γ1- and γ2-Syntrophins, Two Novel Dystrophin-binding Proteins Localized in Neuronal Cells*

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Dystrophin is the scaffold of a protein complex, disrupted in inherited muscular dystrophies. At the last 3′ terminus of the gene, a protein domain is encoded, where syntrophins are tightly bound. These are a family of cytoplasmic peripheral membrane proteins. Three genes have been described encoding one acidic (α1) and two basic (β1 and β2) proteins of ~57-60 kDa. Here, we describe the characterization of two novel putative members of the syntrophin family, named γ1- and γ2-syntrophins. The human γ1-syntrophin gene is composed of 19 exons and encodes a brain-specific protein of 517 amino acids. The human γ2-syntrophin gene is composed of at least 17 exons, and its transcript is expressed in brain and, to a lesser degree, in other tissues. We mapped the γ1-syntrophin gene to human chromosome 8q11 and the γ2-syntrophin gene to chromosome 2p25. Yeast two-hybrid experiments and pull-down studies showed that both proteins can bind the C-terminal region of dystrophin and related proteins. We raised antibodies against these proteins and recognized expression in both rat and human central neurons, coincident with RNA in situ hybridization of adjacent sections. Our present findings suggest a differentiated role of a modified dystrophin-associated complex in the central nervous system.

Since the identification of dystrophin, the product of the Duchenne muscular dystrophy gene at Xp21, molecular genetics has moved quickly (1, 2). The deficiency of dystrophin in Duchenne muscular dystrophy (DMD)† and its first animal model, the mdx mouse, leads to a dramatic reduction in a group of previously unknown proteins identified as the dystrophin-associated protein complex.

In the last few years, the dystrophin-associated protein complex proteins have been isolated; their genes have been cloned; and the following model of the complex has been hypothesized (3–5). Dystrophin is a large rod-shaped protein, primarily localized beneath the muscle cell membrane. Its actinin-like N terminus binds F-actin (6), whereas its C terminus is anchored to the transmembrane protein, β-dystroglycan, which is linked through α-dystroglycan to the extracellular merosin (laminin-2) (7). Then, this complex bridges the muscle membrane from the cytoskeleton to the extracellular matrix. In addition, dystroglycan is the receptor for agrin, a protein with a pivotal role in the clustering of acetylcholine receptors at the neuromuscular junction (8–10) and a fundamental element of the basal lamina (11). At the muscle membrane, this complex is associated with the hydrophobic sarcospan DAP25 (dystrophin-associated protein; A5) (12) and the sarcoglycan complex, which is composed of at least four interacting transmembrane glycoproteins: α-sarcoglycan (DAG50 (dystrophin-associated glycoprotein), A2, adhalin) (13, 14), β-sarcoglycan (DAG43, A3b) (15, 16), γ-sarcoglycan (DAG35, A4) (17), and δ-sarcoglycan (18). Mutations in the laminin-α2 gene are responsible for congenital muscular dystrophy (19); mutations in the γ-, α-, β-, and δ-sarcoglycan genes cause limb-girdle muscular dystrophies 2C, 2D, 2E, and 2F (13, 15–17, 20, 21), respectively; and mutations in the caveolin-3 gene cause a form of autosomal dominant limb-girdle muscular dystrophy (22, 23). In addition, animal models have been identified (24) or created by homologous recombination to establish the role of each component of the dystrophin-associated protein complex (25–28).

The extreme C terminus of dystrophin, which lies beneath the muscle membrane, is associated directly with a group of cytoplasmic peripheral membrane proteins known as syntrophins (29–34). A similar interaction has been demonstrated with utrophin, the autosomally encoded dystrophin-related protein (35), and with some dystrobrevin isoforms (36–38). The three known syntrophin isoforms, α1, β1, and β2, are encoded by distinct genes with specific expression (33). α1-Syntrophin is most abundant in skeletal muscle, where it is located close to the sarcolemma together with β1-syntrophin. In contrast, β2-syntrophin is largely concentrated at the neuromuscular junction, but is barely detectable at the sarcolemma. Syntrophins bind directly to the C-terminal domain of dystrophin, in the region encoded by exons 74 and 75 (34, 39–41). This region is

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¶¶ The abbreviations used are: DMD, Duchenne muscular dystrophy; PH, pleckstrin homology; SU, syntrophin unique; EST, expressed sequence tag; bp, base pair(s); RT-PCR, reverse transcription-polymerase chain reaction; GST, glutathione S-transferase; PBS, phosphate-buffered saline; kb, kilobase pair(s); PZD, postsynaptic density 95/discs large/zona occludens-1; UTR, untranslated region.
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... was assayed by the Bradford method (Bio-Rad protein assay). The antigen was heated at 100 °C in the presence of Laemmli buffer for 5 min. After separation by SDS-PAGE, the proteins were transferred to nitrocellulose membranes and used as probes for the immunoblot analysis. The membranes were blocked with 5% defatted milk in PBS for 2 h before being incubated with primary antibodies diluted 1:2000 at 4 °C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated antibodies (Amersham Pharmacia Biotech), diluted 1:50000 in PBS. The protein bands were detected using ECL reagents (Amersham Pharmacia Biotech). A Ponceau S stain was used to verify equal loading of proteins. The antigenic bands were detected by exposure to X-ray film for 2–3 days.

**Immunofluorescence**: Human skeletal muscle cryo-sections were prepared as described previously (18). The sections were incubated in 3% bovine serum albumin, 0.3% Triton X-100, and 0.05% NaN3, followed by the primary antibody diluted 1:200 in PBS. The sections were incubated with Alexa Fluor 594-conjugated secondary antibodies (1:500) for 1 h at room temperature. The sections were then washed and counterstained with DAPI (1:5000). The sections were visualized using a confocal microscope (Olympus, Japan). The images were processed using Image J software (National Institutes of Health, USA).

**Western Blotting**: SDS-PAGE gel electrophoresis and Western blot analysis were performed as described previously (18). The blots were incubated with primary antibodies. The membranes were exposed to X-ray film for 3–5 days. The bands were detected by exposure to X-ray film for 1–2 days.

**Immunoprecipitation**: The immunoprecipitation was performed using rabbit polyclonal antibodies against the GST fusion proteins. The immunoprecipitates were analyzed by Western blotting. The blots were incubated with primary antibodies. The membranes were exposed to X-ray film for 2–3 days.

**Immunohistochemistry**: Immunohistochemical staining was performed using rabbit polyclonal antibodies against the GST fusion proteins. The sections were incubated with the primary antibody, followed by the secondary antibody conjugated to a fluorophore. The sections were visualized using a confocal microscope (Olympus, Japan). The images were processed using Image J software (National Institutes of Health, USA).
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The former cDNA, initially named syn4, is 1898 bp long and includes an open reading frame of 1554 nucleotides (GenBankTM/EBI accession number AJ003030). It encodes a protein of 517 amino acids with a predicted molecular mass of 57,932 Da and a calculated isoelectric point of 6.24. The latter cDNA, initially named syn5, is 1938 bp long and contains an open reading frame of 1620 nucleotides (GenBankTM/EBI accession number AJ003029). It encodes a protein of 539 amino acids with a predicted molecular mass of 60,066 Da and a calculated isoelectric point of 7.59. Some differences have been observed for a few syn4 and syn5 clones. These are likely due to alternative splicing. For both cDNAs, the sequence flanking the first ATG is in accordance with the Kozak consensus for translational start sites (53). In agreement with other investigators, we have renamed syn4 and syn5 as 1-y-syntrophin and 2-y-syntrophin, respectively.

Peptide similarity (40–44%) to the human syntrophins spanned the entire open reading frames. Similarity to a Caenorhabditis elegans protein (U49829) is higher (46–52%). The 1- and 2-syntrophins share a 73% amino acid similarity, thus suggesting that the two proteins are much more closely related and probably derived from a common single syntrophin precursor.

Computational Analysis of Protein Sequence—The syntrophin sequences share distinctive conserved motifs: two PH domains and one PDZ domain, with the first PH domain split by a glycine-rich loop. The PH domain is not easy to recognize since it includes a series of relatively poorly conserved peptide interspersed with less conserved linker sequences. These may vary from a few amino acids to 100 or more, often containing other functional domains. Nevertheless, the C-terminal 15 or so amino acids contain only an invariant Trp residue, and the six N-terminal residues from this amino acid frequently include two or more negatively charged residues as well as glutamic acid (46). The alignment shows that both the C-terminal Trp residues of the PH1 and PH2 domains are present in 1- and 2-syntrophins (Fig. 1), suggesting that both domains can be conserved.

The SU domain is less homologous, with only a weak similarity to the other three syntrophins. The proposed secondary structure of the SU domain is composed of three to five β-sheets separated by as many turns (33). Using the PHDsec algorithm (50), the predicted secondary structure of γ1- and γ2-syntrophins is mainly arranged into an α-helix.

We analyzed γ1- and γ2-syntrophins using the PROFILES-CAN program. A PDZ domain was identified in both proteins (residues 57–140 for γ1-syntrophin and residues 73–156 for γ2-syntrophin) and only one PH domain, corresponding to the PH2 domain of the other syntrophins (residues 283–390 for γ1-syntrophin and residues 296–421 for γ2-syntrophin) (Fig. 1). No other domain was found. In addition, we have also identified, in both proteins, an ATP/GTP-binding site motif A (P-loop) (54) (residues 440–448 for γ1-syntrophin and residues 471–479 for γ2-syntrophin). This motif is a glycine-rich region that typically forms a loop between a β-strand and an α-helix. This loop interacts with one of the phosphate groups of the nucleotide. Many potential phosphorylation sites have also been identified with no clear relationship to the other syntrophins.
Phylogenetic analysis of all syntrophins establishes a common origin of the syntrophins with an early separation into two groups: the first including α1-, β1-, and β2-syntrophins, and the second including γ1- and γ2-syntrophins. The two C. elegans syntrophins are final confirmation.

Tissue Distribution of γ1- and γ2-Syntrophin Expression—The expression of γ1- and γ2-syntrophins in human and rat adult tissues was assayed by Northern blotting, using fragments of the respective cDNAs as probes.

Expression of γ1-Syntrophin Is Brain-specific in Humans and Rats—In man, only an ~7.0-kb transcript was observed using a 426-bp cDNA probe encoding the last 126 amino acids (Fig. 2a). Conversely, the rat cDNA probe (701 bp), corresponding to amino acids 29–290 of human γ1-syntrophin, hybridized with three equally abundant transcripts of ~2.6, 3.4, and 7.5 kb (Fig. 2b). The different probes may reflect the different expression pattern. The mRNAs are much longer than the coding sequence, and a long 3'UTR and/or 5'UTR is probably present. The expression of further γ1-syntrophin isoforms most likely originates by alternative splicing. For example, exon 18 is in-frame spliced out in clone 49263, and so is the fourth exon in another γ1-syntrophin cDNA clone. Other alternatively spliced products were identified by RT-PCR (data not shown).

Conversely, γ2-syntrophin has a broader but weaker expression. A clearer picture of γ2-syntrophin comes from rat Northern blots (Fig. 2c). In addition to brain with two transcripts of ~2.1 and 2.3 kb and a third very weakly hybridizing transcript of 2.5 kb, a mRNA of 2.1 kb was also observed in testis. Weak signals at 0.8, 2.2, and 2.5 kb were also present in kidney and lung, as well as a weaker 1.6-kb transcript in heart.
These different transcripts are probably related to isoforms of this gene. Evidence for this hypothesis comes from cDNA library screening and RT-PCR of human brain cDNA. In particular, we have characterized two isoforms. In the first, exons 3–5, corresponding to the first half of the PDZ domain, are in-frame spliced out; and in exon 9, a putative 5′-donor splicing site at bp 693 leads to in-frame deletion of the last 27 bp of this exon, corresponding to amino acids 830–839. Interestingly, the consensus of the protein kinase C phosphorylation site can be recognized in this fragment. In the second isoform, exons 3–6 are equally in-frame spliced out, and the PDZ domain is nearly entirely eliminated.

Genomic Structure and Chromosomal Mapping—Genomic library screening and long-range and vectorette PCR were used.

### Table I

| Exon | Exon-intron boundaries | Exon length | Regions/domains | Splicing phase |
|------|------------------------|-------------|-----------------|---------------|
| 1    | TTCCTTAATGGATGAAAGTACCTCTCC ... GCTCGGCAGgtaagtgatagcgctattat | >400 | 5′-UTR |             |
| 2    | tttttattctctcgctctagAGTGCCTCTCT ... TAATTCAGtaaagaccattacgtgcattat | 75 | 5′-UTR |             |
| 3    | cctttttctgtcatctcagCGACATCC ... TGGAGAAGgtagtaacagctctctctgc | 54 | 5′-UTR, includes 1st Met | 0 |
| 4    | ttttttttttaatctcagAAACAGACA ... TATTAGGAGgtaagactttataaagaata | 135 | PDZ domain | 0 |
| 5    | taaaaatctctgtctctcagAAAGAAGCG ... AGCATAAAggtagccctctctctctct | 57 | PDZ domain | 0 |
| 6    | atatgttttttcctttctcagGCAGGAGCA ... AAAAAAGAgtaatatgtagkagatgg | 58 | PDZ domain | 1 |
| 7    | ttgtctttttctctcagCGCAATTCT ... ATCTCAGAgtatcttttatctata | 44 | PDZ domain | 0 |
| 8    | tgtttttttttctctcagAGTTAAGGCC ... GAGAAGTAggtgtagtttctttctctaa | 42 | PDZ domain | 0 |
| 9    | cttttttttttctctcagGTAGCATCT ... ATTGTGCAGgtagactttataaagaata | 103 | PDZ domain | 1 |
| 10   | tagtgattttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 83 | PDZ domain | 0 |
| 11   | gatattttttttctttctcagAGACACATTA ... TTTGAGCAGgtgtagctgctttctct | 131 | PDZ domain | 2 |
| 12   | ttgtctctgacatctcagAGCAATGCC ... AAGCAGCATgaatagatctcagga | 130 | PDZ domain | 0 |
| 13   | ttatttatttatttcgatctcagATTTAAAAA ... AACCAGAGgtagataaactcagacttg | 39 | PDZ domain | 0 |
| 14   | tggattttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 117 | PH domain | 0 |
| 15   | ttattttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 72 | PH domain | 0 |
| 16   | tattttttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 153 | PH domain | 0 |
| 17   | attagttttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 93 | PH domain | 0 |
| 18   | gttttttttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | 111 | ATP/GTP-binding site motif A (P-loop) | 0 |
| 19   | cgattttttttttctctcagAGTGCCTCAA ... AACAATACAgtagataaactcgtgtagaa | >400 | Stop codon 3′-UTR | |
### Table II

Genomic organization of γ2-syntrophin

| Exon | Exon-intron boundaries | Exon length | Regions/domains | Splicing phase |
|------|------------------------|-------------|-----------------|---------------|
| 1    | ACCACGGCGACGGGCGGCA AGTGGGACACC ... CTCGCAGCG ... | >210 5'-UTR, includes 1st Met | 0               |
| 2    | tttcacttctcgacagAGGAAAACCG ... | 138         | 0               |
| 3    | tttttacgtcttcaggGGAAGGCTTCT ... | 57 PDZ domain | 0               |
| 4    | cagacttccctcggagcAGTCGACAAG ... | 58 PDZ domain | 1               |
| 5    | taatattgcacttcttcagGTAATGCG ... | 44 PDZ domain | 0               |
| 6    | tttttggctatattgacagGTGCATCTG ... | 42 PDZ domain | 0               |
| 7    | cttctctgttggtcagagGTTCCCAGG ... | 88 PDZ domain | 1               |
| 8    | tttctctcgatattgacagGTGCATCTG ... | 92         | 0               |
| 9    | tttctctccttgcttcagcAGCCCCTAG ... | 128        | 2               |
| 10   | agactttctctccccagcAGTGAAGTG ... | 130        | 0               |
| 11   | cttctgctctctcagacaAGTAAGATG ... | 39         | 0               |
| 12   | taatatttcaccccctctcagGTTGGCAG ... | 117        | PH domain       |
| 13   | Undefined GTGAGCACA ... | 72         | PH domain       |
| 14   | atcccagctcttggtctagCTCAGACAAG ... | 93         | 0               |
| 15   | aaatgtagatctttctcagTCCAAGAACA ... | 207        | PH domain       |
| 16   | tttatattttcttctcagcAATGTTGCTC ... | 111        | ATP/GTP-binding site motif A |
| 17   | gatctgatattttcctcagGAACTCGAG ... | >310       | Stop codon 3'-UTR |

To determine exon-intron boundaries of these genes, the **γ1**-syntrophin gene (SNTG1) has 19 exons (Table I). The first two exons contain the 5'-UTR, and the first methionine is in the third exon. The **γ2**-syntrophin gene (SNTG2) has at least 17 exons (Table II). The first exon includes the first methionine. For both genes, the termination signal in the last exon is followed by a 3'-UTR colinear with the genomic sequence. The exon structure and splicing sites are conserved in the coding sequences between **γ1**- and **γ2**-syntrophins (Tables I and II), suggesting a common origin.

Two methods were employed for chromosomal mapping. For **γ1**-syntrophin, PCR primers, designed from genomic sequences around exons 1 and 19, were used to screen a yeast artificial chromosome clone (Sntg1-539) and purified on glutathione-agarose beads. The full-length coding sequences of **γ1**- and **γ2**-syntrophins were then cloned, as sense and antisense, into the pCT plasmid and transfected into COS-7 cells. The protein extracts were incubated with the bead-bound fusion proteins and, after extensive washing, analyzed by Western blotting.

To detect **γ1**- and **γ2**-syntrophins, we used immunopurified rabbit antibodies raised against GST-**γ1**-syntrophin-(195–302) and GST-**γ2**-syntrophin-(209–299) fusion proteins. The **γ1**- and **γ2**-syntrophins were affinity-purified by the GST-dystrophin/dystrobrevin bead-bound fusion proteins, and no signals were observed in control lanes (Fig. 3).

**In Vivo and in Vitro Assays of Binding with Dystrophin**—The α1-, β1-, and β2-syntrophins have been shown to bind dystrophin, utrophin, and dystrophreins in vitro (34, 35, 39, 40, 55). To verify whether **γ1**- and **γ2**-syntrophins can also bind dystrophin or related proteins, assays of protein-protein interaction were used. In addition, we performed a GST pull-down assay. The C terminus of dystrophin or α- or β-dystrobrevin was fused to glutathione S-transferase protein in the pGEX-2TK plasmid, expressed in *E. coli* cells, and purified on glutathione-agarose beads. The full-length coding sequences of **γ1**- and **γ2**-syntrophins were then cloned, as sense and antisense, into the pCT plasmid and transfected into COS-7 cells. The protein extracts were incubated with the bead-bound fusion proteins and, after extensive washing, analyzed by Western blotting.

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The two-hybrid system is a yeast-based genetic assay to detect *in vivo* protein-protein interaction (56, 57). In the assay, one protein is fused with the DNA-binding domain, and the other with the transcription activation domain of GAL4. Should an interaction occur, the resulting dimer induces reporter gene activation (*HIS3* and *lacZ*). To confirm this interaction *in vivo*, the dystrophin C terminus (residues 3194–3685) corresponding to exons 66–79 and regions of α-dystrobrevin (residues 422–517) and β-dystrobrevin (residues 444–606), all with the syntrophin-binding site, were fused to the DNA-binding domain of GAL4 in the pGBT9 plasmid (pGBT9-DYS, pGBT9-DTNA, and pGBT9-DTNB, respectively), whereas γ1-syntrophin (residues 19–517) and γ2-syntrophin (residues 1–539) were fused to the activating domain of GAL4 in the pGAD plasmid (pGAD-G1SYN and pGAD-G2SYN, respectively). In addition, human α1-syntrophin (residues 173–505), fused to the activating domain of GAL4 in the pGAD plasmid (pGAD-A1SYN), was used as a positive control of interaction with dystrophin and related proteins. The pGBT9-DYS, pGBT9-DTNA, and pGBT9-DTNB plasmids were cotrans-
formed in yeast strain YRG-2 with pGAD-G1SYN, pGAD-G2SYN, and pGAD-A1SYN on selective Leu2 and Trp2 plates and then streaked on His2 plates to test the activation of the HIS3 reporter gene.

The g1- and g2-syntrophins interacted with dystrophin in vivo (Fig. 4, a and b). For g1-syntrophin, we also used an isoform without exon 18 (pGAD-G1SYN D18) that includes the ATP/GTP-binding site. This isoform did not interact with dystrophin, confirming that the dystrophin-binding site is located at the C terminus, presumptively in the last 80 amino acids.

\( \alpha \)-Dystrobrevin interacted with g2-syntrophin, but only weakly with g1-syntrophin (Fig. 4c). Conversely, both syntrophins bound to \( \beta \)-dystrobrevin (Fig. 4d). These findings were confirmed by testing for \( \beta \)-galactosidase activity (data not shown).

In Situ Hybridization in Rat Central Nervous System Tissues—g1- and g2-syntrophin mRNAs were detected by in situ hybridization in all rat central nervous system regions examined; and in particular, they were highly expressed by neuronal cells. The expression of syntrophin transcripts was mainly restricted to cells with neuronal morphology. Hybridization grains for the g1- and g2-syntrophin mRNAs were localized in the perikaryon and proximal portion of the neuronal processes. Strong hybridization signals were localized in the hippocampus, neuron-rich dentate granule cells, and pyramidal cell layers (Fig. 5). Intense labeling was observed in neurons of the cerebral (parietal and frontal) cortex (Fig. 5G). g1- and g2-syntrophin mRNAs were also expressed in the cerebellar cortex, deep cerebellar nuclei, thalamus, and basal ganglia (data not shown). A very strong mRNA signal was present within neurons of both anterior and posterior horns of the spinal cord, whereas a lower signal could be detected in the white matter (Fig. 5 C and E). No specific signal over the background was detectable after hybridization of adjacent sections with the sense strand probe (data not shown). This confirmed the specificity of hybridization. Moreover, RNA studies were concordant with the distribution of the g1- and g2-syntrophin immunoreactivities. Using polyclonal antibodies raised against g1- and g2-syntrophins, we observed reactivities in the same cell populations containing abundant levels of the corresponding mRNAs (Fig. 5, B, D, F, and H), thus confirming that g1- and g2-syntrophin gene products are indeed highly expressed in the central nervous system. The widespread, albeit uneven, distribution of g1- and g2-syntrophin transcripts and proteins throughout different cerebral and spinal areas suggests that g1- and g2-syntrophin genes play an important housekeeping role in neurons.

FIG. 3. In vitro assay of interaction with dystrophin and related proteins by GST pull-down assay. In both Western blots (to the left), the specificity of affinity-purified polyclonal antibody was tested on a panel of GST fusion proteins. COS-7 protein extracts in which the expression of g1-syntrophin (a) or g2-syntrophin (b) had been induced were incubated with GST bead-bound fusion proteins: GST alone (lane A), GST-DYS (lane B), GST-DTNA (lane C), and GST-DTNB (lane D).

FIG. 4. In vivo assay of interaction with dystrophin and related proteins using the yeast two-hybrid system. The g1- and g2-syntrophins were tested with dystrophin (a and b) and with \( \alpha \)- and \( \beta \)-dystrobrevins, respectively (c and d). In the table, the cotransformed plasmid pairs are indicated for each sector of plates. Dyn, dystrophin; DtnA, \( \alpha \)-dystrobrevin; DtnB, \( \beta \)-dystrobrevin; Syn, syntrophin.
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Immunofluorescence of Human Muscle—No signal was found with anti-γ1-syntrophin antibody (Fig. 6A). γ2-Syntrophin showed a strong sarcolemmal immunoreactivity in all muscle fibers (Fig. 6B). In DMD patients with absence of dystrophin, γ2-syntrophin was absent or severely reduced (Fig. 6C); In contrast, a normal plasmalemmal signal was observed in patients with neurogenic atrophy (Fig. 6D). Upon immunofluorescence, γ2-syntrophin was not selectively localized at the neuromuscular junctions and was present on the membrane of cardiomyocytes in two biopsy specimens (data not shown).

DISCUSSION

Originally identified in postsynaptic membranes of T. californica (29), syntrophins are intracellular peripheral membrane proteins of ~58 kDa that, in man and mouse, exist in three highly conserved but distinct isoforms (α1, β1, and β2) encoded by different genes (30, 31, 33). The name “syntrophin” was introduced to indicate that this group of proteins accompanies dystrophin. Further studies have demonstrated that syntrophins also accompany all the other proteins of the dystrophin family such as utrophin (dystrophin-related protein 1) (35), dystrophin-related protein 2, and dystrobrevins (α and β) (37, 38). Binding is mediated by amino acid sequences that are homologous to the cysteine-rich domain and C-terminal region of dystrophin encoded by exon 74 (34, 39, 40).

The role of syntrophins and their requirement for muscle and nerve cell function are still obscure. To date, no human disease has been associated with a syntrophin gene mutation. Likewise, no gross histological changes in the skeletal muscle of α1-syntrophin knockout mouse (the first syntrophin-deficient animal model) have been reported (58). This may be due to a redundancy of the genes of the syntrophin family. The lack of function of one gene could be replaced by other members of the family, coexpressed in the same tissues. In man, the α1-syntrophin transcript is predominantly expressed in skeletal and cardiac muscle, whereas the β1- and β2-syntrophin transcripts are expressed in a wide variety of tissues (31, 33, 37). In addition, histochemical studies with specific antibodies revealed that the three syntrophins are all present at the neuromuscular junction. α1-Syntrophin is also found at the sarcolemma, whereas β1-syntrophin occurs at the sarcolemma of fast twitch muscle fibers (37). We indicate here that the syntrophin gene family should include at least two other members,
which are less related to the primary sequences of known syntrophins, but retain the property of dystrophin binding. γ1- and γ2-syntrophins should be considered a separate entity because of their relatedness (73% similarity), the common C. elegans ancestor gene, and the genomic organization. In particular, these two genes are split into 17–19 exons by long introns at corresponding positions that are different from the exon-intron boundaries found in the other syntrophins. In addition, we observed a complex pattern of alternatively spliced products, with the presence, at least for γ2-syntrophin, of both translated and nonfunctional transcripts.

Expression data indicate that γ1-syntrophin is restricted to neurons. γ2-Syntrophin has a broader expression and a more complex pattern of splicing and is presumably included in the dystrophin-associated complex beneath the muscle membrane at the sarcolemma. In fact, some DMD patients show a second-dystrophin-associated complex pattern of splicing and is presumably included in the same complex. In vivo, γ2-syntrophin has a broader expression and a more complex pattern of with the presence, at least for γ2-syntrophin, of both translated and nonfunctional transcripts.

The ability of γ1- and γ2-syntrophins to bind dystrophin and dystrobrevins (α and β) has been confirmed in vivo and in vitro. Binding is mediated by the last 80 amino acids, a region with weak similarity to the SU domain, the putative dystrophin-binding site. The homologous cysteine-rich domain and C-terminal region of dystrophin and related proteins include the dystrophin-binding site and several potential binding domains (59, 60). In particular, a coiled-coil motif, flanking the syntrophin-binding site, links dystrophin to the same motif in the C terminus of dystrobrevin. Therefore, dystrophin interacts with dystrophin-glycoprotein complexes via the cysteine-rich domain and heterodimerizes with dystrobrevins via the coiled-coil motif (61). Together, dystrophin and dystrobrevin or can recruit two syntrophins. This model seems to be confirmed by the observation that dystrophin complexes are highly enriched in α1- and β1-syntrophins, whereas utrophin complexes contain mostly β1- and β2-syntrophins (37). Different pairings are possible between the syntrophins: γ1- and γ2-syntrophins increase the possible combinations in brain.

DMD and Becker muscular dystrophy patients with point mutations or deletions localized in the 3′-region of the dystrophin gene in addition to the progressive muscle wasting show a higher incidence of mental retardation, with learning disorders and speech difficulties (62). In contrast, mutations localized in other parts of the gene are usually not associated with mental retardation. There is no explanation for this clinical observation because dystrophin is absent or severely reduced in DMD patients, regardless of where the primary nonsense mutation occurs. The presence of additional promoters located in certain dystrophin gene introns could still generate functional mini-dystrophin 3′-transcripts (apodysphins), unless the mutation does not directly involve the 3′-exons. The latter part of the dystrophin gene encodes the region endowed with the dystroglycan-binding site (exon 65) and the syntrophin-binding site (exons 73–74).

In the brain, γ1- and γ2-syntrophins as well as β-dystrobrevin can bind dystrophin isoforms Dp71 and Dp140 (63). In common with dystrophin and β-dystrobrevin, γ1- and γ2-syntrophins are found in the cortex and hippocampal formation. This data provide evidence that the composition of the dystrophin-associated protein complex in the brain differs from that in muscle (64). A mouse transgenic overexpressing apodysrophin-1/Dp71 (exons 63–79) in dystrophin-deficient animals (mdx mice) could not restore the normal muscle phenotype (65, 66). This suggests that this dystrophin fragment alone has no influence on the muscle disease progression. It is possible that the presence of an intact C-terminal fragment could be important for the associated mental disorders. The apodysaphrin-dystroglycan complex in the central nervous system can bind the γ1- and γ2-syntrophins. Further studies are needed to determine the role of these novel syntrophins in neuron signaling processes and whether their concomitant lack affects learning.
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