure of the 14th repeat (Czogalla et al. 2008; Fig. 12.6). The current structural model of the ankyrin-dependent lipid binding site of β spectrin suggests that one of the tryptophan residues within the 3_{10}/α-helix is slightly shifted outside the hydrophobic core of the helix bundle and thus could initiate interactions with membranes (Wolny et al. 2011). Another spectrin region that interacts with phospholipids is the actin binding domain (ABD). Binding of PIP_2 triggers a conformational switch from a closed to

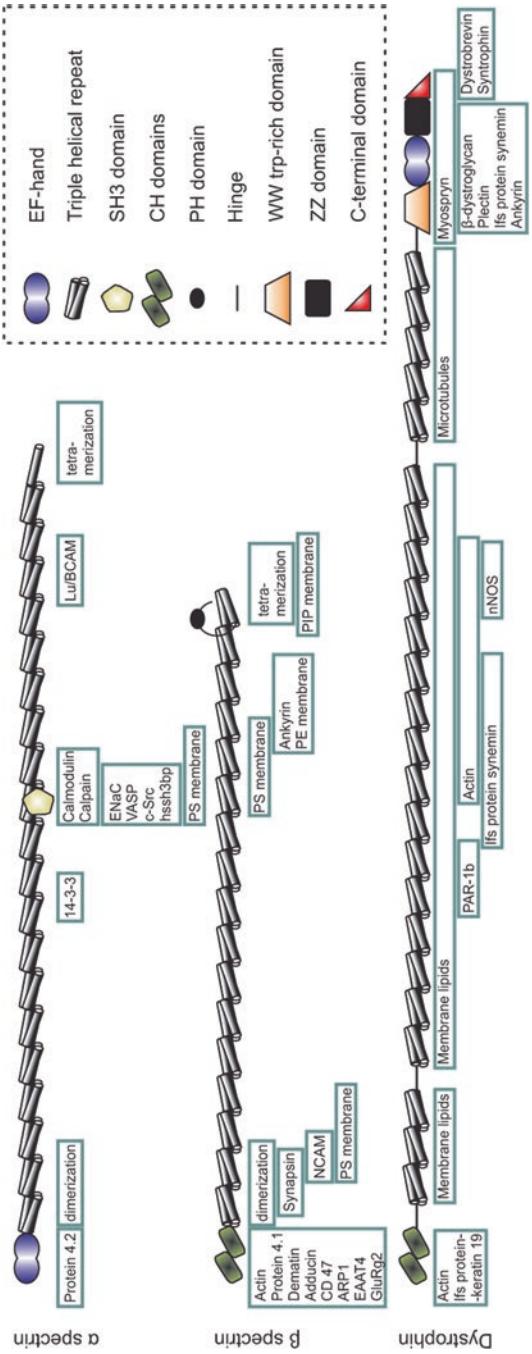

Fig. 12.5 Interaction partners of spectrin and dystrophin. Interacting molecules are included in the rectangles with their locations roughly corresponding to sites of interactions

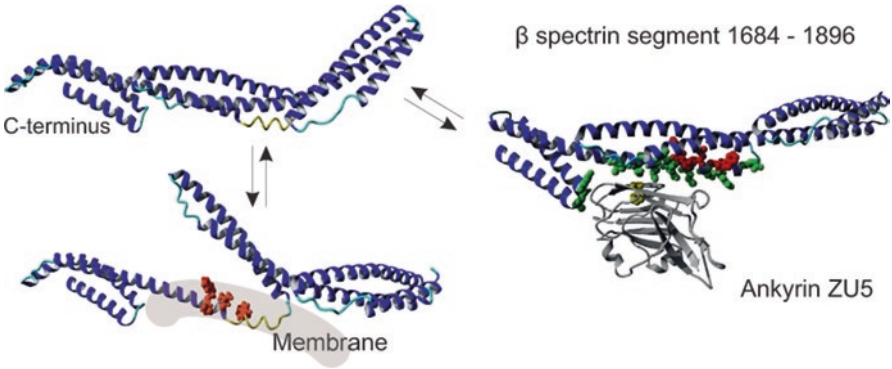

Fig. 12.6 The ankyrin-lipid binding site of spectrin. The triple helical repeats are represented by the ankyrin-sensitive lipid-binding domain within the 14th segment of β-spectrin (ribbon representation of segments 13–15). Membrane binding results in the "open" conformation (amino-acid residues essential for lipid binding in *red*: W1771, L1775, M1778, W1779, all within helix 14C; the 3_{10} helix is *yellow*). Alternatively, steric interference upon the interactions of spectrin with the ZU5 domain of ankyrin (*gray*) blocks the "opening" of the triple helical bundle – PDB ID: 3KBT (amino-acid residues crucial for ankyrin binding in *green*, residue F917 of ZU5 in *yellow*) (Adapted from Boguslawska et al. 2014b)

an open conformation of the CH1-CH2 tandem. This does not influence binding of actin, but enhances interactions with the second target for ABD, namely protein 4.1 (An et al. 2005). On the other hand, structural studies on the CH domains of α-actinin suggest that opening of the domains influences their affinity for actin (Galkin et al. 2010). Out of the various domains of spectrin, the plecstrin homology domains present in some of the isoforms of β subunits seems to be the best candidates for lipid membrane anchors. However, the PH domain of spectrin binds weakly to phosphatidylinosites without specificity towards these lipids, in contrast to the PH domains from some other proteins (Lemmon et al. 2002). Provided that multiple spectrin tetramers form a network containing many lipid binding domains, interactions with membranes may be very strong due to the resulting multivalent character.

12.3.2 Examples of Protein Partners of Dystrophin and Spectrin

The first protein partner of dystrophin to be deswcribed is β-dystroglycan (Campbell and Kahl 1989), which together with dystrophin and other interacting proteins forms the dystrophin-glycoprotein complex (DGC: Fig. 12.7; Ervasti and Campbell 1991), (b). Among muscle membrane proteins dystrophin only interacts with β-dystroglycan, which in turn interacts with intrinsic membrane proteins such as the sarcoglycans. Sarcoglycans and β-dystroglycan interact with proteins of the

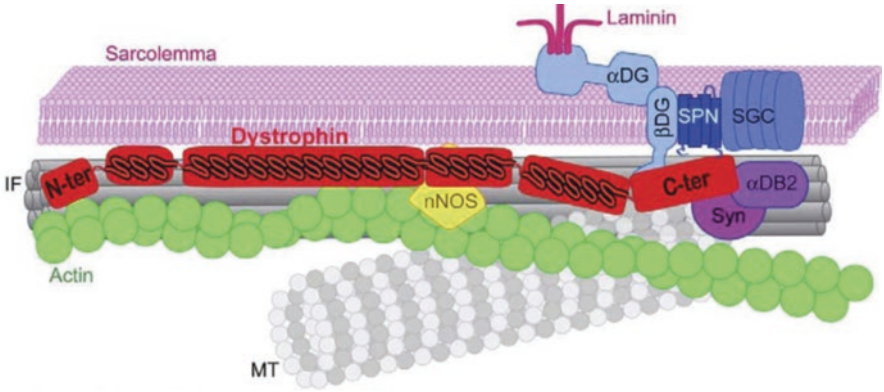

Fig. 12.7 Schematic view of the dystrophin associated complex (DGC). Illustration of the scaffolding role of the dystrophin (in *red*) resulting in the link of sarcolemmal components (in *blue*) to the cytoskeletal protein network. (*MT* microtubule, *IF* intermediate filaments, *DG* dystroglycan, *Syn* syntrophin, *DB* dystrobrevin, *SGC* sarcoglycans)

extra-cellular matrix, including α-dystroglycan and laminins (Ervasti and Campbell 1993a). Intriguingly, dystrophin interacts with three different types of cellular filaments; filamentous actin (Ervasti et al. 1997), intermediate filaments (Bhosle et al. 2006) and microtubules (Prins et al. 2009), which together form a crucial filamentous network for striated muscles. Other proteins involved in signalling or physiology of the muscle or with other unknown functions are also important partners of dystrophin. These include neuronal Nitric Oxide Synthase (nNOS), the polarity associated protein PAR1-b, dystrobrevin, syntrophin and myosprin (Fig. 12.5). The nNOS–dystrophin interaction has been the most intensively studied since it was established that loss of nNOS localization at the sarcolemma was related to an increase in muscle weakness or damage (Sander et al. 2000; Sato et al. 2008) and to a more severe phenotype in patients with mutated dystrophin (Gentil et al. 2012). The primary nNOS binding site was first localized in the dystrophin central domain, involving both repeats R16 and R17 (Lai et al. 2009, 2013). Work combining biochemical experiments, structural data and molecular modelling revealed the association mode of the nNOS PDZ subdomain with the R16-R17 repeats (Molza et al. 2014, 2015). Since PDZ subdomains form classical homodimers, the non-canonical association of PDZ with a three-helix coiled-coil structure seems at first to be similar to the structural organization of the spectrin-ankyrin complex (a beta sheet lying on a three-helix bundle filament bearing a kink; Fig. 12.8). However, detailed analysis of the two structures reveal as many similarities as differerences. The kink in the coiled-coil axis observed at the linker region between successive repeats involved in the interaction with the partner is present in both structures and seems essential for the stabilization of the association. But topologically, as ankyrin binds the spectrin filament at the linker region between two repeats, its contact with the beta sheet is mainly achieved with the end of the helix C of the first repeat and with the loop bridging helices B and C of the second repeat. In contrast, nNOS-PDZ binds

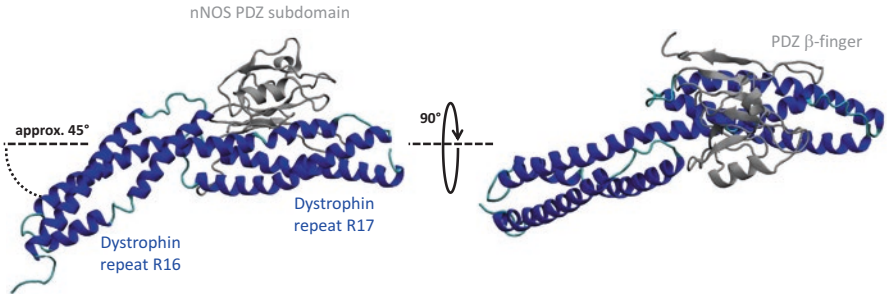

Fig. 12.8 Structural model for nNOS-PDZ–dystrophin R16–17 complex based on biochemical mapping of the interface. Three-helix coiled-coil repeats R16 and R17 of dystrophin are shown in backbone representation colored in *blue* and the nNOS-PDZ subdomain is shown as a *grey* backbone representation. Principal features established to be involved in the specificity of the interaction are highlighted: the filament kink between successive dystrophin repeats and the β-finger contact with the R17 helix C

dystrophin on the other side of the linker region, namely with the begining of the helix A of the second repeat and with the loop bridging helices A and B of the first repeat. In addition, whereas a large extended hydrophobic patch is involved at the nNOS recognition interface of dystrophin (Giudice et al. 2013), specific recognition of ankyrin by spectrin is mainly driven through electrostatics (Ipsaro and Mondragon 2010).

Apparently, the spectrin repeats evolved to gain functional specialization within the conserved three-dimensional fold. Here, the most thoroughly explored example is the ankyrin-binding site which includes highly conserved regions within the 14th and 15th repeats of β spectrin (Czogalla and Sikorski 2010). Moreover, this region also includes an ankyrin-sensitive binding site for PE-rich membranes (Wolny et al. 2011). All these features are hidden in a structurally common tandem of triple-helical spectrin repeats, which was confirmed both in crystal structures (PDB IDs: 3EDU, 3F57, 3EDV; Stabach et al. 2009; Ipsaro et al. 2009; Davis et al. 2009) and in solution (Czogalla et al. 2007). The ankyrin-docking site is formed by conservative anionic amino acid residues on one of the facets of helix C of the 14th repeat and some residues within the inter-repeat linker and the loop between helices B and C of the 15th repeat (Fig. 12.6). Formation of the ankyrin-spectrin complex requires shape complementarity rather than induced fit (Ipsaro et al. 2009), and further studies indicated that the inter-repeat kink is one of the determinants of spectrin-ankyrin interactions (LA-Borde et al. 2010). On the other hand, sensitivity of the spectrin repeats to mechanical stress might be transduced into alterations in ligand (e.g. ankyrin) binding, which could provide selective plasticity of the membrane scaffold and a mechanical switch that links membrane deformations and spatial organization of receptors or signal-transducing molecules (Stabach et al. 2009). The ankyrin-dependent lipid-binding site may be involved in preventing the aggregation of the spectrin tetramers that are linked to some transmembrane proteins via ankyrin. As the number of ankyrin molecules and spectrin tetramers in erythrocytes is similar

and each spectrin tetramer contains two ankyrin/PE binding sites, it seems that the lipid-binding activity of these sites may play a key role in the appropriate spatial arrangement of individual ankyrin-transmembrane complexes (Chorzalska et al. 2010).

12.4 Dystrophin and Spectrin Mutations Related to Pathologies

The two devastating diseases Duchenne and Becker muscular dystrophies (DMD and BMD) occur after mutation of the *DMD* gene (Koenig et al. 1989). The main types of mutation resulting in dystrophinopathies are deletions of one or several exon(s), while there are ~10 % of duplications of exons and ~15 % of single point mutations (Tuffery-Giraud et al. 2009; Flanigan et al. 2009). According to the Monaco rule (Monaco et al. 1988), if the deletion in the *DMD* gene leads to a reading frame shift, there is a total absence of dystrophin in the muscle and severe phenotypes are observed in patients, usually classified as DMD. If the exon deletions do not shift the reading frame, the presence of an internally truncated dystrophin is detected and this is related to a large and variable range in the severity of symptoms as typified by the BMD phenotype. In certain cases, exceptions to the Monaco rule appear where a DMD phenotype with an in-frame mutation is observed. This is particularly prominent when mutations involve the N-terminal actin-binding-domain or the Cys-rich domain affecting the binding of dystrophin to F-actin or β-dystroglycan, respectively.

DMD is characterized by a progressive muscle weakness involving all skeletal and cardiac muscles and is accompanied by highly elevated creatine kinase blood levels (Kohler et al. 2005; Davies and Nowak 2006; Rahimov and Kunkel 2013). The early clinical signs leading to a DMD diagnosis are severe difficulties to walk and to climb stairs in the second or third year of childhood. DMD young patients are never able to run and are confined to a wheelchair before the age of 12 years (main criteria for DMD diagnosis). Progressively, respiratory and cardiac impairments are observed and DMD patients mainly die in their early thirties (Wein et al. 2015). All these specific DMD clinical signs are similarly observed in BMD patients, nevertheless with a very broad range of time courses and severity. Some BMD patients are considered as asymptomatic, while others become wheelchair confined around 16 years of age. Some BMD patients survive to very old ages while others die prematurely from heart failure (Bushby and Gardner-Medwin 1993; Bushby et al. 1993). Histological analyses of DMD muscles show cycles of fiber necrosis and regeneration. The regeneration process is however overtaken by fiber loss mechanisms and fibrosis, as adipose tissue replacement is also increased. All these features are vary markedly during DMD evolution or in different types of BMD. For instance the fibrosis and fatty infiltration increase with the age in DMD patients. Finally, electron microscopy studies have revealed lesions of the plasma

membrane in the case of DMD indicating that the maintenance of plasma membrane integrity is the essential role of dystrophin in muscle cells (Petrof et al. 1993).

From a structural point of view, the effect of exon deletion occurring in the central domain of dystrophin and associated with the BMD disease depends on the maintainance of the repeat phasing. These deletions disrupt repeat coding, but this results in main two cases. Indeed, after exon deletion which maintains the reading frame a new junction is created in the shortened dystrophin. This new junction either restores a hybrid repeat comprising a three-helix coiled-coil or it constitutes a new structural motif not in a coiled-coil fold, but leading to the formation of a fractional repeat. The first structural evidence for these rules was obtained through the computation of BMD shortened dystrophin fragments (Nicolas et al. 2012) and has recently been confirmed by the experimental determination of the SAXS-based models for a dystrophin fragment bearing the most frequent BMD deletion of exons 45–47 (Δ45–47; Delalande et al. to be published). Analysis of this model based on low-resolution experimental data clearly shows that the structural organization of the coiled-coil filament is strongly disturbed at the newly created junction. It is important to notice that, with the changed exon phasing, this alteration of the structure leads to the loss of the previously characterized binding site of nNOS. In addition, we showed that the two types of structure of the internally deleted dystrophins produced in these BMD patients could partly explain the differences in the clinical severity of the patients (Nicolas et al. 2015).

The most promising strategies for DMD therapy are to transform a DMD patient into a BMD patient with an asymptomatic phenotype by using exon-skipping and gene or cell therapies.

Exon-skipping aims to transform a DMD patient with an out-of-frame deletion into a BMD patient by deleting additional exons to restore the reading frame (Aartsma-Rus and Van Ommen 2007; Goyenvalle et al. 2004). The most valuable deletions may be those restoring a hybrid repeat at the new junction and the studies reporting the correlation between dystrophin structure and clinical outcomes of BMD patients are highly valuable.

Gene therapy aims to deliver DNA sequences expressing the most important parts of the protein into skeletal and heart cells. The *DMD* gene is the largest human gene and it is not possible to put the entire cDNA sequence into a unique vector that can reach all muscle cells. Therefore, the concept of micro-dystrophin has emerged (Harper et al. 2002). Truncated gene coding sequences (micro-dystrophins), inspired by the truncated dystrophin coding sequences observed in mild BMD patients (England et al. 1990), have been designed (Seto et al. 2012; Fairclough et al. 2013; Jarmin et al. 2014; Mcgreevy et al. 2015). These highly simplified micro-dystrophins only consist of the N- and Cys-rich domains with two hinges and several repeats (Gregorevic et al. 2008; Foster et al. 2008). However, they do not replicate all the functions of dystrophin (Seto et al. 2012) and further improvements are needed based on our knowledge about the structure and function of the central domain of dystrophin (Wilton et al. 2015).

The spectrin-based membrane skeleton is a prerequisite for maintaining the integrity and flexibility of cells. This is particularly important for erythrocytes which experience strong mechanical stresses during high speed flow in the circulation and major deformations due to their passage through capillaries of diameter much smaller than the size of the cells. Thus, defects in the components of the spectrin-based skeleton are reflected in the fragility, fragmentation and premature destruction of red blood cells. Such disorders lead to hemolytic anemias, including hereditary spherocytosis (HS), elliptocytosis (HE), pyropoikylocytosis (HPP) and stomatocytosis (HSt). In the case of the former, most of the molecular defects concern membrane skeleton components involved in vertical interactions, namely spectrin, ankyrin, anion exchanger 1 and protein 4.2. On the other hand, HE and HPP are characterized by molecular defects of proteins involved in horizontal interactions within the membrane skeleton, namely spectrin and protein 4.1 (Gallagher 2004b). Very little is known about the molecular background of HSt, a rare red cell disorder resulting from altered intracellular cation content and some cell volume alterations (Bruce 2009; Boguslawska et al. 2010).

Hereditary spherocytosis (HS) is the most widely spread inherited disease linked to the red blood cell membrane, affecting more than one per two thousand individuals (Da Costa et al. 2013). In three-quarters of cases the inheritance is dominant. The decreased deformability and spheroidal shape of erythrocytes is associated with the loss of membrane surface area in relation to intracellular volume (Eber and Lux 2004). Such cells are sequestered in the spleen and subsequently phagocytosed by macrophages, which results in anaemia and splenomegaly. Manifestation of spherocytosis may vary substantially from patient to patient, starting from very few spherocytes in approx. 35 % of mild cases of HS. Minor to moderate forms of the disease are characteristic of defects in the *SPTB* gene. These cases comprise 15–30 % of the HS population and the transmission is autosomal dominant, although sporadic mutations have also been described. More than 25 mutations elucidated in this gene include ten nonsense or non-coding sequences, ten null mutations and five missense mutations (Bolton-Maggs et al. 2012). The latter group is represented by spectrin Atlanta and spectrin Kissimmee, where the mutation is located in the conserved region that plays a role in interactions with protein 4.1. More severe forms of HS have their sources in mutations in the *SPTA1* gene, although these are relatively rare (<5 % of HS population). In these cases, homozygous or compound heterozygous mutations lead to the development of the disease and can be related to the fact that in erythroid cells α-spectrin is produced in large excess over β-spectrin. An interesting example is the low expression allele α^{LEPRA} (low expression allele of *SPTA1* gene, 3.3 %) that contains a C-to-T transition at position −99 of intron 30. The mutation activates an alternative acceptor site 70 nucleotides upstream from the usual site, which leads to mis-splicing and a frameshift in pre-mRNA. α^{LEPRA} is supposed to be broadly involved in recessive or non-dominant HS, although it is silent in normal individuals. Severe hemolytic anaemia is a result of its combination in *trans* with the α-spectrin Prague mutation (Wichterle et al. 1996). The Prague mutation leads to exon skipping, frameshifts and the production of a truncated protein.

Hereditary elliptocytosis (HE) is another common disease related to mutations in genes encoding membrane skeleton proteins. In general, HE is distributed worldwide, ranging from 1 to 2 cases per ten thousand Caucasians and up to 2 % of the population in malaria endemic regions (Glele-Kakai et al. 1996; Dhermy et al. 2007). HE individuals have a resistance to the parasite *P.falciparum*, as the absence of protein 4.1 and mutations in the spectrin tetramerization site impede binding of the parasite to the host cells (Waller et al. 2003). The hallmark of HE is a mechanically unstable cell membrane, mostly due to mutations in spectrins (90 % of cases). The shape of erythrocytes becomes elliptic, although the cell morphology and clinical severity is heterogeneous. While the majority of HE cases are asymptomatic, homozygous and compound heterozygous individuals suffer from mild-to-severe anaemia, jaundice and splenomegaly (Gallagher 2004a). Moreover, a few cases of fatal hydrops fetalis were reported (Gallagher et al. 1995). Greater membrane fragility, reflected by the presence of numerous poikilocytes, together with thermal sensitivity of erythrocytes, was initially considered as a distinct type of disorder called hereditary pyropoikylocytosis (HPP), but in fact it corresponds to severe cases of HE (Lecomte et al. 1987). The vast majority of HE cases are related to the disturbed ability of the spectrin dimers to self-aggregate into functional tetramers, which are crucial for mechanical stability of the red blood cell membrane (Lecomte et al. 1993). Indeed, most of the defects are linked to missense mutations located at or in the vicinity of the spectrin tetramerization sites; in the N-terminal helix C and first spectrin repeat of the α subunit or within the two C-terminal helices of the β subunit. Some mutations lead to truncations of the C-terminus of β spectrin. In general, location of the molecular defects determines the clinical phenotype of the disease (Lecomte et al. 1993). When mutations are present within the helices critical to the formation of the spectrin tetramer (Nicolas et al. 1998), and they involve amino acid residues that play a key role in stabilization of the spectrin repeat restored after self-association (Zhang et al. 2001; Gaetani et al. 2008), patients experience a more severe form of HE. It is worth underlining that more than 25 mutations related to HE are located within the *SPTA1* gene, while the *SPTB* gene is less affected. This is related to the issue that another factor that determines the severity of HE is the existence of a low expression α allele in *trans*. For example, the α^{LELY} (Low Expression Lyon) allele has a C-to-T mutation at nucleotide −12 of intron 45 which leads to deletion of a few amino acid residues within the 20th repeat of α-spectrin. As a consequence, αβ dimer formation is compromised (Wilmotte et al. 1997).

The fact that up to 3 % of the total protein in brain is represented by spectrin reflects how important this protein is in neuronal cells where it interacts with numerous membrane proteins (Davis and Bennett 1983; Machnicka et al. 2014). In neuronal axons spectrin forms a regular network of spectrin tetramers connected to junctional complexes (Xu et al. 2013). Of particular importance is the involvement of non-erythrocytic β spectrins in the docking of synaptic vesicles to the presynaptic membrane, mostly through interactions with synapsin (Sikorski et al. 1991). The latter binds to a roughly 25 amino acid residue segment of the β subunit, and this process is critical for neurotransmission (Sikorski et al. 2000; Zimmer et al. 2000). Deletion mutations of α or β spectrin in *D. melanogaster* result in a severe distortion

of synaptic transmission and disrupt the spatial arrangement of synaptic components (Featherstone et al. 2001).

West syndrome is a relatively rare epileptic disease affecting infants. The basis of the disease apparently relates to distortions in neurotransmitter function. Most recently it was shown that some mutations in αII-spectrin are related to the West syndrome (Saitsu et al. 2010; Writzl et al. 2012). The observed mutations lead to severe cerebral hypomyelination, decreased white matter, brain atrophy and reduced corpus callosum. A deletion within the helix linker between repeats 19 and 20 and duplication within repeat 20 of α-spectrin affect the region of the protein responsible for the formation of spectrin heterodimers. This may disturb β-spectrin-mediated stabilization of membrane proteins and axonal transport (Holleran et al. 2001; Lorenzo et al. 2010). In this context it is also worth mentioning that αII-spectrin plays an important role in the maintenance of the integrity of myelinated axons, and is enriched at the paranodes which flank Ranvier's node (Ogawa et al. 2006; Voas et al. 2007).

Mutations in the *SPTBN2* gene have been connected with spinocerebellar ataxias (SCAs; Ikeda et al. 2006). Such disorders are characterized by degeneration of cerebellum, spinal cord and brainstem. A 39 nucleotide deletion within exon 12 of the the gene coding βIII-spectrin, causing deletion of amino acid residues 532–544, was found in an American family suffering from spinocerebellar ataxia type 5. Similarly, a 15 nucleotide deletion in exon 14, that leads to deletion of amino acid residues 629–634 and introduction of a Trp residue, was found in a French family. Both mutations refer to spectrin repeat 3, which plays a role in the dimerization of α and β subunits. Another example is a T-to-C transition in exon 7 resulting in a substitution (Leu253Pro) in the highly conserved region of the calponin homology domain. βII-spectrin is mainly expressed in Purkinje cells, where it plays a role in the stabilization of membrane proteins including the glutamate transporter EAAT4 (Ohara et al. 1998; Jackson et al. 2001). Mutations of spectrin prevent proper localization of EAAT4 and GluRg2 on the plasma membrane. Loss of these proteins at the cell membrane may lead to glutamate signalling abnormalities that provoke Purkinie cell death and eventually lead to ataxia. βII-spectrin is also associated with Golgi and vesicles (Stankewich et al. 1998) and binds to dynactin, which may suggest a role of spectrin in vesicular transport (Holleran et al. 2001). Mutations in the CH domain of βII-spectrin may alter its ability to bind to the actin skeleton and thus affect the intracellular transport and/or stabilization of membrane proteins.

12.5 Conclusion

As they share a common structural organization – i.e. a multi-domain filament that includes actin-binding, lipid-binding and EF subdomains and a succession of coiled-coil repeats forming a central domain – Dystrophin and Spectrin are two proteins presenting highly similar topologies and interaction networks. All these specificities are closely related to their scaffolding role, which has been revealed to

be essential for the maintenance of the architecture of the cell. Nevertheless, in contrast to spectrin, dystrophin is coded by a single gene, it is non-ubiquitous and monomeric in solution. The comparison of these two proteins is also limited by the focus on the very different pathologies that emerge from the mutation of their encoding genes. Recent structural studies dedicated to the filamentous coiled-coil central domain of both dystrophin and spectrin have provided meaningful details in order to highlight their specific role in the cell and are promising in the pursuit towards understanding the organization of the large macromolecular assemblies managed by these two dissimilar sisters of the same family.

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