REVIEW

Syntrophins entangled in cytoskeletal meshwork: Helping to hold it all together

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Abstract

Syntrophins are a family of 59 kDa peripheral membrane-associated adapter proteins, containing multiple protein-protein and protein-lipid interaction domains. The syntrophin family consists of five isoforms that exhibit specific tissue distribution, distinct sub-cellular localization and unique expression patterns implying their diverse functional roles. These syntrophin isoforms form multiple functional protein complexes and ensure proper localization of signalling proteins and their binding partners to specific membrane domains and provide appropriate spatiotemporal regulation of signalling pathways. Syntrophins consist of two PH domains, a PDZ domain and a conserved SU domain. The PH1 domain is split by the PDZ domain. The PH2 and the SU domain are involved in the interaction between syntrophin and the dystrophin-glycoprotein complex (DGC). Syntrophins recruit various signalling proteins to DGC and link extracellular matrix to internal signalling apparatus via DGC. The different domains of the syntrophin isoforms are responsible for modulation of cytoskeleton. Syntrophins associate with cytoskeletal proteins and lead to various cellular responses by modulating the cytoskeleton. Syntrophins are involved in many physiological processes which involve cytoskeletal reorganization like insulin secretion, blood pressure regulation, myogenesis, cell migration, formation and retraction of focal adhesions. Syntrophins have been implicated in various pathologies like Alzheimer's disease, muscular dystrophy, cancer. Their role in cytoskeletal organization and modulation makes them perfect candidates for further studies in various cancers and other ailments that involve cytoskeletal modulation. The role of syntrophins in cytoskeletal organization and modulation has not yet been comprehensively reviewed till now. This review focuses on syntrophins and highlights their role in cytoskeletal organization, modulation and dynamics via its involvement in different cell signalling networks.

1 | INTRODUCTION

Syntrophins are a heterogeneous group of membrane-associated adaptor proteins, originally identified in the post-synaptic membrane of torpedo electric organ.¹ Syntrophins act as scaffolding proteins

containing multiple protein-protein and protein-lipid interaction domains, interacting with various signalling molecules and kinases like ion channels,^{2,3} aquaporin4 (AQP4),^{4,5} ankyrin repeat-rich membrane spanning (ARMS) protein,⁶ α 1D-adrenergic receptor,⁷ growth factor receptor bound 2 (Grb2),⁸ phosphoinositides,^{9,10} microtubule-associated serine/threonine kinase (MAST),¹¹ syntrophin-associated serine/threonine kinase (SAST),¹² diacylglycerol kinase- ζ ,¹³ stress-activated

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TABLE 1 Characterization of Syntrophin protein family isoforms

Syntrophin protein family						
	α -1 Syntrophin	β -1 Syntrophin	β -2 Syntrophin	γ -1 Syntrophin	γ -2 Syntrophin	
Chromosome location	20q11.21	8q24.12	16q22.1	8q11.21	2p25.3	
Number of exons	9	8	7	19	17	
Number of amino acids	505	538	540	517	539	
Molecular weight, kDa	55.5	59.1	59.4	56.8	59.2	
Tissue specificity	Ubiquitous high expression in skeletal and cardiac muscles	Ubiquitous expression in different tissues	Ubiquitous expression in different tissues	Exclusively expressed in neurons of brain and CNS	Brain, CNS and testis	
Subcellular localization and interacting domains	Sarcolemma via PH2 and SU, Cell junction, Neuromuscular junction via PH1 and PDZ, Cytoskeleton via PH and SU	Plasma membrane via PH2 and SU, Neuromuscular junction folds via PH1 and PDZ, Synapse via PDZ, Cell junction, Cytoskeleton via PH and SU	Plasma membrane via PH2 and SU, Microtubules via PH2 and PDZ, Focal adhesion via PDZ, and SU, Synapse via PDZ, Cytoplasmic vesicle via PDZ	Nucleus, E.R, Cytoskeleton via PH and SU, Plasma membrane via PH2 and SU	Cytoskeleton via PH and SU, E.R, Cytoplasmic side of Sarcolemma via PH2 and SU, Membrane proteins via PDZ	

protein kinase (SAPK).¹⁴ Syntrophins, thus, form multiple functional protein complexes, ensuring proper localization of signalling proteins and their binding partners to specific membrane domains and providing appropriate spatiotemporal regulation of signalling pathways. Interaction of syntrophins with multiple signalling molecules is mediated by two PH domains, a PDZ domain and a conserved SU domain. The PH2 domain and the SU domain are involved in the interaction between syntrophin and the dystrophin-glycoprotein complex (DGC). Syntrophin recruits signalling proteins to DGC, thus linking extracellular matrix to internal signalling apparatus via DGC.

The different domains of syntrophin are crucial for cytoskeletal dynamics as well as for signalling. Syntrophins associate with various cytoskeletal proteins (like actin, intermediate filaments and microtubules) within the cell and induce diverse cellular responses by modulating cell cytoskeleton. Syntrophins are involved in key physiological processes which involve cytoskeletal reorganization like insulin secretion, blood pressure regulation, myogenesis and cell migration.¹⁵⁻¹⁷ In pancreatic β -cells, the PDZ domain of β 2-syntrophin regulates the release of insulin and helps in maintaining the blood glucose level. When the protein is phosphorylated on ser90, it activates actin filaments to clamp the secretory insulin granules and inhibits the release of insulin from cells. When Cdk5 phosphorylates the β 2-syntrophin on ser75 near the PDZ domain, it decreases its binding to insulin granules and increases its motility and exocytosis.^{17,18} α -1 syntrophin has a role in myoblast differentiation in early stages of muscle development. It has been found that α -1 syntrophin in association with histone methyltransferase MLL5 increases expression of myogenin, a key regulatory factor of muscle differentiation, in myoblasts.¹⁶

Syntrophins have a pivotal role in cell migration and are involved in formation and retraction of focal adhesion. The PH1 domain of α -1 syntrophin binds to the phosphoinositol-4,5-bisphosphate (Ptdins(4,5)P2) which regulates signalling proteins crucial for migration.¹⁹ Phosphatidylinositol phosphate 5 kinase (PTEN) synthesizes Ptdins(4,5)P2 at membrane ruffles inducing actin polymerization and focal adhesion formation at these sites.²⁰⁻²² In skeletal muscles, α -1 syntrophin links calcium signalling to DGC via heterotrimeric G-protein($G_{\alpha\beta\gamma}$). The PDZ domain of α -1 syntrophin binds to $G_{\beta\gamma}$ subunit of heterotrimeric G-protein ($G_{\alpha\beta\gamma}$) in presence laminin. This interaction decreases active G_{α} subunit and inhibits Ca^{2+} influx through calcium channels. However, in absence of laminin-dystroglycan complex, G_{α} subunit gets activated and $G_{\beta\gamma}$ subunits dissociate from α -1 syntrophin leading to dihydropyridine receptor (DHPR) mediated Ca^{2+} influx in skeletal muscles.²³⁻²⁵ The increase in intracellular Ca^{2+} ion concentration activates calcium-regulated enzymes like calcineurin (phosphatase) and calpain (protease) which mediate focal adhesion disassembly.²⁶⁻³⁰ In presence of calcium, the SU domain of syntrophin interacts with calmodulin and plasma membrane Ca^{2+} /calmodulin dependent ATPase (PMCA).^{31,32} Syntrophins are also phosphorylated by calcium-calmodulin-dependent kinase II (CAMK II) which is required for vascular smooth muscle cell migration.^{33,34} Syntrophins, thus, help in the retraction of tail end at the trailing edge of migrating cell by inducing focal adhesion disassembly in a calcium-dependent manner. The Syntrophin scaffold has been

implicated in the pathophysiology of various diseases including brain edema,^{35,36} muscular dystrophy,^{9,37-39} long QT syndrome⁴⁰ and cancer.^{38,41} Owing to the importance of syntrophins in cell physiology and disease pathology, in this review we have discussed in detail the role of syntrophin in cytoskeletal organization and remodelling.

2 | THE LOCALIZATION AND STRUCTURE OF SYNTROPHIN PROTEIN FAMILY

Syntrophins are a multigene family of cytoplasmic 59 kDa peripheral membrane scaffolding adapter proteins, containing multiple protein-protein interaction domains. The syntrophin family consists of five different homologous isoforms (α -1, β -1, β -2, γ -1 and γ -2) that exhibit specific tissue distribution, distinct sub-cellular localization and unique expression patterns implying their diverse functional roles.⁴²⁻⁴⁵ α -1 syntrophin shows predominant expression in skeletal and cardiac muscles, distributed over entire sarcolemma and throughout folds of the neuromuscular junction. β -1 and β -2 syntrophins are ubiquitously expressed in different tissues including liver, kidney, brain, testis, skeletal muscles and neuromuscular junction. The distribution of β syntrophin is mainly restricted to neuromuscular junction folds.^{46,47} γ -1 and γ -2 syntrophins show strong expression in brain, central nervous system (CNS) and testis, localized to endoplasmic reticulum and sarcolemma.⁴⁸ The subcellular localizations of the various syntrophin isoforms are stringently regulated in response to their distinctive functional roles within cells. For example, in skeletal muscles, α -1 syntrophin has been shown to be predominantly over the sarcolemma and throughout the folds of neuromuscular junction where they interact with membrane proteins and cytoskeletal elements providing mechanical support to sarcolemma during contractions and also provide appropriate signalling scaffold at synapses for

the targeting signalling proteins like nNOS, dystrophin/utrophin to the post-synaptic membrane increasing the efficacy of the signalling cascade at neuromuscular junction. Similarly, β -2 syntrophin via its PDZ domains localizes to insulin secretory granules and microtubules regulating the release of insulin and microtubule organization respectively. γ -1 and γ -2 syntrophins regulate the subcellular localization of DGK- ζ protein and are also present at synaptic junction involved in synaptic functions of cell (Table 1).

All the members of the syntrophin protein family share similar domain organization, containing two pleckstrin homology (PH1 and PH2) domains at amino-terminus, a single PDZ domain and highly conserved domain unique to syntrophin at the carboxyl terminus. The PH domains are modules of 120 amino acids found in various signalling and cytoskeletal proteins (like β -spectrin, Phospholipase C- γ etc) playing diverse roles in signal transduction and cytoskeletal organization.^{49,50} The N-terminal PH1 domain of syntrophins dissociates into two halves (PH_N and PH_C) due to the intrusion of 80 amino acid long (Post-Synaptic Density Protein-75, Drosophila Discs Large Protein, and the Zona Occludens Protein 1) PDZ domain. Biochemical and structural studies on α -1 syntrophin demonstrated that the split PH domain enfolds into canonical PH domain with or without the PDZ domain embedded.^{51,52} The C-terminal PH2 domain exists as an intact PH domain; PDZ domain insertion does not split it into two halves. The Syntrophin Unique (SU) domain is highly conserved 57 amino acid sequence present at the C-terminal end and is specific to all syntrophin isoforms (Figure 1).

3 | SYNTROPHINS IN THE DGC NETWORK

All five isoforms of syntrophin have a role to play in cytoskeletal organization and maintenance. Different domains of syntrophins

FIGURE 1 Common Domain organization of Syntrophin Protein Family and their interaction with various signalling and structural proteins regulating cytoskeletal dynamics. A, α and β Syntrophin. B, γ Syntrophin

are important for cytoskeletal dynamics as well as signal transduction. Since none of these domains contains any intrinsic enzyme activity, syntrophins are therefore thought to function as adaptors that serve to target their binding partners to specific locations on the cell membrane and localize them for signal transduction⁴⁶. Syntrophins interact with dystrophin-glycoprotein complex (DGC) primarily via its PH2 domain and the C-terminal syntrophin-specific SU domain and recruit various cytoskeletal and signalling proteins to DGC thereby providing membrane stabilization and maintaining integrity of cell membrane.^{42,45,53,54} Dystrophin-glycoprotein complex (DGC) is a multi-component and multifunctional protein complex localized onto the plasma membrane, composed of extracellular, transmembrane and intracellular components, expressed in myriad tissues (muscle and non-muscle).^{55,56} Syntrophins are integral components of this complex along with dystroglycans (α and β), dystrobrevins (α), sarcoglycans (α , β , γ and δ), sarcospan, caveolin-3 and NO synthase that are in strong association with the dystrophin, that is, the central core protein of this complex. The components of DGC serve mechanical role maintaining the structural integrity of skeletal muscle fibres by mediating interaction between extracellular matrix, transmembrane and cytoskeleton proteins. The binding of α -dystroglycans to extracellular matrix proteins (laminin, argin, perlecan and neurexin) and dystrophin to actin filaments forms a continuous framework linking the cytoskeletal elements with peripheral membrane proteins.⁵⁷⁻⁶¹ Thus, DGC acts as a strong functional link physically coupling cell cytoskeleton and extracellular matrix proteins. The anomalies in DGC have been linked with several forms of muscular dystrophies and cardiomyopathy.^{62-64,66,67} Apart from the structural role, DGC also interacts with various signalling molecules, ion channels and kinases including Grb-2, calmodulin, neuronal nitric oxide synthase, stress-activated protein kinase 3, diacylglycerol kinase- ζ , muscle voltage-gated sodium channels SkM1 and SkM2, cardiac voltage-gated sodium channels $\text{Na}_v1.5$, the heterotrimeric G-protein and the TRPC1 stretch-activated calcium channel, thereby regulating various biological processes like cell growth and survival, cytoskeletal reorganization, calcium homeostasis and cell migration.^{8,14,23,31,33,68-71} Association of syntrophins with different members of DGC has been reported in various studies. Syntrophins are known to interact with dystrophin, utrophin (dystrophin homolog) and dystrobrevin members of membrane-associated cytoskeletal proteins of dystrophin family.⁷²⁻⁷⁴ Interaction of syntrophin with dystrophin protein family is mediated by SU domain and adjacent PH domain localized at the distal half of syntrophin. In skeletal muscle, four potential syntrophin binding sites are present in each dystrophin complex: two on dystrobrevin and two on dystrophin or utrophin.⁷⁵ The presence of adjacent syntrophin binding sites in DGC complex suggests that syntrophin acts as an adaptor protein which facilitates the recruitment and organization of multiple signalling molecules together into functional complexes at plasma membrane, linking the cytoskeletal

elements with the plasma membrane.⁷⁶ This co-localization increases specificity and efficiency of signalling cascade.

4 | MODULATION OF CYTOSKELETAL FRAMEWORK BY THE DGC-SYNTROPHIN SCAFFOLD

Dystrophin, 427 kDa cytoskeletal protein, member of spectrin superfamily of actin-binding proteins, is organized into four distinct domains, an N-terminal actin-binding domain, a central rod region, a cysteine-rich (CR) domain and a C-terminal domain.⁷⁷ The N-terminal domain of dystrophin has a pair of Calponin Homology (CH) modules harbouring actin-binding motif (ABD1) that directly binds to actin.⁷⁸ ABD1 also connects the dystrophin to contractile apparatus in skeletal muscle by interacting with the costamere-enriched intermediate filament protein cytokeratin 19 (K19).^{79,80} The central rod domain composed of 24 spectrin-like repeats (SLRs) and four hinge regions conferring it properties for shock resistance, elasticity and flexibility.⁸¹ The rod domain also bears second actin-binding motif (ABD2) that in conjunction with ABD1 forms a strong lateral association with actin filaments.⁸² The CR region consists of a WW anti-parallel β -sheet motif containing two highly conserved tryptophan residues; two EF-hand-like motifs having helix-loop-helix fold and a ZZ module containing two zinc finger motifs.^{83,84} The WW motif interacts with β -dystroglycan and recognizes proline-rich sequences/phosphorylated linear peptide/PPxY motif in ligand.⁸⁵ The EF-hands along with ZZ modules bind to calmodulin in a calcium-dependent manner. The cysteine-rich domain also binds to ankyrin and intermediate filament synemin which further strengthens the link between cytoskeleton and sarcolemma via dystrophin.^{86,87} The C-terminal region contains two α -helical motifs (α -helical coiled coil) interrupted by a short proline linker that is necessary for full dystrophin function and interacts with molecules with similar regions such as syntrophin, dystroglycan.⁸⁸⁻⁹⁰ Dystrophin has two syntrophin binding sites in the C-terminal region that are flanked by coiled-coil motifs.⁹¹ Protein overlay assays, studying the association of syntrophin with dystrophin fusion protein; outlines the syntrophin binding site on GST fused human dystrophin protein. This study confined the syntrophin binding motif to terminal 251 amino acid residues (amino acids 3447-3481) encoded by exon 73 and 74 of dystrophin, directly upstream coiled-coil motif.^{74,91} The interaction of syntrophin with dystrophin was further confirmed in vivo using yeast two-hybrid system.⁹² Utrophin, like dystrophin, engrosses in similar association with syntrophin; however, its localization is restricted to zone enriched with acetylcholine receptor in neuromuscular junction.⁹³⁻⁹⁵ In dystrophin/utrophin protein complex, syntrophins act as an adaptor interacting with muscle voltage-gated sodium channels SkM1 and SkM2, cardiac voltage-gated sodium channels $\text{Na}_v1.5$ and neuronal nitric oxide synthase (nNOS) via its PDZ domain.⁹⁶⁻⁹⁸ Syntrophins thus link voltage-gated sodium channels to the actin cytoskeleton and extracellular matrix through dystrophin/utrophin and DGC complex.^{99,100}

FIGURE 2 Dystrophin-glycoprotein complex linking the contractile apparatus to Extracellular matrix by interacting with cytoskeletal and matrix proteins

Syntrophin proteins have also been shown to have a role in cell synapse modulated via the PDZ domain interactions with several proteins containing PDZ domains.^{44,52} The SU domain is primarily responsible for linking syntrophins to the membrane via the DGC by interacting with proteins like dystrophin and utrophin.^{53,72,101,102} There is an additional C-terminus amino acid tail in γ -syntrophins that encodes for a P-loop. This P-loop is considered to be a potential nucleotide (ATP/GTP) binding site.^{103,104} γ -syntrophins bind to the dystrophin protein in DGC via this P-loop, and the splice variant of γ -1 syntrophins that lack the P-loop fail to bind dystrophin.^{103,104}

α -dystrobrevin, 87 kDa membrane-associated protein, a critical member of DGC that interacts directly with α -1 syntrophin and dystrophin.^{1,89} α -dystrobrevin shows predominant expression in muscle cells (skeletal and cardiac) and in brain.¹⁰⁵ The domain organization of this protein is similar to that of dystrophin containing EF domain with two EF-hand motifs involved in calcium binding, a ZZ zinc finger domain binding to calmodulin, a syntrophin binding domain encoded by exon 13 and 14 (amino acids 427–480), and a coiled-coil domain consisting of two alpha helices interacts dystrophin/utrophin. α -dystrobrevin also has a unique C-terminal tail containing tyrosine residues that are phosphorylated under *in vivo* conditions.⁸⁹ α -dystrobrevin links DGC to intermediate filament network that are the key components of cell cytoskeleton via syncoilin, β -synemin/desmulin, dysbindin and plectin^{75,106–108} (Figure 2).

5 | BRIEF OVERVIEW OF CYTOSKELETAL ORGANIZATION

The cytoskeleton is composed of interconnected meshwork of filaments: actin microfilaments, microtubules and intermediate filaments that provide structural support to cells and maintain organization of multicellular tissues. The cytoskeletal proteins also participate in signal transduction pathways integrating various intracellular and extracellular signals received at membrane and thereby facilitating distinct cellular responses to the different signals. Cytoskeleton is critical for life, as it regulates many aspects of cell physiology including cellular division, cell migration, cell morphology, cellular and tissue integrity, extracellular matrix patterning and cell polarity. The actin filaments are highly conserved indispensable building block of cytoskeleton that are constantly being remodelled between two forms of actin, globular and monomeric G-actin and filamentous and polymeric F-actin. This transition between the two forms of actin is tightly regulated by different signalling, scaffolding and actin-binding proteins. Microtubules are also important components of cytoskeleton that organize the interior of cell by positioning the organelles in cytoplasm. These hollow cylindrical filaments composed of α - and β -tubulin are dynamic in nature and are involved in cell division, motility, intracellular transport, cell signalling etc. These are maintained by various microtubule-associated proteins (MAP). The dynamic remodelling of cytoskeleton is

crucial for most of cellular processes and malfunctioning or defective regulation of cytoskeletal proteins leads to serious consequences resulting in various human diseases such as cancer, Alzheimer's disease, Parkinson's disease and a myriad of other ailments.

6 | CYTOSKELETAL MODULATION BY SYNTROPHIN SIGNALPLEX

DGC and syntrophins act as a scaffold to promote the formation of a signalling complex that regulates the remodelling of the actin cytoskeleton. Syntrophins play a central role in organizing signalplex anchored to the dystrophin scaffold. The DGC provides structural stability to the cell membrane by forming a link between the actin cytoskeleton and the extracellular membrane in skeletal muscles. DGC is also pivotal in providing stability against the forces of contraction or relaxation in muscle cells.¹⁰⁹ DGC is also involved in modulating the actin cytoskeleton by recruiting various proteins, including syntrophins, involved in actin cytoskeletal organization to the membrane.¹¹⁰⁻¹¹² Syntrophins have been shown to have a pivotal role in executing the cytoskeletal modulating role of the DGC. The sub-cellular localization of syntrophins in muscle cells is controlled through the cytoskeletal reorganization in these cells. In addition to this, syntrophins themselves have been characterized as actin-binding proteins with α -1, β -1 and β -2-syntrophins been shown to bind F-actin.¹¹³ The second PH domain and the carboxy-terminal SU domain are primarily involved in the binding of syntrophins to actin. α -1 syntrophin has four actin-binding sites, three of which are present in the PH2 domain (amino acids 317-328, 349-357 and 497-505) and fourth in the SU domain. The amino acid sequences at these sites are highly conserved among syntrophin isoforms. The syntrophin-actin interaction further strengthens mechanical coupling between sarcolemma and actin filaments in costamers during muscle contraction. Syntrophins also help in the actin stabilization at the cell membrane and inhibit the actin-activated myosin ATPase activity.¹¹³ We have recently studied the role of F-actin in the α -1 syntrophin mediated signalling pathway. Loss of α -1 syntrophin tyrosine phosphorylation was observed upon actin depolymerization. This actin depolymerization mediated loss of α -1 syntrophin phosphorylation, in turn, prevents the interaction of Rac1 with α -syntrophin. The loss of α -1 syntrophin-Rac1 interaction further leads to a loss of Rac1 activation, which ultimately causes increased apoptosis, decreased cell migration and a decrease in ROS production. Loss of α -1 syntrophin phosphorylation by actin depolymerization may be responsible for all these downstream effects in breast carcinoma cells.⁴¹

Pleckstrin homology (PH) domains of syntrophins have been shown to play important roles in the cytoskeletal organization.⁵⁰ PH domains target several PH-containing proteins to the cell membrane. These are involved in lipid signal transduction by functioning as lipid-binding modules.^{114,115} Along with the PH domains, the PDZ domain of syntrophins recruits several membrane proteins to the cell membrane by acting as an adaptor.⁴⁶ α -1 syntrophin interacts

with a highly conserved COOH-terminal domain of Microtubule-Associated Protein 1 family (MAP1A, MAP1B, MAP1S) via its PH2 and PDZ domain.¹¹⁶ MAP1 proteins contain structurally and functionally conserved domains in their light and heavy chains that mediate heavy-light chain interaction, binding to microtubule and F-actin.^{117,118} MAP1 proteins interact with microtubules and various signalling proteins that maintain the stability, integrity and dynamics of microtubules as well as modulate the access and activity of microtubule-dependent motor proteins. The PDZ domain of β -2-syntrophin binds to the microtubule-associated serine/threonine kinase-205 (MAST205).¹¹ This binding involves a PDZ-PDZ domain interaction. MAP1 proteins and MAST205 kinase link the dystrophin/utrophin proteins in DGC with microtubule filaments via syntrophins, thus linking the DGC with the microtubule network.^{11,116}

Syntrophins have also been shown to bind lipid proteins like phosphoinositol-3-phosphate kinase (PI3K) and diacylglycerol kinase- ζ (DGK- ζ) on the inner surface of the membrane. These proteins are involved in regulating the lipid signalling pathways and actin cytoskeletal organization.^{110,119} The role of plasma membrane lipids in the regulation of actin dynamics is well documented.¹²⁰ Diacylglycerol kinase- ζ (DGK- ζ) phosphorylates membrane phospholipid phosphatidylinositol yielding Phosphatidyl inositol 4,5-bisphosphate (PI(4,5)P2). PI(4,5)P2 controls activity of various actin-organizing proteins by increasing their association with membrane proteins and subsequently triggering the signalling pathways that promote actin polymerization.^{121,122} Phospholipase C (PLC), a membrane-bound enzyme, hydrolyses PI(4,5)P2 into diacylglycerol (DAG) and inositol-3-phosphate (IP₃), which further contribute to changes in actin dynamics. IP₃ triggers the release of calcium ions from the endoplasmic reticulum, thereby, altering activity and function of many actin remodelling proteins.¹²³ DAG, an Allosteric activator of protein kinase C (PKC), binds to the cysteine-rich C1 domain of PKC and activates it. This DAG-mediated PKC activation causes dramatic remodelling of the actin cytoskeleton. In addition to this, DAG has been found to be an important intermediate in phospholipids and triglyceride synthesis.^{110,124} DAG also gets phosphorylated to phosphatidic acid (PA), activating various proteins involved in actin reorganization like p21 activated kinase 1 (PAK1), Raf-1, phosphatidylinositol-4-phosphate-5-kinase (PIP5K) and PKC ζ .^{52,74,125-127} PA also invigorates actin stress fibres assembly.^{128,129} DGK- ζ in association with syntrophin significantly regulates the state of actin organization. In skeletal muscles, PDZ domain of Syntrophin binds to the C-terminal PDZ binding sequence of DGK- ζ and forms a complex. PKC dependent phosphorylation of DGK- ζ MARCKS homology domain regulates the translocation of this complex from cytosol to plasma membrane. The membrane localization of DGK- ζ plays an important role in the regulation of DAG concentration and production of phospholipids at the membrane, which is critical for proper control of lipid signalling pathways regulating actin organization¹³ (Figure 3). γ -1 syntrophin has been found to co-localize with DGK- ζ and F-actin in the peripheral ruffles and lamellipodia in skeletal muscle cells.^{13,112} Co-localization DGK- ζ with Rac-1, a member Rho family of small GTPases that regulates organization

FIGURE 3 Syntrophin modulating cell cytoskeleton by regulating Ca^{2+} signalling networks in skeletal muscles

actin cytoskeleton, has also been reported. DGK- ζ in association with syntrophin forms a complex with Rac1 controlling actin reorganization in membrane ruffles and lamellipodia.^{110,130}

PH domain-containing proteins are recruited to the sites of receptor activation at the plasma membrane by signalling phosphoinositides such as phosphoinositol-3,4-bisphosphate [PI(3,4)P₂] and phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P₃].¹¹² These lipid signalling proteins tightly regulate changes in actin organization. They regulate actin cytoskeletal organization and reorganization in response to growth factor stimulation. PI3K being a component of many important signalling pathways regulates many important processes like cell survival, cell growth and motility.^{111,131} PI3K also plays an important role in the production of phosphatidylinositols. These phosphatidylinositols, in turn, lead to the activation of various downstream signalling pathways by recruiting PH domain-containing signalling proteins to the membrane. One of these PH domain-containing proteins is TAPP1 (Tandom PH domain-containing proteins1) adapter protein. TAPP1 is recruited to the plasma membrane of cells when PI3K is activated by growth factors like PDGF. PI-(3,4)-P₂ interacts with TAPP1 protein and translocates it to the plasma membrane. PI(3,4)P₂ binds to the TAPP1 protein via the C-terminal PH domain of TAPP1 protein.¹³² Reorganization of the actin cytoskeleton is one of the responses these signalling proteins initiate at the plasma membrane after they are activated. Hence TAPP1 functions as an actin reorganizing protein. TAPP1 has been shown to interact with syntrophins and has been shown to regulate the actin cytoskeletal organization in conjunction with the syntrophins.¹³²⁻¹³⁶ The intracellular

localization of a TAPP1 protein is determined by its binding to syntrophins. The C-terminal PDZ binding sequence of TAPP1 binds to the PDZ domain of α -1-, β -2- and γ -1-syntrophin.¹³² The signalling pathways that are initiated after the stimulation of PI3K by PDGF induce a rapid reorganization of the actin cytoskeleton, and it has also been shown that various components of DGC also have a role in the regulation of actin organization in response to PDGF signalling.^{75,99,137} In addition to this, TAPP2 proteins also link PI3K signalling with cytoskeleton by interacting with β -2-syntrophin and utrophin.¹³⁸

7 | SYNTROPHIN-ASSOCIATED PROTEINS AS MODULATORS OF CELL CYTOSKELETON

The syntrophin isoforms and the DGC in conjunction organize signalling components which lead to the reorganization of the actin cytoskeleton. Up to four different syntrophin proteins associate with the DGC at a time and recruit proteins to the plasma membrane. These signalling proteins bind via the PDZ binding motifs to the circular ruffles on the membrane which are induced by PDGF.^{75,139} One of the complexes comprises of the DGC, syntrophins, TAPP1 and the other downstream effectors. These protein complexes which include syntrophins stabilize the association of TAPP1 with the plasma membrane.^{112,137} Therefore, syntrophins play a pivotal role in the modulation of the cytoskeleton in the cell by controlling the localization and activity of the proteins like PI3K, PI(3,4)P₂ and DGK- ζ , TAPP1 and Rac1.

FIGURE 4 Phosphorylation of transcriptional factor c-Jun at Ser65 by Syntrophin signalling network leads to cell cycle progression. The inhibition of the phosphorylation can lead to cell cycle defects and apoptosis

α -1-syntrophin interacts with Grb2 via its proline-rich regions. This interaction is important for the activation of Rho family GTPase Rac1.⁸ The matrix laminin on the outside is linked to Grb2 binding to α -1-syntrophin on the inner surface on the sarcolemma and forms a signalling pathway via -Sos1-Rac1-PAK1-JNK, further leading to the phosphorylation of c-jun on Ser65.^{140,141} α -dystroglycan serves as receptor for laminin and binds to LG4-5 globular domain of laminin. The binding LG4-5 region of laminin to α -dystroglycan induces syntrophin phosphorylation at tyrosine residue and recruits SOS1/2 to Grb2¹⁴¹ (Figure 4). Rac1 protein has been shown to get immuno-precipitated with α -1-syntrophin.⁴¹ Rac1, in turn, is known to control many cellular processes one of which is the actin and microtubule cytoskeletal organization.^{142,143} The stability of the Rac1, PAK and RhoA signalling is important for driving the actin cytoskeleton dynamics and motility.¹⁴⁴ There must be the highly coordinated interplay between Rac1 and RhoA activities for cells to migrate effectively.^{145,146} High activity of one of these proteins warrants the low expression of the other one in order to maintain conflict-free actin cytoskeletal organization. Rac1 activity is high at the leading edge, and RhoA activity is high at the trailing edge.¹⁴²

8 | DISCUSSION AND CONCLUSION

Many functions of syntrophin proteins have been elucidated, and it has been seen that these membrane-associated adaptor proteins play a major role in recruiting various signalling molecules into signalling complexes. These signalling complexes are further involved in maintaining the proper functioning of the cell. Any disruption in any of these signalling pathways can lead to disturbances that can lead to a myriad of unwanted events in cell functioning. One of the unexplored tasks vis-à-vis syntrophins is their role in cytoskeletal organization. Syntrophins, associated with the DGC complex, have been shown

to be involved in the organization and maintenance of the cytoskeletal integrity of the cell. The components of DGC (which includes the syntrophin proteins) serve mechanical role maintaining the structural integrity of skeletal muscle fibres by mediating interaction between extracellular matrix, transmembrane and cytoskeleton proteins. Syntrophins also link voltage-gated sodium channels to the actin cytoskeleton and extracellular matrix through dystrophin/utrophin and the DGC complex. Syntrophins play a pivotal role in the modulation of the cytoskeleton in the cell by controlling the localization and activity of the actin reorganizing proteins like PI3K, PI(3,4)P2 and DGK- ζ , TAPP1 and Rac1. Syntrophins associate with various cytoskeletal proteins within the cells, and are, therefore, involved in key physiological processes like myogenesis, calcium homeostasis, cell migration and regulation of focal adhesions. Understanding the relationships between syntrophin proteins and cytoskeletal structures will pave the way to further the information about the functioning of syntrophins and their role in various signalling pathways. Further knowledge in this field will help us to understand how syntrophins help in the organization and maintenance of cellular cytoskeleton and other cellular events and molecular processes like cell growth, cell migration, proliferation and synapses, in addition to the understanding of various pathologies in which syntrophins are implicated like cancer, muscular dystrophy and Alzheimer's. A thorough understanding of the functioning of syntrophins and their role in cytoskeletal organization can pave the way for the design of novel therapeutics, where then can be used as markers or these can also be potentially used as therapeutic targets which can prove helpful in the prevention of pathologies such as cancer.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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