Fate of Dystrophin-associated Glycoproteins in Dystrophic Muscle Fibres

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Abstract: The trans-sarcolemmal dystrophin-glycoprotein complex is proposed to protect the muscle fibre periphery from mechanical stress since it provides a linkage between the extracellular matrix and the actin membrane cytoskeleton. Following the initial biochemical identification of dystrophin-associated proteins, recent molecular studies have now focused on a refined analysis of the spatial configuration of the complex. Besides these new insights into muscle biology, overwhelming evidence exists that abnormalities in individual components of this complex are implicated in the molecular pathogenesis of several muscular dystrophies. Analysis of mutations in dystrophin, alpha-sarcoglycan, beta-sarcoglycan, gamma-sarcoglycan, as well as in merosin have led to the unifying hypothesis that pathophysiological changes in the sarcolemmal dystrophin-glycoprotein complex trigger Duchenne/Becker muscular dystrophy, various forms of limb-girdle muscular dystrophy and congenital muscular dystrophy. How exactly secondary and tertiary molecular changes cause overall mechanical instability of dystrophic muscle fibres and furthermore induce abnormalities in calcium homeostasis and signal transduction remains to be determined.

Key words: Dystrophin; Sarcoglycan; Dystroglycan; Dystrophin-glycoprotein complex; Duchenne muscular dystrophy.

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Abnormalities in the actin-binding protein dystrophin are associated with severe Duchenne Muscular Dystrophy (DMD) and the milder Becker muscular dystrophy [13]. Several excellent reviews describe in detail the discovery and molecular characterisation of the human DMD gene and its protein product dystrophin, as well as the existence of manifold dystrophin isoforms [1, 2, 6, 65]. For an indepth description of the complex association of dystrophin with several integral, peripheral and cytoskeletal proteins at the muscle cell periphery, consult recent articles focusing on the molecular organisation of the dystrophin-glycoprotein complex [8, 16, 49, 66]. This article instead provides a brief review of the involvement of components of the dystrophin-glycoprotein complex in numerous muscular dystrophies and outlines how these findings have influenced our current understanding of the molecular pathogenesis of dystrophinopathies.

Dystrophin-associated glycoproteins

As a basis for the below description of the fate of dystrophin-associated glycoproteins in dystrophic muscle fibres, this section will briefly summarise recent findings on the spatial configuration of the dystrophin-glycoprotein complex. Although the identification of the individual components of the dystrophin-glycoprotein complex and its basic structure have been known for several years now [15], the original model had to be slightly revised to take into account results from refined experiments on the molecular organisation of this sarcolemmal complex [17, 27, 29, 36, 61, 63, 73, 76]. Dystrophin binds with its amino-terminal region to cortical actin filaments [33, 71] and with its carboxy-terminal, cysteine-rich region to an integral glycoprotein complex [29, 36, 61]. While the cytoplasmic syntrophins of apparent 59 kDa bind to discrete regions of the carboxy-terminus of dystrophin [63, 74, 75], the other dystrophin-associated components are represented by transmembrane proteins of 25 kDa, 35 kDa, 50 kDa, as well as a 43 kDa-glycoprotein doublet [15]. Jung et al. [29] have localised the beta-dystroglycan and dystrophin interaction domains to the carboxy-terminus of the 43 kDa dystrophin-associated glycoprotein and a unique binding site in the cysteine-rich domain of dystrophin. In addition, beta-dystroglycan interacts with the highly glycosylated, extracellular laminin-binding protein alpha-dystroglycan of 156 kDa [27]. Hence, this complex association of extracellular, peripheral, integral and membrane cytoskeletal proteins appears to provide an indirect linkage between laminin and F-actin. Furthermore, Belkin and Burridge [5] described a novel dystrophin-associated, cytoskeletal protein named aciculin. Dystrophin interaction with this 60

kDa protein might provide an additional cytoskeletal-matrix transmembrane linkage. See Table 1 for a list of identified dystrophin-associated proteins. The most simplistic model of this trans-sarcolemmal connection hypothesizes that it confers a high degree of flexibility to the muscle fibre periphery and thus stabilise the surface membrane during contraction. However, this does not exclude a multi-functional purpose of the dystrophin-glycoprotein complex, i.e. a role in signal transduction [28, 35].

Dystrophin-glycoprotein complex and muscular dystrophy

Skeletal muscle dystrophin-glycoprotein complex in Duchenne muscular dystrophy

It is well established that the expression of dystrophin-associated proteins is severely affected in dystrophin-deficient muscle fibres. Extensive studies into the fate of the dystrophin-glycoprotein complex in dystrophic muscle demonstrated that the abundance of all of the dystrophin-associated proteins is greatly reduced in skeletal muscle biopsy specimens from patients afflicted with Duchenne muscular dystrophy [14, 25, 40, 48, 49]. In dystrophin-deficient skeletal muscle cells, the expression of spectrin is not affected and can thus be employed as a positive control for immunoblotting procedures and immunofluorescence microscopy [48, 49]. In contrast to the deficiency in dystrophin-associated glycoproteins, the overall expression of wheat germ agglutinin-positive glycoproteins present in the dystrophic muscle periphery is comparable to normal human muscle [48, 49]. Thus, the reduction in dystrophin-associated glycoproteins is most likely to be primarily due to a deficiency in dystrophin and not caused secondarily by general muscle cell necrosis which might eventually affect all surface glycoproteins. Without proper anchoring by a subsarcolemmal network of dystrophin molecules [31], the otherwise dystrophin-associated surface proteins might not be targeted to the right plasma membrane domains and/or become quickly degraded. In analogy to the findings in DMD muscle, similar results were obtained in the analysis of *mdx* muscle fibres before, during and after segmental necrosis [46]. With the exception of extraoccular and toe skeletal muscle cells [37], *mdx* bulk body muscle exhibited a dramatic reduction in all dystrophin-associated proteins [14, 46].

Cardiac muscle dystrophin-glycoprotein complex in Duchenne muscular dystrophy

As can be seen in the immunoblot analysis of Fig. 1, a cardiac muscle sample from a DMD patient also shows a severe reduction in the expression of all of the dystrophin-associated proteins in dystrophin-deficient heart muscle.

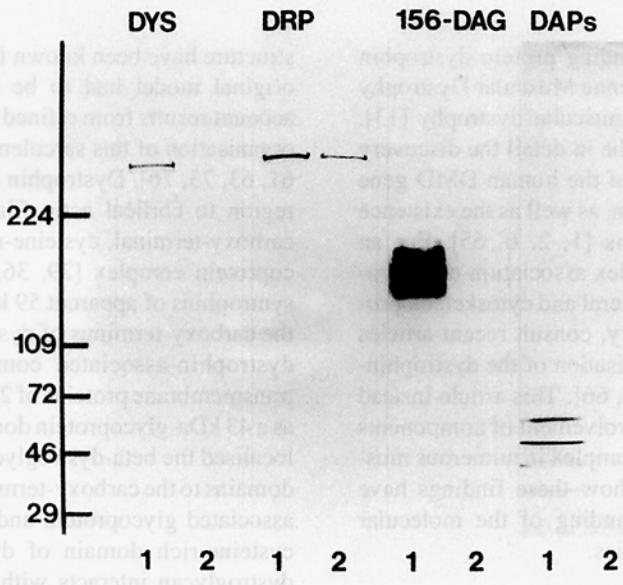

Figure 1: Severe reduction in all of the dystrophin-associated proteins in dystrophin-deficient cardiac microsomal membranes from a patient afflicted with Duchenne muscular dystrophy. Immunoblot analysis of crude microsomal membranes isolated from control (lane 1) and dystrophic cardiac muscle fibres (lane 2) was performed by standard methods [47]. While dystrophin (DYS) is completely absent, the dystrophin-related protein utrophin (DRP) is reduced in its expression in cardiac DMD muscle. Labelling for the dystrophin-associated glycoprotein alpha-dystroglycan of 156 kDa (156DAG), as well as the other dystrophin-associated proteins of 35 kDa to 50 kDa (DAPs) is dramatically reduced in cardiac DMD muscle. Molecular weight standards (M_r x 10^{-3}) are indicated on the left.

Table 1: Established components of the matrix-cytoskeletal linkage via dystrophin-associated proteins

Component	Molecular weight	Localisation
Peripheral F-Actin	(43)n kDa	Membrane cytoskeleton
Dystrophin	427 kDa	Membrane cytoskalaton
Syntrophins	59 kDa	Membrane cytoskeleton
Alpha-Sarcoglycan	50 kDa	Sarcolemma
Beta-Sarcoglycan	43 kDa	Sarcolemma
Gamma-Sarcoglycan	35 kDa	Sarcolemma
25DAP (A5 band)	25 kDa	Sarcolemma
Beta-Dystroglycan	43 kDa	Sarcolemma
Alpha-Dystroglycan	156 kDa	Extracellular
Laminin Heterotrimer	850 kDa	Extracellular matrix

The expression of the dystrophin-related protein, utrophin, is much less affected in cardiac DMD muscle (Fig. 1). In analogy to the hypothesised susceptibility of skeletal muscle to necrosis due to dystrophin deficiency [48], reduction in dystrophin-associated glycoproteins might also cause a disruption of a trans-sarcolemmal matrix-cytoskeleton linkage in cardiac muscle. This might compromise the integrity of the cardiac muscle surface membrane and thus trigger the destructive pathway finally leading to muscle fibre weakness and cell death. In contrast to cardiac DMD muscle, *mdx* cardiac muscle fibres, although deficient in dystrophin, exhibit a reduction only in the 59 kDa dystrophin-associated glycoprotein [74]. Possibly, upregulation of utrophin might prevent the loss of the other dystrophin-associated proteins, which was previously described for extraoccular and toe skeletal muscle of the *mdx* mouse [37].

Dystrophin-glycoprotein complex and muscular dystrophies

Several extensive reviews by Campbell and coworkers [8, 18, 40, 41] describe the possible role of individual components of the dystrophin-glycoprotein complex in the molecular pathogenesis of muscular dystrophies. Recently, additional mutations in components of the dystrophin-glycoprotein complex were described as the underlying genetic cause for Duchenne-like muscular dystrophies [7, 24, 34, 45, 56, 58]. The analysis of gene mutations and protein abnormalities in dystrophin [2, 4, 26], alpha-sarcoglycan of 50 kDa [9, 19, 38, 56, 58, 59], beta-sarcoglycan of 43 kDa [7, 34], gamma-sarcoglycan of 35 kDa [45] and merosin [24, 67] has led to the unifying hypothesis that abnormalities in the dystrophin-glycoprotein complex trigger Duchenne/Becker muscular dystrophy, various forms of limb-girdle muscular dystrophy and congenital muscular dystrophy. Table 2 summarises the proposed linkage between mutations in individual components of the dystrophin-glycoprotein complex and the various forms of muscular dystrophy.

Table 2: Primary defects in components of the dystrophin-glycoprotein complex and linkage to muscular dystrophies

Component	gene locus	Muscular dystrophy
Dystrophin	Xp21	Duchenne muscular dystrophy
Dystrophin	Xp21	Becker muscular dystrophy
Alpha-Sarcoglycan	17q21	Limbgirdle muscular dystrophy (type 2D)
Beta-Sarcoglycan	4q12	Limbgirdle muscular dystrophy (type 2E)
Gamma-Sarcoglycanl	3q12	Limbgirdle muscular dystrophy (type 2C)
Laminin/Merosin	6q22	Congenital muscular-dystrophy

While complete deficiency in dystrophin or mutations in critical carboxy- and amino-terminal domains result in severe dystrophic phenotypes [8], the milder Becker muscular dystrophy is characterised by a partially dysfunctional dystrophin molecule [4, 26] and a corresponding mild reduction in dystrophin-associated glycoproteins [39]. Mutations in the gene encoding for alpha-sarcoglycan of 50 kDa, previously also named adhalin, cause some cases of limb-girdle muscular dystrophy [9, 56]. However, muscular dystrophies which exhibit progressive weakness in the pelvic and shoulder girdle muscle groups are a heterogeneous group of disorders. Newly identified mutations in dystrophin-associated proteins, namely beta-sarcoglycan [7, 34] and gamma-sarcoglycan [45], were also found to be involved in some forms of limb-girdle muscular dystrophy (Table 2). Interestingly, mutations in beta-sarcoglycan led not only to a specific loss in the dystrophin-associated glycoprotein of 43 kDa, but also caused a dramatic decrease in the expression of alpha-sarcoglycan of 50 kDa and gamma-sarcoglycan of 35 kDa. Hence, the previously biochemically described sarcoglycan-subcomplex [76], might play an essential role in secondary pathophysiological changes in certain forms of autosomal recessive limb-girdle muscular dystrophy [7, 34, 44, 45]. Major abnormalities in DMD muscle fibres include mechanical instability [43], as well as changes in calcium homeostasis [20] and disturbed signal transduction [23]. The sequence of these pathophysiological changes is most likely extremely complicated and which major factor(s) finally induce necrosis is not yet understood.

The *mdx* mouse and muscular dystrophy

Dystrophin-deficient *mdx* mouse muscle is an established animal model of muscular dystrophy [68] and especially the *mdx* diaphragm exhibits histopathological changes similar to human DMD muscle fibres [60]. As already mentioned above, the expression of all components of the dystrophin-glycoprotein complex is severely re-

duced in *mdx* skeletal muscle fibres [14, 46] making it a relatively good model system to study experimental therapy approaches. Recent studies by Tidball *et al.* [64] indicate that apoptotic events precede necrosis in dystrophin-deficient *mdx* muscle. Whether these two phenomena are strictly consecutive in nature or whether a complex interrelationship exists between apoptosis and necrosis during the progression of muscular dystrophy is currently unknown.

Many insights into the organisation of the dystrophin-glycoprotein complex and the molecular consequences of specific mutations have come from the analysis of transgenic mice. Greenberg et al. [22] and Cox et al. [11] could show using *mdx* mice made transgenic for the dystrophin isoform Dp71 that both the carboxy- and the amino-terminal domains of dystrophin are absolutely essential for normal dystrophin function. Dp71 contains the carboxy-terminal and cysteine-rich domains of the main 427 kDa dystrophin isoform but lacks central spectrin-like repeat domains and the amino-terminal actin-binding domain [32]. Expression of this isoform in transgenic *mdx* muscle sarcolemma restored the anchoring of dystrophin-associated proteins but did not alleviate symptoms of skeletal muscle degeneration due to the lack of interaction between F-actin and Dp71 [11, 22]. In contrast, over-expression of the full-length 427 kDa isoform of dystrophin in transgenic *mdx* mice reversed histopathological symptoms without causing cytotoxic side effects [10], indicating that in principle gene therapy is feasible. In addition, normal calcium homeostasis appears to be restored in *mdx* mice made transgenic for dystrophin [12], which emphasises the potential role of increased cytoplasmic calcium levels in fibre destruction in muscular dystrophy [20, 69].

Since skeletal muscle fibres from *mdx* mice exhibit mechanical instability [53, 54], an increased sensitivity to osmotic shock [42] and abnormalities in calcium homeostasis [69] aside from certain histopathological changes [60], a reversal in these parameters can be used as an indicator for the success of experimental therapeutic approaches in *mdx* mice. Although many problems are associated with gene and myoblast transfer therapy in patients with neuromuscular disorders, experiments with *mdx* mice indicate that both techniques have the potential to be useful in reversing dystrophic symptoms. Firstly, injection of normal myoblasts into *mdx* mouse muscle causes a transformation from dystrophin-deficient to dystrophin-containing skeletal muscle fibres [50,70]. Secondly, with respect to somatic gene therapy, it was demonstrated that intramuscular injection of dystrophin DNA constructs results in dystrophin expression in *mdx* mice [3]. More recently, several studies showed that the expression of truncated minidystrophins in transgenic *mdx* mice results in normal muscle function and the elimination of dystrophic symptoms [21, 55, 57, 72]. Thus, using a suitable viral vector system, somatic gene therapy might be feasible in the treatment of muscular dystrophies.

Prospects

In conclusion, great progress has been made in the elucidation of the primary genetic defects in several forms of muscular dystrophy. The clinical relevancy of mutations in components of the dystrophin-glycoprotein complex as the molecular basis of pathological muscle destruction has many implications for prognosis and possibly novel therapeutic approaches. See recent review articles [30, 51, 52] on the feasibility of gene and myoblast transfer therapy in the treatment of muscular dystrophy. However, many unanswered questions remain especially with respect to secondary and tertiary pathophysiological changes actually leading to muscle weakness and fibre wasting. The missing link between a specific mutation in a muscle gene on the one hand and final muscle fibre destruction on the other hand are the secondary molecular mechanisms underlying muscle cell necrosis. Since the components and the structural organisation of the dystrophin-glycoprotein complex have basically been identified, a refined analysis of the secondary changes actually causing overall muscle weakness are essential for our understanding of the aetiology of muscular dystrophy. The exact cellular mechanisms that result in abnormalities in calcium homeostasis, signal transduction and mechanical stability remain to be determined.

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