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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 viewed 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. Guest Editor: Jean Claude Hervé.

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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 cysteine-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 cysteine-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 noncovalently 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 crinin [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 cysteine-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 proposed 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–Rac 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

act as a scaffold interacting with components of the ERK–MAPK cascade. A role of dystrophin–dystroglycan complex in MAPK signalling in skeletal muscle was indirectly explored in *mdx* dystrophic mice lacking dystrophin. Some studies reported a higher ERK1/2 activation after a mechanical stimulation [49] or following a chronic treadmill exercise [50], suggesting that the loss of dystrophin–dystroglycan complex is responsible for an altered mechanotransduction. It was also reported that disruption of laminin binding to α -dystroglycan induced apoptosis in dystrophic myotubes through a decreased Akt activity [51]. This may account for a role of dystrophin–glycoprotein complex in survival signal. However Akt was reported to be increased in two other studies on *mdx* muscle deficient in DAPC [52,53]. Dystroglycan is also involved in various cellular processes including neuromuscular junction formation through, in part, interaction of β -dystroglycan with rapsyn [54], a peripheral protein required for nicotinic acetylcholine receptor (AChR) clustering. Rapsyn and dystroglycan interact in the postsynaptic membrane reinforcing the notion that dystroglycan could be involved in synaptogenesis. In vitro studies have also suggested that β -dystroglycan interacts with growth factor receptor bound protein 2 (Grb2), when non-associated with dystrophin [55]. The Grb2/ β -dystroglycan association is mediated through β -dystroglycan proline-rich domains and Grb2 src homology 3 domains. This interaction may be of biological importance in transducing signals arising from the binding of dystroglycan to extracellular matrix proteins [56] or in transferring information between the dystroglycan complex and other signalling pathways [57]. Recent studies support a model whereby dystroglycan serves as a receptor essential for the initial binding of laminin on the cell surface [58]. New findings with in neurons reveal a fundamental role for dystroglycan in organizing axon guidance cue distribution and function within the extracellular matrix [59]: It was found that glycosylated dystroglycan binds directly to the axon guidance cue Slit to organize its protein distribution in the floor plate and the basement membrane, thereby regulating Slit-mediated axon guidance. A nuclear import pathway for β -dystroglycan was also recently reported and new findings imply that β -dystroglycan is a nuclear scaffolding protein involved in nuclear organization and nuclear envelope structure and functions in myoblasts [60]. More generally, dystroglycan is viewed as a cell surface receptor for extracellular matrix proteins, which is involved in cell polarity, matrix organization and mechanical stability of tissues [61–63]. Several studies documented the loss of dystroglycan protein expression and glycosylation in a variety of cancer types [64,65], and it was recently proposed to be caused by a down regulation of LARGE2 resulting in hypoglycosylation of α -dystroglycan and loss of its ability to bind to laminin-111 [66].

2.2. Sarcoglycans

The sarcoglycan complex is composed of α , β , γ and δ -sarcoglycan isoforms encoded by separate genes [67–70] and of sarcospan [71]. Sarcoglycans are single transmembrane glycoproteins with N-terminus oriented extracellularly for α -sarcoglycan and intracellularly for β , γ and δ -sarcoglycans [67–70]. On the contrary, sarcospan is constituted of four transmembrane-spanning segments, which are homologous to the tetraspanin family [71]. Two other sarcoglycan have been identified, ϵ -sarcoglycan, with homology to α -sarcoglycan [72], and ξ -sarcoglycan, most homologous to γ and δ -sarcoglycan isoforms [73]. The function of the sarcoglycan complex is not fully understood, but it appears to strengthen interaction of β -dystroglycan with α -dystroglycan and dystrophin [74]. Mutations in any of these four glycosylated single-pass transmembrane proteins result in autosomal recessive limb-girdle muscular dystrophy (LGMD-2C-2 F). In the absence of δ -sarcoglycan, such as in LGMD-2 F, the remaining sarcoglycan members (α , β and γ) cannot assemble and are quickly degraded before transport from the Golgi [75,76]. The absence of δ -sarcoglycan has also been shown to reduce nNOS levels (neuronal nitric oxide synthase,

which in turn regulates vasodilation during exercise), and increases its displacement from the sarcolemma [77].

Biglycan was shown to be a ligand for two members of the sarcoglycan complex and regulates their expression at discrete developmental ages [78]. Small leucine-rich repeat (LRR) proteoglycan biglycan binds to α - and γ -sarcoglycan. Both biglycan proteoglycan as well as biglycan polypeptide lacking glycosaminoglycan (GAG) side chains are components of the dystrophin glycoprotein complex isolated from the adult skeletal muscle membranes. Biglycan is an extracellular component of signalling pathways that was shown to play a role in the formation of stress fibres in cultured cells in a manner that is dependent on small GTPases [79] and also in muscle regeneration [80,81]. Sarcoglycans could thus be a pathway in skeletal muscle for mediating the effects of biglycan during myogenesis and muscle regeneration. Since defects in the sarcoglycan complex were also associated with muscle dystrophies, one may also hypothesise that biglycan binding to sarcoglycan is involved in transduction of cell survival signals.

Several studies on sarcoglycan function suggested a role in intracellular signal transduction. For instance the cytoplasmic domain of γ -sarcoglycan displays five tyrosine residues, which may be involved in bidirectional signalling with integrin [82]. In another hand, the α -sarcoglycan was reported to display ecto-ATPase activity [83], which suggests that α -sarcoglycan may modulate the activity of P2X receptors by buffering the extracellular ATP concentration. Sarcospan, a 25-kDa transmembrane protein, was the last component to be identified [71] and its function in skeletal muscle has been elusive. As reviewed by Marshall and Crosbie-Watson [84], recent works highlighted new signalling functions for sarcospan. Sarcospan improves cell surface expression of the dystrophin- and utrophin-glycoprotein complexes as well as α 7 β 1 integrin, which are the three major laminin-binding complexes in muscle. Moreover, sarcospan was proposed to modulate utrophin protein levels at least in part through Akt/p70S6K signalling pathways [85].

2.3. Syntrophins

Syntrophins are multigene family of intracellular membrane-associated adaptor proteins. The syntrophin family consists of five homologous isoforms, α 1-syntrophin, β 1-syntrophin, β 2-syntrophin, γ 1-syntrophin and γ 2-syntrophin [86–89]. The different isoforms of syntrophin have different cellular and sub-cellular localization suggesting a distinct functional role. The α 1-syntrophin linked to the DAPC and distributed over the entire sarcolemma of skeletal muscle fibres is mediating the anchoring of neuronal nitric synthase (nNOS) to the sarcolemma and the dystrophin complex [90,91]. The β 2-syntrophin is exclusively restricted to the neuromuscular junction [92]. The γ 1-syntrophin has been shown to be involved in cellular synaptic function by binding and regulating sub-cellular localisation of diacylglycerol kinase ξ [93], which catalyses the conversion of diacylglycerol (DAG) to phosphatidic acid (PA). Both DAG and PA are lipid second messengers with roles in actin organization for instance. This could confer a role in cell shape regulation through actin modelling and also in cell migration.

These scaffold proteins are characterized by the presence of a N-terminal PH-1 domain (Plekstrin Homology) split in two halves (PH_N and PH_C) by insertion of a PDZ domain (Post Synaptic Density protein-95, *Drosophila* discs large protein, and the *Zona occludens* protein 1), a second Plekstrin Homology domain (PH-2) and a C-terminal domain unique to syntrophins (SU). [87,94]. The SU domain and PH-2 domain interact with the carboxy terminus of dystrophin [95,96]. There are two syntrophin binding sites in the carboxy terminus of dystrophin and additional two sites on α -dystrobrevin [97], cytoplasmic proteins sharing significant homology with the carboxy-terminal domains of dystrophin [98]. The presence of binding domains like PH, SU and PDZ domain allow these scaffold proteins to interact with several other proteins and lipids leading to the building of multi-protein/lipid signalling

complexes. This confers DAPC a role in regulating various intracellular signalling pathways.

2.3.1. Syntrophins and signaling pathways

Syntrophins were shown to be involved in signalling pathways necessary for the development and building of the neuromuscular junctions: $\alpha 1$ -syntrophin-knockout in mice shows that the scaffolding protein plays an important role in neuromuscular junction maturation [99,100], and during muscle regeneration [101], and the density of acetylcholine receptors at the neuromuscular junction is reduced in $\alpha 1$ -syntrophin^{-/-} mice [99]. The $\alpha/\beta 2$ -syntrophin null mice displays neuromuscular junctions that are structurally more aberrant than those lacking only of $\alpha 1$ -syntrophin [102].

The PH and PDZ domains of syntrophins were shown to bind various proteins, which may implicate syntrophins in numerous signalling pathways and cell function. For instance, the NH2 terminus of PH1 domain and the NH2 terminus of PDZ domain were shown to bind calmodulin [103,104], a calcium binding protein transducing calcium signal in the cell by interacting with downstream target proteins. Signalling pathways dependent on heterotrimeric G-proteins may also interact with syntrophins. The PH_N halfdomain was shown to interact with multiple isoforms of G α subunits [105], such as G α s, G α i, G α o and G α q subtypes. The binding of G α subunit of heterotrimeric G protein with syntrophin suggests the scaffolding protein may regulate signalling pathways dependent on G-coupled transmembrane receptors. For instance, in COS-7 cells, down regulation of $\alpha 1$ -syntrophin resulted in an enhanced cAMP production [105]. PH domains of other proteins were shown to bind the $\beta\gamma$ -subunits of the heterotrimeric G proteins and proposed to be involved in localization of key signalling proteins to appropriate membrane compartment. Isolated syntrophin was also demonstrated to bind brain G $\beta\gamma$ -subunits [106], and the PDZ domain-containing sequence of recombinant α -syntrophin was required for binding G $\beta\gamma$ -subunits. This binding was shown to be dependent on laminin- α -dystroglycan binding in skeletal muscle, and to affect interaction of G $\beta\gamma$ with α -syntrophin [106]. Interestingly, this interaction decreases the amount of active G $\beta\gamma$ and was suggested to inhibit Ca²⁺ through calcium channels. In addition, $\alpha 1$ -syntrophin interacts through the PDZ domain with the C-terminal domain of $\alpha 1$ -adrenergic receptors [107], which are G protein-coupled receptors mediating physiological function in cardiovascular apparatus. Moreover, mutation of the PDZ domain was shown to decrease inositol phosphate formation in response to norepinephrine and to decrease $\alpha 1$ -adrenergic receptor binding and expression [107]. These observations provided additional arguments about the role of syntrophins in modulating G protein-coupled receptor function. Moreover, the syntrophin-dystrophin complex in mouse aortic smooth muscle cells was shown to associate with $\alpha 1$ -adrenergic receptor for building a signalplex, playing an essential role in the regulation of $\alpha 1$ -adrenergic receptor function and of vascular tone and blood pressure [108]. Dystrophin, syntrophin, dystrobrevin and utrophin are interacting proteins for $\alpha 1$ -adrenergic receptor, and the knock-out of multiple syntrophin isoforms results in the complex loss of $\alpha 1$ -adrenergic receptor function in mouse aortic smooth muscle cells.

Syntrophin PDZ domain also binds an ETTF motif in neuronal nitric oxide synthase (nNOS) that forms a β -finger [109]. In skeletal muscle, interaction of nNOS with $\alpha 1$ -syntrophin is mediated by PDZ domain [110], and nNOS in brain also interacts with $\alpha 1$ -syntrophin in specific neurons [111]. Alpha1-syntrophin gene disruption results in the absence of nNOS at the sarcolemma of skeletal muscle fibres [112], and an in vivo dominant-negative approach indicated that the PDZ domain is not required for plasma membrane association of $\alpha 1$ -syntrophin, but is necessary for the sarcolemmal localization of nNOS [91]. It is likely that $\alpha 1$ -syntrophin PDZ domain is required for proper function of nNOS in skeletal muscle, and it was shown that localization of the enzyme to the sarcolemma was important for regulating adrenergic-vasoconstriction in muscle during activity [113,114]. Moreover, nNOS and $\alpha 1$ -syntrophin were

shown to be parts of a macromolecular protein complex containing the sarcolemmal calcium pump (PMCA) in cardiomyocytes [115]. A ternary interaction between PMCA, $\alpha 1$ -syntrophin and nNOS was proposed, where the COOH-terminal tail of PMCA interacts with the PDZ domain of nNOS and the linker region between the PH2 and SU domains of $\alpha 1$ -syntrophin are involved in binding an intracellular loop of PMCA. The PMCA was shown to be a negative regulator of nNOS-dependent NO production [115,116], and $\alpha 1$ -syntrophin and PMCA were proposed to synergistically moderate nNOS-activity. NO signalling is involved in different functions in the heart including contractility [117] and inward sodium currents [118]. In addition to NOS interaction, syntrophins have been implicated in the regulation of various ion channels of the plasma membrane such as voltage-operated sodium channels, and this usually involved the PDZ domain of syntrophin.

2.3.2. Syntrophins and ion channels and membrane transporters

The PDZ domain from $\alpha 1$, $\beta 1$ and $\beta 2$ syntrophins was shown to bind the C-terminal 10 amino acids of voltage-gated sodium channels from the skeletal (SkM1) and cardiac (SkM2) muscles [119]. This interaction was shown to be mediated by the direct interaction of the PDZ domain to the (S/T)XV C terminus of the sodium channel. The cardiac voltage-gated channel Nav1.5 was also reported to associate through C-terminus with dystrophin, and this interaction was mediated by α and β -syntrophins in a PDZ-dependent manner [120]. The study of deficient *mdx* mice suggested that the disruption of the dystrophin/syntrophin/Nav1.5 channel resulted in the alteration of expression and function of sodium channels, with consequences on ECG properties. Interestingly, inward sodium currents of cardiomyocytes were described to be modulated by the level of S-nitrosylation of Nav1.5 sodium channels, which was dependent on association of the channels with the PMCA-nNOS complex through interaction with $\alpha 1$ -syntrophin [121]. An A390V mutation of $\alpha 1$ -syntrophin selectively disrupted the association of PMCA with the complex and increased nitrosylation of Nav1.5 sodium channels, likely through an increased NOS activity. The disruption of the complex increased amplitude of peak and late sodium currents and was associated with a long QT syndrome [121], which can be due in rare case to $\alpha 1$ -syntrophin mutation. Altogether, these studies show that the scaffold of $\alpha 1$ -syntrophin is required for maintaining a PMCA/nNOS/Nav1.5 complex, which is a key regulator of sodium currents in cardiomyocytes and as a consequence of cardiac rhythm.

This study, together with the work of Ueda and collaborators [121], highlighted the central role of syntrophin in maintaining a signalling complex anchored to dystrophin scaffold and necessary for proper expression and function of voltage-gated sodium channels. These channels linked to syntrophin are thus incorporated in a signalplex containing PMCA and nNOS, which regulates voltage-gated sodium currents. The absence of dystrophin also modifies the expression level and gating properties of Nav1.4 channels in *mdx* skeletal muscle, leading to an increased Na⁺ concentration under the sarcolemma [122]. In *mdx* muscle, the analysis of Nav1.4 distribution suggested that syntrophin is an important linker between dystrophin and Nav1.4. The idea that dystrophin/syntrophin complex interacts with ion channels and regulates the channel function was also proposed for non-voltage-gated calcium channels in skeletal muscle. Native TRPC1 channels were shown to be associated to the dystrophin/syntrophin complex [123], as well as TRPC4 channels [124], and $\alpha 1$ -syntrophin was shown to play a key role through PDZ domain on regulation of store-operated calcium entry [124], supported by TRPC1 and TRPC4 in skeletal myotubes. Through a VTTRL motif, TRPC4 binds to the PDZ domain of the scaffolding protein Na⁺/H⁺ Exchanger Regulatory Factor, NHERF [125]. This motif present in TRPC4 could account for the association between the TRPC tetrameric channel and the PDZ domain of $\alpha 1$ -syntrophin, which was shown to capture TRPC1 and TRPC4 in pull-down assays [123,124]. Moreover, $\alpha 1$ -syntrophin was essential for a PLC-dependent-regulation of store-operated calcium entry in mouse and human

skeletal myotubes [126,127], and PLC γ was shown to be associated with α 1-syntrophin [126]. These observations provided the idea that TRPC1/C4 channels association with syntrophin and PLC γ could constitute a signalling complex anchored to dystrophin at costameric membrane domains, which regulates the function of the TRPC channel. In accordance with a costameric DAPC including TRPC1 at the sarcolemma, Gervasio and collaborators reported the association of TRPC1 with the scaffolding protein caveolin-3, by showing co-localization at the sarcolemma and co-immunoprecipitation of endogenous proteins [128]. Moreover, this work suggested by FRET assay and coexpression of TRPC1-CFP with caveolin-3-YFP, that the latter is necessary for the localization of TRPC1 at the plasma membrane of myoblasts. The regulation of the TRPC signalplex by DAPC may have a critical role in maintaining sub-plasma membrane calcium homeostasis. Regulated calcium entry through TRPC may create a calcium microdomain underneath the plasma membrane, which will regulate for instance the activity of membrane-associated enzymes such as calpain. This can explain calcium alteration observed in dystrophin deficient skeletal muscle cells with elevated calcium entry, high calcium concentration sub-membrane microdomain and higher proteolysis activity [129]. However, no myopathy is associated with syntrophin depletion [99,100] suggesting either this function is compensated by another signalplex, or this pathway is not crucial for striated muscle survival. However, over expression of a TRPC channel and increase in associated calcium entry in mouse expressing dystrophin was shown to be sufficient for inducing dystrophic phenotype [130].

The PDZ domain of syntrophin was also shown to interact directly with other non-voltage gated channels such as mechanosensitive Na⁺ channels [131], as shown by pull-down experiments using the PDZ domain of γ 2-syntrophin and the C-terminus of the mechanosensitive Na⁺ channel. Interaction with γ 2-syntrophin and its PDZ domain was shown to regulate sodium currents in human jejuna circular smooth muscle cells, and was proposed to determine mechanosensitivity and

current availability. The presence of a consensus PDZ domain binding motif (SNV) at the C-terminal domain of the potassium channel Kir4.1 was also shown to be involved in interaction with the PDZ domain of α -syntrophin [132]. This interaction mediates the binding of the inwardly rectifying potassium channel to the dystrophin-associated complex at glial cells plasma membrane, and is required for correct distribution at precise membrane subdomains. This may have physiological consequence since potassium channels in glial cells are known to be implicated in extracellular potassium homeostasis in the central nervous system [133]. Syntrophin and its PDZ domain were also implicated in the plasma membrane distribution of Aquaporin-4 (AQP4), a channel protein mediating water flows. AQP4 c-terminal domain contains the sequence Ser-Ser-Val (-SSV), which potentially binds to PDZ domain [134], and expression of AQP4 is drastically reduced in skeletal muscle cells of dystrophin-deficient *mdx* mice [135]. The polarized distribution of AQP4 in the brain is altered in α -syn^{-/-} mice, and AQP4 expression is markedly reduced in astrocytes endfeet membrane adjacent to blood vessels in the cerebellum and cerebral cortex [136]. This study showed that subcellular localization of AQP4 depends on association with the dystrophin complex, through a (-SSV)-PDZ-mediated interaction with α -syntrophin. In the brain, the polarized expression of AQP4 in astrocytes may be important for water homeostasis and works in concert with inwardly rectifying K⁺ channels to allow maintaining potassium homeostasis during high neuronal activity. The syntrophin/dystrophin complex interacting with both channels may thus play a critical role for these functions supported by astrocytes.

3. Conclusive remarks

Since the discovery of dystrophin and its associated complex, number of studies highlighted the role of dystrophin-associated complex, especially in striated muscle, in mechanoprotection of plasma membrane. It is now well established that dystrophin-associated complex

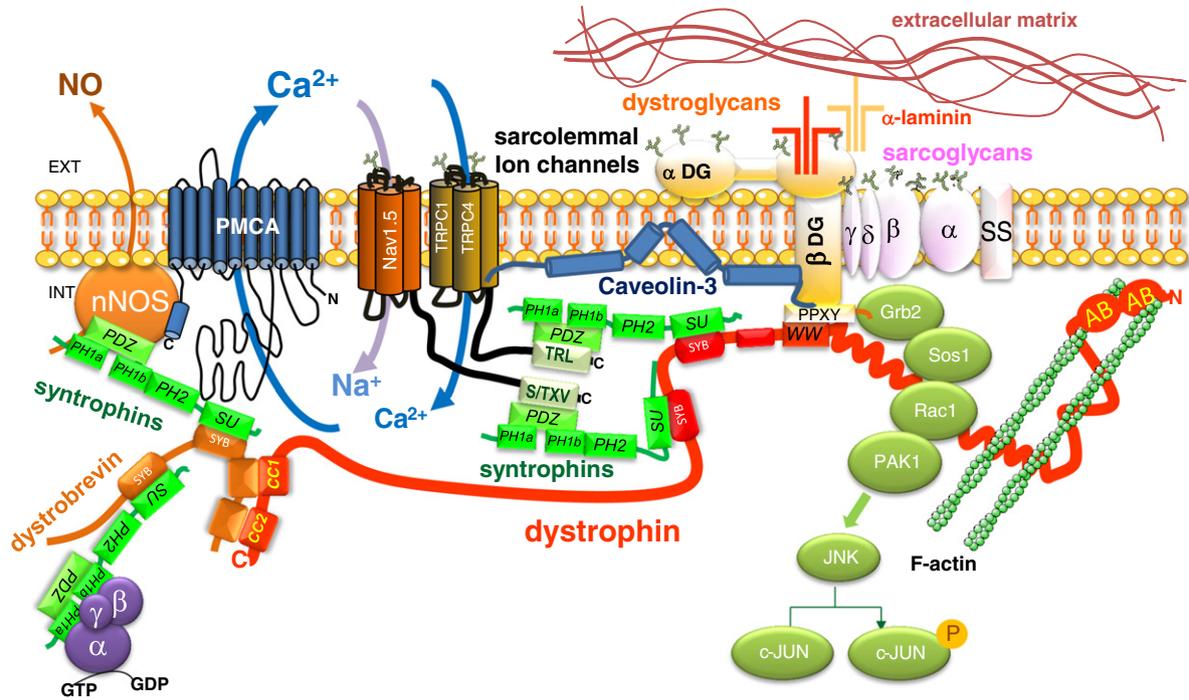


Fig. 1. Different interactions between the dystrophin complex and signalling molecules organize a signalplex anchored to the subsarcolemmal dystrophin. Dystroglycan complex is involved in binding extracellular components and binds to dystrophin through the C-terminal domain of β -dystroglycan. This latter through interaction with Grb2 and Sos1 can be involved in the activation of Rac1 and PAK1 and of JNK/c-Jun pathways. Syntrophin can also interact with this intracellular signalling pathway. Syntrophins, bound to dystrophin through SU domain can also regulate sodium homeostasis through the binding of Nav1.5 voltage-gated sodium channels via its PDZ domain, and calcium homeostasis through interaction with TRPC1/TRPC4 channels and also PMCA. The calcium pump PMCA is also interacting with nNOS, regulating the activity of the enzyme, which is anchored and targeted by syntrophin through interaction with the PDZ domain. The PDZ domain of α -syntrophin regulates the activity of SCN5A sodium channels, and of calcium influx through TRPC1/TRPC4 channels which also interact with caveolin-3. Voltage-gated sodium channels linked to syntrophin are thus incorporated in a signalplex containing PMCA and nNOS, which may also interact functionally with TRPC1/TRPC4 channels. Heterotrimeric G-proteins also interact with syntrophin, which may regulate G protein-coupled receptor function and have consequences on calcium homeostasis also.

plays a crucial role for numerous signalling pathways, and that the adaptor proteins syntrophin contribute to recruit and regulate various signalling proteins such as ion channels, into a macromolecular complex (Fig. 1). 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 syntrophins through the 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 G protein-coupled receptor, plasma membrane calcium pump, and NOS, to enable an efficient and regulated signal transduction and ion transport.

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