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Review

Dystrophin: More than just the sum of its parts

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ABSTRACT

Dystrophin is one of a number of large cytoskeleton associated proteins that connect between various cytoskeletal elements and often are tethered to the membrane through other transmembrane protein complexes. These cytolinker proteins often provide structure and support to the cells where they are expressed, and mutations in genes encoding these proteins frequently gives rise to disease. Dystrophin is no exception in any of these respects, providing connections between a transmembrane complex known as the dystrophin–glycoprotein complex and the underlying cytoskeleton. The most established connection and possibly the most important is that to F-actin, but more recently evidence has been forthcoming of connections to membrane phospholipids, intermediate filaments and microtubules. Moreover it is becoming increasingly clear that the multiple spectrin-like repeats in the centre of the molecule, that had hitherto been thought to be largely redundant, harbour binding activities that have a significant impact on dystrophin functionality. This functionality is particularly apparent when assessed by the ability to rescue the dystrophic phenotype in *mdx* mice. This review will focus on the relatively neglected but functionally vital coiled-coil region of dystrophin, highlighting the structural relationships and interactions of the coiled-coil region and providing new insights into the functional role of this region.

1. Introduction

Dystrophin is a skeletal muscle protein encoded by the Duchenne muscular dystrophy (DMD) gene. The gene located on the X chromosome and spanning 2.5 Mb is the largest human gene. The gene sequence comprises over 79 exons coding for a 427 kDa protein of 3685 residues [1]. DMD gene mutations lead to a functional absence of dystrophin in patients suffering from DMD giving rise to severe and progressive muscle wasting disease [2].

Dystrophin comprises four essential regions spanning from the N-terminal end which displays actin-binding activity, through a very long central coiled-coil region of 24 spectrin-like repeats, to a cysteine-rich domain and a C-terminal coiled-coil domain. The cys-rich region itself comprises a WW domain, two EF hands and a cys-rich domain (for a review, see [3]).

Although the N-terminal actin-binding and the cys-rich domains are well characterised and there has been much discussion of these domains, the role and partners of the large central coiled-coil region are less well known. The purpose of this review is to focus on the role of, and to propose new hypotheses for, the physiological function of this central region. The relative lack of information on this central coiled-coil region arises in part from observations of human mutations and correlation with their phenotypes. These observations have lead to the notion that the central coiled-coil region is functionally redundant and hence not important.

Duchenne muscular dystrophy (DMD) is the second most common inherited disorder with an incidence of around 1:3,500 male births [4,5]. The resulting muscle wasting phenotype leads to sufferers with a life span reduced to about 20 years. The muscles of DMD sufferers display a total absence of the 427 kDa dystrophin molecule. By contrast, a lower frequency allelic condition leads to Becker muscular dystrophy (BMD) where sufferers have less severe and/or highly variable phenotypes associated with the presence of dystrophin containing internal deletions but remaining partly functional. In these cases, the mutations consist mainly of one or several exon deletions that do not disrupt the reading frame and therefore, dystrophin containing partial deletions (which we will call “deleted dystrophin”) is stably produced [6]. The vast majority of these deletions subtract several spectrin-like repeats of the central rod region [7]. In BMD therefore, we have the notion of a lower severity phenotype compared to DMD, associated with a dystrophin molecule largely devoid of the central rod region, leading to the view that the central rod region is functionally redundant. This explains to some extent why the central region has appeared as dispensable for dystrophin function and why fewer studies have focused on this region.

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In this review, we will provide a comprehensive expose on the structure, function and interactions of the spectrin-like repeats of dystrophin and what has been learned both from human pathology and from transgenic studies with the *mdx* mouse. From this we will expound further hypotheses concerning the functions of the dystrophin coiled-coil region.

2. Structural analysis of the dystrophin repeats

The dystrophin coiled-coil region is made up of 24 spectrin-like repeats separated into three sub-regions by so-called 'hinges' (Fig. 1). This picture comes largely from the analysis of the primary sequence of the repeats [8,9] which shows some homology with spectrin and α-actinin repeats [10], which with the conservation in the N-terminal actin-binding domains leads to the concept of these proteins belonging to the same family [11]. The same argument applies for utrophin, a ubiquitously expressed autosomal homologue of dystrophin, produced in muscle cells at earlier stages of development and differentiation [12]. Indeed careful analysis of the coiled-coil repeats from this family of proteins shows a likely evolution from an ancestral α-actinin molecule through a series of gene duplications to yield first an ancestral protein containing ABD, spectrin repeats and EF hands [13,14]. This ancestor subsequently undergoes gene fission and further duplication to yield spectrins and separately acquires new domains through gene fusion to yield the ancestor of dystrophin and utrophin [15]. Moreover, antibody studies of dystrophin from various vertebrate species suggest that the length and overall structure of dystrophin has remained relatively constant since at least the emergence of extant vertebrates [16].

As early as 1988, the appearance of repeated structures in the primary sequence of dystrophin was noted [8,17] leading to the hypothesis of a rod shaped structure in the dystrophin central region. Further examinations of the sequences showed that it was possible to align the repeated sequences according to a heptad motif and that they might fold into three alpha-helices which constitute left-handed, antiparallel triple-helical coiled coils [9–11]. 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. 1) [9]. The alpha-helical nature of the repeats was experimentally confirmed by the pioneering work of the Gratzer group [18]. However, they demonstrated that the dystrophin second repeat could not fold as a simple unit but that the folding is observed when the repeat extends some residues into the adjoining sequence repeat (Fig. 2) [19,20].

These studies led to the idea that the repeats may fold in a nested manner [21]. This is a challenging question which is not easy to resolve. Several studies of other repeats of dystrophin show that the folding is well ordered when the constructs are extended by several residues of the flanking repeats [22–26]. For example, in the recent work of Saadat et al. [22] it was noted that repeats 2 and 3 were not expressed to a significant extent in *E. coli* but must be expressed as extended or "big" motifs flanked by 9 additional residues. They applied the same strategy for repeats 16, 17 and 18 which were extended by 8 residues from their two flanking repeats [26]. Whether these experimental data imply that the repeats fold in a nested manner or rather indicate that we do not know where each repeat starts and ends, remains unanswered. Indeed, two repeat alignments are still in common use even though they differ somewhat from each other [9,11]. In agreement with the last hypothesis, repeat 4 was expressed with no extension [22].

Certain combinations of repeats from repeat 8 to 14 are rather difficult to produce *in vitro* as highly purified or as well-folded proteins often due to irregularities or insertions in the spectrin-like repeat structure of dystrophin that are not seen in spectrin [11,25]. We were unsuccessful in expressing repeat 10–12 or 14–16 [25] while larger proteins spanning repeat 11–15 [27] or 11–17 [28] or 14–17 [29] were expressed in *E. coli*. However, the two last proteins showed several additional bands on Coomassie staining indicating an overall susceptibility to proteolysis. This could be due to the presence of an extra sequence of 20 residues between repeat 15 and 16 representing a potential small additional hinge as suggested by Menhart [29]. Similarly, it was impossible to produce a tandem protein spanning repeat 3–4 [22]. However, in this case, the hinge 2 separating the two repeats could have precluded the folding of this construct. It is also noteworthy that these studies concerned repeats lacking tryptophan residue i.e. repeats 10, 11 and 14. Two conserved Trp residues in particular are situated at the 17th and 90th positions in the repeats, and are involved in spectrin repeat stability. The absence or the mutagenesis of these residues in the spectrin repeat is accompanied by a very low stability [30,31] as well as a modification of the 3D structure [32] compared to repeats containing the two Trp residues. It is likely that folding could be precluded by the presence of Trp lacking repeats in several of our constructs. Moreover, repeats 11, 13, 15 and 17 display theoretical isoelectric points higher than 7 compared to the others, which all have an isoelectric point lower than 7 as noted previously [27,28] (Fig. 3). These properties could have a role in the folding of the molecule and these features deserve further experimental investigation.

Therefore, these results highlight the complexity of the rod region properties and the fact that repeats are not simply interchangeable. This was also the conclusion of the work of Bhasin where spectrin-like repeats from dystrophin were stretched to unzip using atomic force microscopy (AFM) [33]. In some cases, individual bundles open up one at a time while in other cases, the linker between two tandem repeats unzipped at lower stretch force before the repeats unzipped. Therefore, whether these repeats are structured in nested units or not remains to be fully elucidated.

Repeats from spectrin itself by contrast, can be consistently expressed and produced with the exact edges of each repeat [34,35] and the folding pathway of a single repeat is not affected by its neighbouring repeat [36] even though a tandem repeat is thermodynamically more stable than a single repeat [31,37]. However, the folding pathway has recently been shown to be highly different for several of these repeats even though they have similar structures and stabilities [38].

To date, there is no three dimensional structural data on dystrophin repeats, probably because of their high conformational flexibility. Our experience is that there are too few cross-peaks in 2D NMR data from repeats 2 and 23 showing that the structure is highly flexible and that the determination of 3D structure remains highly challenging to achieve. By contrast, repeats from spectrin have been consistently studied by NMR [39,40] and by X-ray crystallography of single, tandem or multiple repeats [32,41–44]. These studies demonstrated that the repeats constitute small bundles largely in agreement with the coiled-coil structure hypothesis. The same rules apply for α-actinin repeats [45–47]. These experimental data largely imply that the triple-helical coiled-coil structure is a common feature of repeats from proteins of the spectrin family. In agreement with that, molecular modelling of an individual dystrophin repeat has been achieved with good alignment and homology with these experimental structures [48].

However, crystals of spectrin as well as α-actinin repeats are head to tail dimers, suggesting that their stabilities are dependent upon dimerisation. Whether dystrophin could dimerise or not has been the subject of much debate and in the beginning of the story, dystrophin was assumed to be a dimer [49]. However, the large variation in the

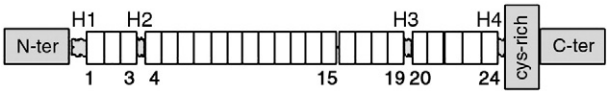

Fig. 1. Domain structure of dystrophin. H1 to H4, hinges; numbers below are the numbers of the repeats.