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Mini-Review

The biochemical and mass spectrometric profiling of the dystrophin complexome from skeletal muscle

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ABSTRACT

The development of advanced mass spectrometric methodology has decisively enhanced the analytical capabilities for studies into the composition and dynamics of multi-subunit protein complexes and their associated components. Large-scale complexome profiling is an approach that combines the systematic isolation and enrichment of protein assemblies with sophisticated mass spectrometry-based identification methods. In skeletal muscles, the membrane cytoskeletal protein dystrophin of 427 kDa forms tight interactions with a variety of sarcolemmal, cytosolic and extracellular proteins, which in turn associate with key components of the extracellular matrix and the intracellular cytoskeleton. A major function of this enormous assembly of proteins, including dystroglycans, sarcoglycans, syntrophins, dystrobrevins, sarcospan, laminin and cortical actin, is postulated to stabilize muscle fibres during the physical tensions of continuous excitation-contraction-relaxation cycles. This article reviews the evidence from recent proteomic studies that have focused on the characterization of the dystrophin-glycoprotein complex and its central role in the establishment of the cytoskeleton-sarcolemma-matrisome axis. Proteomic findings suggest a close linkage of the core dystrophin complex with a variety of protein species, including tubulin, vimentin, desmin, annexin, proteoglycans and collagens. Since the almost complete absence of dystrophin is the underlying cause for X-linked muscular dystrophy, a more detailed understanding of the composition, structure and plasticity of the dystrophin complexome may have considerable biomedical implications.

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Contents

1. Introduction

Following the establishment of the mass spectrometry-based draft of the human proteome and its variation in different tissues [1–3], a new emphasis of proteome-wide studies is the detailed elucidation

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of genotype-phenotype relationships at the level of interactome networks [4]. The systematic application of target proteomics and the detailed characterization of complexomes promise new insights into proteome-wide alterations due to developmental processes, physiological adaptations, pathological insults or natural aging [5–7]. A large number of bioinformatics tools are available to assess proteome-wide predictions of protein-protein interaction patterns in health and disease [8–12]. In skeletal muscle proteomics, comprehensive studies focusing on the systematic cataloguing of the protein constituents of contractile tissues have been carried out over the last decade and established thousands of distinct protein species being present in the most abundant type of tissue in the mammalian body [13–18]. Building on these proteomic maps, it is now possible to determine the specific arrangement and latent plasticity of protein-protein interaction patterns within large protein complexes from skeletal muscles. Contractile fibres contain considerable numbers of extremely high-molecular-mass proteins and many membrane-associated supramolecular protein complexes. Such protein species are difficult to study using standard biochemical and biophysical techniques. However, the extraordinary advances made in large-scale protein separation methods and the development of highly sensitive mass spectrometers has drastically improved the capabilities of studying very large proteins and multi-subunit complexes [19].

The giant class of muscle proteins is exemplified by titin, nebulin and obscurin, crucial molecular players that provide fibre elasticity, stretch response and sarcomeric organization [20]. Large protein complexes from skeletal muscles are represented by the ryanodine receptor calcium release channel of the triad junction, the dihydropyridine receptor of the transverse tubules and the dystrophin-associated glycoprotein complex of the sarcolemma [21,22]. This makes skeletal muscle an ideal system for the study of the formation and stabilization of very large protein complexes, as well as determining the potential susceptibility of supramolecular protein structures to proteolysis and degradation under pathophysiological conditions. This review presents an overview of recent proteomic investigations that have focused on the mass spectrometric analysis of dystrophin and its associated glycoprotein complex. Since the results of comparative proteomic studies of muscular dystrophy and the determination of secondary effects downstream of dystrophin deficiency have previously been reviewed [23–25], these aspects of the proteomic analysis of the dystrophin complex will not be covered in detail. Instead, this article provides a comprehensive account of the mass spectrometric analysis of the dystrophin-associated complexome and its central role in the trans-sarcolemmal linkage between the basement membrane and the intracellular actin cytoskeleton.

2. Dystrophin

The positional cloning strategy that was used in the molecular genetic analysis of X-linked muscular dystrophy resulted in the ground-breaking discovery of the dystrophin gene [26]. The *Dmd* gene represents the largest identified gene in the human genome [27]. It contains 79 exons and exhibits a highly complex arrangement of 7 promoters that drive the expression of 3 full-length Dp427 isoforms and 4 shorter isoforms named Dp260, Dp140, Dp116 and Dp71 [28]. The tissue-specific dystrophin species are Dp427-M in striated muscle fibres, Dp427-B in brain, Dp427-P in Purkinje neurons, Dp-260-R isoform in retina, Dp-140-B/K in brain and kidney tissues and Dp-116-S in Schwann cells [29]. The smallest dystrophin isoform Dp-71-G is ubiquitous with high levels in the central nervous system [30].

The molecular structure of the full-length Dp427 protein from skeletal muscle includes a unique carboxy-terminal (CT) domain, a cysteine-rich (CR) domain (including a WW-domain protein-binding motif, a ZZ module and an EF hand Ca^{2+}-dependent region), a central rod domain characterized by 24 spectrin-like repeats (SLR 1-3, SLR 4-19 and SLR 20-24) interspersed by 4 proline-rich hinge regions H1 to H4, and an amino-terminal domain with calponin homology units [31,32]. The Dp427-M isoform contains the major binding sites for cortical actin in the amino-terminus and the central rod domain, as well as interaction zones for neuronal nitric oxide synthase nNOS, β-dystroglycan, syntrophins and dystrobrevins in the rod domain, the most distal hinge region, the cysteine-rich domain and the carboxy-terminal domain, respectively [33].

Striking similarities of large structural domains within the Dp427 molecule with the prototypical cytoskeletal proteins β-spectrin and α-actinin established the principal role of dystrophin as a stabilizing linker at the sarcolemma-actin interface in striated muscles [34]. Dystrophin of 427 kDa has characteristic biochemical properties of a membrane cytoskeletal protein [35] and is recognized as a major member of the spectrin-type super family of actin-binding proteins that are proposed to have originated from a common ancestral α-actinin molecule [36]. Immuno electron-microscopical studies of normal skeletal muscle localized dystrophin to the fibre periphery on the cytoplasmic face of the sarcolemma [37]. The detailed biochemical and cell biological characterization of the protein product of the *Dmd* gene established that full-length dystrophin does not exist in isolation at the sarcolemma membrane, but forms tight interactions with a variety of other muscle proteins [38].

3. Dystrophin-associated proteins

The systematic application of lectin-based methods was of central importance for the detailed biochemical analysis of dystrophin. The plant lectin wheat germ agglutinin is frequently employed for the isolation and characterization of glycoproteins containing N-acetylglucosamine and sialic acid residues [39] and was instrumental for the biochemical enrichment of the dystrophin complex. Since the Dp427 molecule itself is not glycosylated [31], the tight binding of dystrophin to lectin-containing beads was interpreted as indirect interactions via associated glycoproteins [40]. Lectin affinity techniques were used in combination with cell biological, structural and biochemical methods to establish the composition of the dystrophin-glycoprotein complex in both normal and dystrophic muscle fibres [41]. This included the analysis of the dystrophin-glycoprotein complex in affinity-purified sarcolemma vesicles [35,42] and the related utrophin-glycoprotein complex of the neuromuscular junction [43], the biochemical isolation and characterization of the dystrophin-associated glycoprotein complex from detergent-solubilized microsomal preparations [40,44–46] and the purification of skeletal muscle dystrophin to homogeneity [47].

Since the original discovery of the dystrophin-associated glycoprotein complex [44], a large number of studies have characterized the various components attached to dystrophin [48]. Dystrophin-associated proteins can be divided into sarcolemmal proteins (β-dystroglycan, α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, δ-sarcoglycan, sarcospan), cytosolic proteins (dystrobrevins, syntrophins, nNOS) and extracellular proteins (α-dystroglycan, laminin) [41]. This article focuses on the proteomic identification and characterization of the dystrophin complexome. For detailed descriptions of the initial biochemical and cell biological characterization of the dystrophin-glycoprotein complex prior to its proteomic evaluation, please see extensive reviews [22,41,48,49]. Distinct molecular linkages underlie the coupling between dystrophin and the sarcolemmal glycoprotein β-dystroglycan, dystrophin and cortical actin, and dystrophin and the cytoplasmic components of the dystrophin-glycoprotein complex, including syntrophins and dystrobrevins. Domains within the Dp427 molecule with crucial binding sites are represented by the amino-terminal domain, the central part of the spectrin-like repeats, the cysteine-rich domain and the coiled-coil region at the carboxy terminus.

Besides the core members of the dystrophin-associated glycoprotein complex, various physiological receptors, signalling proteins, cytoskeletal elements and extracellular components are indirectly linked to dystrophin forming a large protein network at the

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