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Review

Dystrophin complex functions as a scaffold for signalling proteins ☆

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ABSTRACT

Dystrophin is a 427 kDa sub-membrane cytoskeletal protein, associated with the inner surface membrane and incorporated in a large macromolecular complex of proteins, the dystrophin-associated protein complex (DAPC). In addition to dystrophin the DAPC is composed of dystroglycans, sarcoglycans, sarcospan, dystrobrevins and syntrophin. This complex is thought to play a structural role in ensuring membrane stability and force transduction during muscle contraction. The multiple binding sites and domains present in the DAPC confer the scaffold of various signalling and channel proteins, which may implicate the DAPC in regulation of signalling processes. The DAPC is thought for instance to anchor a variety of signalling molecules near their sites of action. The dystroglycan complex may participate in the transduction of extracellular-mediated signals to the muscle cytoskeleton, and β-dystroglycan was shown to be involved in MAPK and Rac1 small GTPase signalling. More generally, dystroglycan is view as a cell surface receptor for extracellular matrix proteins. The adaptor proteins syntrophin contribute to recruit and regulate various signalling proteins such as ion channels, into a macromolecular complex. Although dystrophin and dystroglycan can be directly involved in signalling pathways, syntrophins play a central role in organizing signalplex anchored to the dystrophin scaffold. The dystrophin associated complex, can bind up to four syntrophin through binding domains of dystrophin and dystrobrevin, allowing the scaffold of multiple signalling proteins in close proximity. Multiple interactions mediated by PH and PDZ domains of syntrophin also contribute to build a complete signalplex which may include ion channels, such as voltage-gated sodium channels or TRPC cation channels, together with, trimeric G protein, G protein-coupled receptor, plasma membrane calcium pump, and NOS, to enable efficient and regulated signal transduction and ion transport. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters.

Contents

1. Introduction : Dystrophin and dystrophin-related proteins

Dystrophin is a 427 kDa cytoskeletal protein expressed from the DMD gene defective in Duchenne muscular dystrophy [1,2]. The transcription of the DMD gene is controlled by three independent promoters, the Brain (B), muscle (M) and Purkinje (P) promoters reflecting the tissue distribution of dystrophin expression [3]. The M promoter drives high level of expression in striated skeletal and cardiac muscles [4]. The DMD gene has also four internal promoters (R for retinal, B3 for Brain3, S for Schwann cells, G for General) that give rise to shorter transcripts encoding for truncated COOH-terminal isoforms. Splicing at a unique first exon generates dystrophin isoforms of 260 kDa (DP60), 140 kDa (DP140), 116 kDa (DP116), and 71 kDa (DP71) [5–7]. These COOH-

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terminal dystrophin proteins contain some binding sites allowing interaction with a number of dystrophin-associated proteins (DAP).

The 427 kDa dystrophin is a member of the β-spectrin/α-actinin protein family [8]. Based on sequence homology, this cytoskeletal protein is thought to be organized into four distinct domains: (*i*) The amino-terminal domain contains pair of calponin homology (CH) modules binding filamentous actin [9]; (*ii*) Adjacent to this region, the central rod domain is composed of more than 2800 amino acids building 24 homologous triple helical repeats and four hinge domains [8], which are suggested to confer flexibility to the protein; (*iii*) A third region is composed of a WW domain [10], which is a small β-sheet motif that is usually involved in intracellular signalling through the recognition of proline-rich or phosphorylated linear peptide sequences. The WW domain of dystrophin recognizes a PPxY motif and is involved in the interaction with β-dystroglycan. This WW domain is followed by a cystein-rich domain with two EF-hand motifs [11] and two ZZ modules in series [12] binding to calmodulin in a calcium-dependent manner [13]; (*iv*) The COOH terminus domain, which is unique to dystrophin and related protein [14] contains two regions forming α-helical coiled coils [15] forming the binding site for dystrobrevin. Dystrophin is a sub-membrane cytoskeletal protein, i.e. associated with the inner surface membrane and incorporated in a large macromolecular complex of proteins, the dystrophin-associated protein complex [16,17]. The demonstration that dystrophin is linked through the membrane-spanning protein complex to the extracellular matrix (ECM) and to the actin cytoskeleton through the amino-terminal domain [18], has originally led to the idea that dystrophin played a structural role in ensuring membrane stability and force transduction during muscle contraction. This role was thought to preclude membrane disruptions (micro-ruptures) and non-specific leakages of ions and/or other biological components and led to the "mechanical hypothesis" for DMD, in which the loss of dystrophin, and of the cytoskeleton-ECM linkage, could be the primer of the progressive cellular necrosis (by over-activating calcium-dependent proteases) observed in such a disease. Studies of transgenic mice expressing deleted dystrophin constructs suggested that the cystein-rich domain with amino-terminal domain or portions of the rod domain are minimally required for protecting mouse muscle against dystrophic degeneration [19]. The dystrophin-related protein, utrophin, can functionally compensate for the lack in dystrophin in *mdx* dystrophic mouse and protect the muscle against degeneration [20,21]. Utrophin shows significant sequence homology with dystrophin and structural similarities [14], which can also provide mechanical protection to the skeletal muscle. However, utrophin does not anchor nNOs to sarcolemma and cannot restore this signalling pathway as does the dystrophin/syntrophin complex. Among dystrophin-related proteins with sequence homology to dystrophin, the DRP2 and the dystrobrevins proteins only have sequence similarity to the COOH-terminal regions of dystrophin [22]. Dystrobrevins are encoded by two different genes, α and β, and have significant homology with the cysteine-rich domain of dystrophin [23,24]. Alpha-dystrobrevin is expressed predominantly in muscle and brain whereas β-dystrobrevin is expressed in non-muscle tissues, which is abundant in the brain, kidney, lung and liver [25]. Knockout of α-dystrobrevin results in progressive myopathy suggesting an essential role in striated muscle [26]. Apart from dystrophin, utrophin and DAPC the dystrobrevins have a set of specific binding partners involved in structural integrity: syncoilin; dysbindin; desmuslin (also known as β-synemin) and DAMAGE [25,27]. Dystrobrevins have also been involved in intracellular signalling in muscle and non-muscle tissues, either directly, or through interaction with syntrophin [26,27], and also by interaction with Regulatory Subunit of protein kinase A, and Protein phosphatase 2A [28].

2. Dystrophin-associated protein complex (DAPC) and cell signalling

In addition to dystrophin the DAPC is composed of dystroglycans, sarcoglycans, sarcospan, dystrobrevins and syntrophin. Discovery of DAPC, referred to as the dystrophin–glycoprotein complex (DGC), represented a major advancement in the understanding of the DGC's function in skeletal muscle and provided further support for the contraction-induced sarcolemma injury model underlying DMD pathogenesis. In another hand, the DAPC has also been proposed to constitute a putative cellular signalling complex by conferring the scaffold for numerous signalling proteins. For instance, the ZZ modules in the cysteine-rich domain of dystrophin may represent a functional calmodulin-binding site which could modulate the binding of other dystrophin-associated protein in a calcium-dependent manner. The multiple binding sites and domains present in the DAPC confer the scaffold of various signalling and channel proteins, which may implicate the DAPC in the regulation of signalling processes. The DAPC is thought for instance to anchor a variety of signalling molecules near their sites of action.

2.1. Dystroglycans

The single dystroglycan gene encodes for a precursor protein [29] that undergoes posttranslational proteolytic cleavage, which produces two noncovalantly subunits of the dystroglycan complex, α- and β-dystroglycan [29–32]. In muscle, α-dystroglycan and β-dystroglycan display a molecular mass of 156 kDa and 43 kDa respectively, whereas in the Brain, the molecular mass of α-dystroglycan, identified as cranin [33] is 120 kDa. The α-dystroglycan is an extensively glycosylated extracellular protein [18,34] with two globular domains connected by an extensible portion [35,36]. The glycoepitope of α-dystroglycan mediates the binding of extracellular matrix components [34,37]. The dumbbell-shaped protein α-dystroglycan binds to the laminin G domain in extracellular matrix components such as laminins, agrin and perlecan. Biglycan binding to α-dystroglycan was also demonstrated by coimmunoprecipitation with both native and recombinant α-dystroglycan [38]. The biglycan binding site was mapped to the COOH-terminal third of α-dystroglycan. In muscle, biglycan was detected at both synaptic and nonsynaptic regions. The binding of biglycan to α-dystroglycan could act in concert with, or as an alternative to, binding via the G-protein—containing basal lamina proteins agrin, perlecan, and laminin, but the function is unknown. However, biglycan null mice exhibits a mild dystrophic phenotype and displays a selective reduction in the localization of alpha-dystrobrevin-1 and -2, alpha- and beta1-syntrophin, and nNOS at the sarcolemma [39]. Moreover, Biglycan protein injected into muscle stably associates with the sarcolemma and ECM and restores the sarcolemmal expression of alpha-dystrobrevin-1 and -2, and beta1- and beta2-syntrophin in biglycan null mice. Biglycan binding is thus important for the stability of DAPC in the skeletal muscle. The β-dystroglycan has a single transmembrane domain spanning the plasma membrane and an extracellular amino-terminal extracellular domain binding to the carboxy-terminal globular domain of α-dystroglycan [40,41]. The COOH terminus on the cytoplasmic side contains several proline residues required for the binding to dystrophin, and binds directly to the WW modules and the cystein-rich domain containing the EF and ZZ modules [42–45]. A study indicates that a WW-like domain within caveolin-3 [46] directly recognizes the extreme C terminus of β-dystroglycan that contains a PPXY motif. It was propose that interaction of caveolin-3 with β-dystroglycan may competitively regulate the recruitment of dystrophin to the plasma membrane.

The dystroglycan complex may participate in the transduction of extracellular-mediated signals to the muscle cytoskeleton, and β-dystroglycan was shown to be involved in MAPK signalling. Laminin engagement by dystroglycan is leading to the recruitment of a Grb2–Sos1 complex to dystroglycan [47]. The resulting downstream activation of Rac1, activates JNK, through the Cdc42-Race effector p21 activated kinase 1 (PAK1). Several studies performed in non-muscle cells implicate dystroglycan in the modulation of ERK-MAPK signalling. The interaction of β-dystroglycan with MEK and ERK [48] suggests dystroglycan may

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